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Abstracts of Scientific Posters

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Organizing Committee Note: Posters can be viewed in the Exhibit Hall. All posters will be posted for two and one-half hours. The presenting author will be in attendance during the final hour. Poster numbers not listed in this document have been withdrawn by the authors.

Tuesday, July 26, Poster Sessions

10:00am-12:30pm

2:00pm-4:30pm

Wednesday, July 27, Poster Sessions

10:00am-12:30pm

2:00pm-4:30pm

Thursday, July 28, Poster Sessions

9:30am-12:00pm

Tuesday AM, July 26

Poster Session: 10:00 am - 12:30 pm
Animal Clinical Chemistry

A-01

Validation of the Two-Color Analysis of Mouse Platelet Activation Kit by Emfret Analytics for use in assessing platelet activation in mice

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Under normal conditions, platelets become activated during hemostasis, however, they may also become activated under pathological conditions resulting in clot formation. In order to assess the potential for thromboembolism in preclinical animal studies, an assay was needed to evaluate platelet activation in mice. The Two-Color Analysis of Mouse Platelet Activation Kit is a commercially prepared product manufactured by Emfret Analytics, Eibelstadt, Germany. The kit contains PE labeled JON/A antibody and FITC labeled Wug.E9 antibody. JON/A binds to mouse integrin αIIb/βIIIa upon the conformational change in activated platelets. Wug.E9 binds with mouse P-Selectin (CD62P). Platelet alpha granuoles express P-Selectin and it is transferred to the membrane upon activation. The kit was purchased and validated for use in study work. Blood was collected from the descending aorta of mice with a preloaded syringe containing 3.2% sodium citrate. A 9:1 ratio of blood to anticoagulant was mixed gently by inversion inside the syringe and then transferred to an Eppendorf tube. Equal volumes of JON/A and Wug.E9 antibodies were combined before use. The use of an agonist was essential in validating the kit's ability to detect activated platelets in mice. Convulxin was chosen as the suitable agonist and the appropriate concentration (1.43 µg/ml) was added to the antibody mixture. Blood was diluted 1:25 with modified Tyrodes-Hepes Buffer and Calcium Chloride, and then added to a well of 96 well microtiter plate containing the antibody/agonist mixture for a total volume of 35 µl. The plate was incubated for 15 minutes at room temperature. The blood/antibody mixture contained in the well was transferred to a tube containing PBS to stop the reaction. Samples were analyzed using the FACSCanto II flow cytometer within 30 minutes.

The median fluorescent intensity of JON/A and Wug.E9 was determined from the platelet population. The increase fold induction of fluorescence of activated platelets was compared to that of resting platelets. Platelets stimulated with Convulxin showed an 11 to 23 fold induction in fluorescence with the JON/A PE antibody and a 14 to 51 fold induction in fluorescence with the Wug.E9 FITC antibody. Replicate reproducibility within samples was less than 7%.

An indication that the platelets are in an activated state is demonstrated by the significant increase in fluorescence with both the JON/A and Wug.E9 antibodies.

The Two-Color Analysis of Mouse Platelet Activation Kit, when used with citrated mouse blood and in the in the presence of the agonist, Convulxin, was successful in detecting activated platelets, therefore, the kit will be acceptable for use in assessing platelet activation in mouse studies.

A-02

Modification of an Immunoradiometric Assay to Support Analysis of Low Volume Samples Frequently Encountered in Mouse Safety Assessment

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Background: The domesticated mouse is often used as a robust model in medical research but seldom as the choice rodent in safety assessment. Mice have several advantages over rat being economical to maintain, having short gestation periods and life spans, and are an advantage when test articles are expensive and in short supply. However, their small size precludes them from procedures that require large or repeated samples of blood such as clinical pathology and biomarker analyses. We were recently faced with a study with mouse as the alternate rodent species requiring the validation of a method for the measurement of calcitonin.

Methods: An ALPCO rat immunoradiometric assay (IRMA) test kit (Product #31-CALRT-R100) was used for calcitonin determination. The kit is a two site sandwich assay that uses a monoclonal antibody immobilised to beads and a radiolabelled polyclonal antibody for detection. The level of radioactivity detected is directly proportional to the amount of calcitonin. A Packard Gamma Counter with the Riasmart Software (Version 1.17) was used to conduct the analysis. The manufacturer specifications outlined in the package insert indicates that 200µl of serum or plasma would be sufficient to perform the assay in duplicate. We modified the assay specification to follow a single sample analysis format. The standard curve precision profile, precision, sensitivity, dilutional linearity, matrix effects and stability were investigated. Two quality controls (QCs) provided with the kit and in-house pooled mouse serum was used.

Results: The intra-assay precision was assessed by using the QCs and mouse serum in single replicates of 6 within one run, measurements ranged from 2.7-5.8% coefficients of variance (CV) for QC samples and was 4.7% for endogenous levels in pooled mouse serum. Inter-assay precision was performed by analyzing separate runs against at least three independent standard curves. The measurements ranged from 6.2-11.2% CV for QCs, and was 0.5% for the mouse serum pool. Dilutional linearity results showed consistent recovery of calcitonin in mouse serum at dilutions of 1:2 and 1:4. Matrix effects were none evident and well within acceptance criteria.

Conclusion: Preclinical research laboratories are often challenged with the task of providing multiple tests with small amounts of sample and within rodents where specifically designed kits fail to exist. This summarized approach to calcitonin demonstrates a plausible strategy and problem resolution relative to modifying a kit for use in mouse safety assessment. This particular assay design required duplicate analysis and subsequent reportable results back calculated from a mean. The volume needed for this assay would have ruled out the ability to report this biomarker as required for this study. In all, these results demonstrate that this quantitative method for the measurement of calcitonin using the ALPCO IRMA test kit in mouse serum is fit for purpose in the context of using single replicates for sample analysis.

A-03

Angiotensin-converting enzyme inhibitor curbs exaggerated renal cortical protein tyrosine nitration during the early stage of diabetes mellitus in the rat

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Background: During the early stage of diabetes mellitus (DM), renal cortical production of nitric oxide (NO) and superoxide anion ($O_2^{\bullet-}$) is increased, leading to peroxynitrite formation ($NO+O_2^{\bullet-}\rightarrow ONOO^-$). This oxidant enhances nitration of protein tyrosine residues to form 3-nitrotyrosine (3-NT), a process that may contribute to development of diabetic nephropathy. Angiotensin-converting enzyme (ACE) inhibitors, previously used exclusively for treatment of hypertension, have been recommended recently as an initial therapy for DM to decelerate development of diabetic nephropathy with or without hypertension.

Objective: The first goal of this study was to evaluate the hypothesis that ACE inhibition suppresses the increase in 3-NT production evident in the rat renal cortex during DM. The second goal of this study was to identify renal cortical proteins exhibiting reduced DM-induced nitration during ACE inhibition.

Methods: Four groups of rats (n=6 per group) were examined: 1) STZ group: rats studied 2 wks after induction of DM by streptozotocin injection (STZ, 65 mg/kg, *i.p.*), 2) Sham group: rats receiving the STZ vehicle, 3) STZ+ENAL group: STZ rats treated with enalapril (ENAL, an ACE inhibitor, 20 mg/kg in drinking water for 2 wks), and 4) Sham+ENAL group: ENAL-treated Sham rats. In each rat, we measured blood glucose, blood pressure and urinary creatinine excretion. $O_2^{\bullet-}$, NO and 3-NT production, as well as superoxide dismutase (SOD) activity, were measured in the renal cortex. Renal cortical nitrated protein was identified by proteomic analysis.

Results: Blood glucose levels were higher in STZ and STZ+ENAL groups than in Sham and Sham+ENAL groups (P<0.05), confirming development of DM. Blood pressure did not differ among groups. Urinary creatinine excretion was increased in the STZ group compared with Sham (P < 0.05), and this was prevented by ENAL treatment. Renal cortical $O_2^{\bullet-}$ and 3-NT production was greater in the STZ compared with Sham (each P<0.05); however, ENAL suppressed this phenomenon (each P<0.05 STZ vs. STZ+ENAL). Renal cortical NO production and SOD activity were elevated in the STZ group (each P<0.05 vs. Sham) and further increased in the STZ+ENAL group (each P<0.05 vs. STZ). Agarose 2-dimensional gel electrophoresis and western blotting revealed more than 20 spots with positive anti-3-NT-immunoreactivity in the renal cortex of the STZ group. The use of LC-MS/MS, SEQUEST algorithm and Swiss-Prot protein sequence databases revealed that ENAL conspicuously prevented enhanced nitration of three proteins: aconitase 2 (ACO2), glutamate dehydrogenase 1 (GLUD1) and aldehyde dehydrogenase 6 family, member A1 (ALDH6A1).

Conclusions: ACE inhibition protected against DM-induced 3-NT production and protein tyrosine nitration, effects that may result from suppressed oxidant ($O_2^{\bullet-}$) production and enhanced antioxidant effects (including SOD activation). Interestingly, each of the three proteins found to exhibit ENAL-sensitive nitration during DM resides in mitochondria, with ACO2 known to be sensitive to $O_2^{\bullet-}$ while GLUD1 and ALDH6A1 both have oxidoreductase activity. These observations suggest that preventing protein nitration at tyrosine residues by ACE inhibition may be useful in developing diagnostic biomarkers and therapeutic methods that can be employed during the early stage of DM to prevent or delay development of diabetic nephropathy.

A-05

Ranges of Commonly Evaluated Chemistry and Hematology Parameters from Clinically Healthy Cynomolgus Monkeys of Chinese Origin

W. Cherry, M. Heinitz, M. Trimble. *Covance Laboratories, Chandler, AZ,*

Background: Chemistry and hematology parameters commonly evaluated in toxicology studies were summarized from clinically healthy naïve cynomolgus monkeys (*Macaca fascicularis*) of Chinese origin.

Methods: Samples were collected from naïve monkeys (age 2.5-9.1 years) after fasting overnight using serum separator tubes with no anticoagulant for chemistry and potassium EDTA anticoagulant for hematology. Samples were analyzed for chemistry utilizing a Roche Modular and a Siemens Advia 2120 for hematology.

Conclusion: These ranges are helpful in establishing typical values from clinically healthy naïve cynomolgus monkeys and can serve as a baseline in newly established research laboratories.

Results:

	GLU mg/dL		UN mg/dL		CREA mg/dL		CHOL mg/dL		AST U/L		ALT U/L		ALP U/L	
	Males	Females	Males	Females	Males	Females	Males	Females	Males	Females	Males	Females	Males	Females
N	541	519	541	519	541	519	541	519	541	519	541	519	541	519
Min	39	37	9	10	0.4	0.3	64	81	19	15	13	17	59	89
Max	176	129	37	62	1.6	1.5	219	242	942	377	247	361	2400	711
Median	77	69	19	21	0.9	0.7	125	140	39	38	44	44	315	271
Mean	77	71	20	21	0.9	0.7	128	142	44	42	46	51	333	284
SD	17.8	14.9	3.4	4.4	0.25	0.15	29.3	27.9	43.9	21.1	22.5	30.1	175.1	113.8
	TP g/dL		ALB g/dL		Ca mg/dL		TBIL mg/dL		PHOS mg/dL		TRIG mg/dL		GGT U/L	
	Males	Females	Males	Females	Males	Females	Males	Females	Males	Females	Males	Females	Males	Females
N	541	519	541	519	541	519	540	517	541	519	541	519	541	519
Min	6.5	5.2	2.8	2.3	8.6	8.6	0.1	0.1	3.7	2.8	14	15	23	10
Max	9.4	9.1	5.5	5.6	13.7	14.6	0.5	0.8	13	10.5	119	133	332	311
Median	7.8	7.6	4.6	4.5	10.2	10.1	0.2	0.2	6.6	5.7	38	47	66	54
Mean	7.9	7.6	4.6	4.4	10.3	10.2	0.2	0.2	6.6	5.8	41	49	70	57
SD	0.49	0.52	0.34	0.39	0.70	0.68	0.08	0.10	1.21	1.09	15.6	15.6	28.2	23.9
	Na mmol/L		K mmol/L		Cl mmol/L		AGR		GLOB g/dL					
	Males	Females	Males	Females	Males	Females	Males	Females	Males	Females				
N	541	519	541	519	541	519	541	519	541	519				
Min	137	140	3.7	3.6	94	96	0.8	0.7	2.3	2.2				
Max	169	163	7.6	7.1	113	115	2	2.1	4.7	4.4				
Median	151	148	4.9	4.9	104	105	1.4	1.4	3.2	3.2				
Mean	151	149	5.0	4.9	104	105	1.4	1.4	3.3	3.2				
SD	4.3	3.9	0.61	0.59	2.9	2.7	0.20	0.21	0.38	0.36				

	Red Blood Cell Count (RBC) E6/uL		Hemoglobin (HGB) g/dL		Hematocrit (HCT) %		Mean Corpuscular Volume (MCV) fL		Mean Corpuscular Hemoglobin (MCH) pg		Mean Corpuscular Hemoglobin Concentration (MCHC) g/dL		Platelet Count (PLT) E3/uL			
	Males	Females	Males	Females	Males	Females	Males	Females	Males	Females	Males	Females	Males	Females		
N	548	526	548	526	548	526	548	526	548	526	548	526	548	526		
Min	4.58	3.25	10.10	7.60	35.40	25.70	62.00	58.50	17.30	16.70	24.60	26.50	262.00	186.00		
Max	7.50	8.29	16.90	15.90	57.00	54.10	91.70	85.80	26.90	26.10	33.40	35.40	992.00	1006.00		
Median	5.86	5.58	13.80	13.10	44.95	42.30	76.70	76.40	23.50	23.40	30.70	30.90	485.50	519.00		
Mean	5.89	5.62	13.77	13.05	45.05	42.53	76.57	75.79	23.43	23.28	30.61	30.72	508.89	531.52		
SD	0.45	0.48	0.98	0.90	3.47	3.13	4.18	4.16	1.39	1.47	1.17	1.13	123.67	112.56		

		White Blood Cell Count (WBC) E3/uL		Neutrophils (NEUT) E3/uL		Lymphocytes (LYM) E3/uL		Monocytes (MONO) E3/uL		Eosinophils (EOS) E3/uL		Basophils (BASO) E3/uL		Large Unstained Cells (LUC) E3/uL		Reticulocytes (RETI) E3/uL	
		Males	Females	Males	Females	Males	Females	Males	Females	Males	Females	Males	Females	Males	Females	Males	Females
N		548	526	548	526	548	526	548	526	548	526	548	526	548	526	548	526
Min		4.53	6.03	1.17	1.65	2.45	1.90	0.11	0.07	0.00	0.00	0.01	0.01	0.01	0.02	8.90	14.60
Max		33.64	32.35	25.72	28.29	21.85	15.93	2.17	1.28	1.40	1.01	0.23	0.17	0.79	0.72	414.80	292.40
Median		14.36	12.80	5.39	5.79	7.52	5.95	0.42	0.33	0.11	0.08	0.05	0.03	0.11	0.08	73.45	71.00
Mean		14.84	13.51	6.19	6.53	7.85	6.35	0.47	0.36	0.15	0.12	0.05	0.04	0.12	0.10	78.27	73.46
SD		4.12	4.18	3.55	3.31	2.78	2.43	0.23	0.18	0.16	0.14	0.03	0.02	0.08	0.06	31.27	30.92
				Percent Neutrophils (%)		Percent Lymphocytes (%)		Percent Monocytes (%)		Percent Eosinophils (%)		Percent Basophils (%)		Percent Large Unstained Cells (%)		Percent Reticulocytes (%)	
				Males	Females	Males	Females	Males	Females	Males	Females	Males	Females	Males	Females	Males	Females
		N		548	526	548	526	548	526	548	526	548	526	548	526	548	526
		Min		10.70	16.60	13.10	8.20	0.70	0.70	0.00	0.00	0.10	0.10	0.20	0.20	0.40	0.20
		Max		85.00	87.40	84.80	77.30	12.90	7.10	8.90	6.70	1.40	0.90	4.50	2.20	13.10	7.50
		Median		38.40	47.30	55.55	48.00	3.00	2.55	0.80	0.60	0.30	0.30	0.80	0.70	1.30	1.30
		Mean		40.59	47.51	54.04	47.88	3.18	2.71	1.03	0.92	0.34	0.29	0.83	0.71	1.37	1.33
		SD		15.05	13.67	14.33	13.01	1.31	1.12	1.01	0.95	0.15	0.12	0.41	0.30	0.76	0.64

A-06

Morphological Changes in the Intestine of Rats Fed Phytic Acid Extract from Sweet Potato (*Ipomoea Batatas*)

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Background: Phytic acid is a storage form of phosphorus which is found in plant seeds and in many roots and tubers. It has long been recognized as an anti-nutrient because of its ability to bind to, precipitate and decrease the availability of many important minerals such as calcium, iron, magnesium, zinc etc. Similarly, phytic acid interferes with the activities of some enzymes by binding to them directly. In addition to binding to minerals and some enzymes, phytic acid is also thought to regulate the process of digestion by binding to some digestion products and making them unavailable for absorption. However, it is not clear if there are changes in intestinal morphology associated with phytic acid supplementation that may further contribute to the reduction in blood glucose.

Methods: In this study, the effects of phytic acid extract from sweet potato in zinc deficient and zinc supplemented diets fed to Wistar rats for four weeks was assessed. For comparison, a group of test animals were fed diets supplemented with commercially available sodium phytate salt. After the feeding period, blood glucose, intestinal Na^+/K^+ ATPase and function markers were evaluated.

Results: Blood glucose levels in all the groups fed phytic acid extract from sweet potato or commercial phytic acid were reduced compared to their controls. The supplemented diets significantly reduced Na^+/K^+ ATPase activity in lower intestine compared to controls. Glycocalyx, goblet and paneth cells were adversely affected in rats fed a zinc deficient diet. These parameters were further aggravated in the rats fed sweet potato phytic acid extract.

Conclusion: We theorized that the observed effects on the glycocalyx, goblet and paneth cells may compromise the integrity of gut's immune system and reductions in the metabolic and absorptive capacity of the intestine as demonstrated by the decrease in Na^+/K^+ ATPase which may be beneficial for diabetics. However, due to the adverse effects on the gut's immune and metabolic functions, adequate zinc supplementation is necessary especially if foods rich in phytic acid are included in the diet.

Tuesday AM, July 26

Poster Session: 10:00 am - 12:30 pm
Cancer/Tumor Markers

A-08

Discovery of Serum Biomarkers for Prostate Cancer based on cellular mRNA Differences and Validation with Protein Measurements in Tissue and Blood

E. W. Klee, O. Bondar, M. Goodmanson, R. Dyer, S. Erdogan, E. Bergstralh, R. Bergen, G. Klee. *Mayo Clinic, Rochester, MN,*

Background: Prostate cancer is a leading cause of cancer related death in men. PSA lacks the necessary sensitivity and specificity for accurate screening. Better tests are needed to identify men who have aggressive forms of prostate cancer and benefit most from early diagnosis and treatment. We hypothesized that gene expression data from frozen prostate tissue obtained from men with well characterized prostate disease could guide the identification of novel protein candidates for the development of serum assays for prostate cancer management.

Methods: Frozen prostate tissue samples from 100 consenting patients with prostate cancer with both low and high Gleason scores, high-grade prostatic intraepithelial neoplasia and benign prostate hyperplasia were procured from the Mayo Clinic Prostate SPORE. Epithelial cells were laser capture microdissected and mRNA was extracted, amplified, and measured on Affymetrix U133 Plus 2.0 microarray chips. Novel candidate markers were selected based on specific mRNA expression patterns and the predicted chemical properties of the encoded proteins. Prostate tissue specific expression of the predicted proteins was confirmed by immunohistochemistry (13) and mass spectrometry (5). The concentrations of candidate markers in blood were evaluated using immunoassays (8) and targeted mass spectrometry to measure predicted peptides (9). Rabbit anti-peptide antisera were developed using synthetic peptides conjugated to KLH. Targeted proteins predicted to be glycosylated were extracted from serum with Concanavalin A. Additional sera aliquots were depleted of abundant proteins using MARS14 columns. These samples then were trypsin-digested and immuno-extracted using anti-peptide antisera. The extracted peptides were measured on an API 5000 mass spectrometer along with isotopically labeled peptides.

Results: Thirty-five novel candidate prostate cancer biomarkers were identified (21 extracellular proteins and 9 membrane associated proteins). Thirty showed > 2 fold up-regulated transcript expression in the prostate cancer tissue and an additional 5 had high prostate tissue-specific mRNA expressions. Thirteen markers were analyzed and showed immunohistochemical positivity in the tumor areas of the prostate cancer cases (CXCL9, CDH7, COL2A1, COL9A2, COMP, CXCL14, CXCL9, EFNA4, F5, GPR116, NRN1, PCSK6, PRG3). Targeted mass-spectrometry of two matched cancer/benign extracted prostrate tissue specimens identified 5 of our markers, with three (PGLS, ASPN and RPL22L1) showing increased levels in cancer. Four markers showed elevated levels in 10% of the 50 advanced cancer compared to early cancer and benign disease (APOC1, CXCL11, CXCL9, and F5), using ELISA. F5 and six additional markers showed elevations with mass spectrometry in at least 10% of advanced cases. Overall, 7 novel biomarkers were identified with differential expression in prostate tissue and found to have increased levels in blood of at least 10% of men with advanced prostate cancer (ASPN, CDH7, COL2A1, F5, PCSK6, PGLS, RPL22L1).

Conclusions: Genomic information from frozen prostate tissue samples can be used to discover candidate serum biomarkers. Seven novel markers show potential utility for prostate cancer; however, individually these markers can only identify a portion of the men likely to develop advance disease. Multivariate combinations of these markers may yield better performance, but large studies are needed to evaluate combinations of markers.

A-10

Association between two promoter polymorphisms in interleukin-18 gene and the occurrence and progression of prostate cancer in Chinese Han population

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Background: Interleukin-18(IL-18) has been implicated in a wide variety of cellular functions on the biological response to tumors, which can not only act as an anticancer factor but also promote tumor progression. Compelling evidence suggests that these dual effects exist in the initiation and progression of prostate cancer. However, the evidence coming from *IL-18* gene variants is relatively insufficient. This study was designed to find the association of two promoter polymorphisms -137G/C (rs187238) and -607C/A (rs1946518) in *IL-18* gene with prostate cancer occurrence and progression, including its clinical stage, metastasis and development of hormone refractory prostate cancer (HRPC) in Chinese Han population in order to provide data for molecular diagnosis and clinical practices.

Methods: We used the high-resolution melting (HRM) method on LightCycler 480 machine and GeneScan software to genotype these two single polymorphisms (SNPs) in 234 Chinese Han patients with prostate cancer and 244 matched controls. Statistical analysis was performed by SPSS 13.0. The differences of genotype and haplotype were compared by using the Pearson $\chi 2$ analysis. The calculations of odds ratio (OR) and 95% confidence interval (95% CI) was conducted with the risk option of crosstabs.

Results: No significant differences were observed between cases and controls in the frequencies of two SNP genotypes. But the frequency of allele C in -607C/A was significant higher in patient group ($\chi 2$=16.424, p<0.001, OR= 1.702, 95% CI 1.315-2.203). -607C/-137C haplotype was associated with a significantly decreased risk of prostate cancer (OR=0.478; 95% CI: 0.435-0.526; p=0.013). No significant difference was detected in genotype and allele distributions of both the two polymorphisms in clinical stage and metastasis. However, significant association was observed between the genotypes of -607C/A and HRPC ($\chi 2$=6.538, p=0.038), and the -607C allele was found to be associated with a significantly higher risk of HRPC ($\chi 2$=6.711, p=0.010, OR=1.999, 95% CI 1.176-3.398).

Conclusion: Our data demonstrated the potential role of *IL-18* gene in the risk of prostate cancer and the transition of hormone resistance in Chinese Han population,especially -607C/A.

A-11

Prostate health index: pre-analytical phase

F. Ceriotti, M. Pontillo, G. Pizzagalli. *Diagnostica e Ricerca San Raffaele, Milan, Italy,*

Background: Prostate health index (PHI) is a new parameter proposed by Beckman - Coulter to evaluate the presence of prostate cancer. To calculate the index, the measurements of Total PSA (PSA), Free PSA (fPSA) and [-2]proPSA (p2PSA) are needed. Previous literature data indicate that p2PSA increases when stored at room temperature “on the clot”, and separation of serum is mandatory within 3 hours maximum (Semjonow). Aim of this work is to check alternative pre-analytical approaches to overcome this limitation.

Methods: Two experiments were performed. 1. use of BD Vacutainers with serum separator (SST II), 2. storage of serum in water-ice bath.1. serum was collected from 25 patients both in a plain tube and in a tube with serum separator. The tubes were centrifuged within a maximum of 90 minutes and a first aliquot was taken and frozen at -80 °C. The centrifuged tubes were left at room temperature (21 - 23 °C) for 5 hours and then a second aliquot was taken and frozen at -80 °C. The frozen aliquots were then thawed and analyzed in duplicate on Beckman DxI 800. All the four aliquots of the same patient were analyzed in the same run. 2. From 35 patients serum was drawn in two plain tubes; one was left at room temperature, the second was put in a water-ice bath immediately after the blood drawing. After a maximum of 40 minutes the tubes were centrifuged, a first aliquot was taken and frozen at -80 °C. Then one tube was left at room temperature, the second was placed in a refrigerator (+4 °C), after 5 hours a second aliquot was taken and frozen. All the aliquots were then thawed and analyzed together (single measurement).

Results: The use of SST II tubes introduced a negative bias in the measurement of PSA (-4.6%, p <0.05, Student’s t for paired data) and especially of fPSA (-5.0%, p <0.01), but had no effect on the measurement of p2PSA even after 5 hours storage (p = 0.367). Unfortunately the strong effect on total and especially fPSA introduced a significant positive bias in the calculation of PHI (+8%, p <0.001). Storing the samples at 4 °C was able to block the increase of p2PSA over the 5 hours storage (mean increase -3%, p NS), but not in eliminating the decrease of fPSA. The combination of these two effects lead to an increase of PHI (+3%), even if not statistically significant.

Conclusion: Serum separator tubes cannot be used for the measurement of PSA and fPSA on Beckman DxI, while they are appropriate for the measurement of p2PSA which remains stable for at least 5 hours after centrifugation at room temperature. Refrigerating the serum tubes immediately after blood drawing stabilizes p2PSA that can be left on the cloth for 5 hours or more. fPSA measured on DxI 800 decreases with time (no matter if stored at room or at +4 °C) with an average decrease of 5% over 5 hours.

A-12

Monitoring Epithelial Cancers using microRNAs from Serum

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Background: Recent research has revealed that various types of cells, including tumor cells, release small vesicles called exosomes into the blood that contain different microRNAs depending on the cell of origin. The primary objective of this study was to identify a set of miRNAs that can reproducibly distinguish cancer patients from non-cancer patients and that could be used for monitoring cancer.

Methods: Blood serum submitted for diagnostic testing at ARUP Laboratories was obtained from a total of 160 patients including: subjects with no cancer (30), breast cancer (30), ovarian cancer (30), pancreatic cancer (30), prostate cancer (30) and lung cancer (10). Exosomes were isolated using ExoQuick Exosome Precipitation Solution, followed by RNA extraction using the QIAGEN miRNeasy Kit. The expression of miRNAs within "normal" and the different tumor types was initially analyzed using Agilent miRNA Expression Microarray and pooling samples of each type. Control microRNAs and microRNAs that showed greater than two-fold change were selected for additional validation using RACE (rapid amplification of cDNA ends)-PCR, which provides miRNA specificity through use of a forward primer perfectly complimentary to the miRNA sequence of interest. A set of the most stable control/housekeepers were statistically selected and used to normalize classifier microRNAs.

Results: We identified 10 potential control/housekeeper microRNAs and 24 miRNAs that were dysregulated between non-cancer and cancer samples and/or were differentially expressed between cancer types. Confirmation by RACE-PCR performed on individual samples identified several statistically significant genes ($p<0.05$) for distinguishing cancer from non-cancer and different types of cancer from each other (Table 1).

Table 1	Normal	Breast	Ovarian	Pancreas	Prostate	Lung
Normal	----	----	----	----	----	----
Breast	miR-720 miR-572	----	----	----	----	----
Ovarian	miRs -516a-5p -1225-5p	miR-572 miR-451	----	----	----	----
Pancreas	miR-572 miR-720	miRs -516a-5p -139-3p	miR-202 -516a-5p	----	----	----
Prostate	miR-92a -516a-5p	miR-494 miR-451	miR-1246 miR-638	miR-1246 -516a-5p	----	----
Lung	miR-663 miR-572	miR-663 miR-451	miRs -516a-5p -1225-5p	miR-1246 miR-1290	miR-198 -516a-5p	----

Conclusion: We have identified a unique set of microRNAs that can be used for cancer monitoring, and are potentially useful for early detection.

A-13

Preliminary Investigation of the Correlation Between TNM Stage and Circulating Tumor Cells Count in Lung Cancer Patients

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Background: Enumeration of circulating tumor cells (CTCs) may be valuable for lung cancer treatment and monitoring cancer patient relapse. In this study,we explore the relation of circulating tumor cells (CTCs) to TNM stage and the levels of tumor markers for patients with lung cancer.

Methods: In the prospective, single-center and small sample study, we tested the levels of CTCs in 42 patients with emerging or recurrent lung cancer from Beijing Shijitan Hospital. And some tumor markers such as Cyfra21-1, NSE, CA199, CA125, CA153, CEA and AFP were also measured. The tumor-node-metastasis (TNM) staging was performed based on the results of imageology and pathology.

Results: CTC count was 1~2 in 50%(4/8) of patients with T1stage, while the CTC counts of ≥3 was majority at the rate of 46.2% (6/13), 62.5% (5/8) and 60% (6/10) in the patients with T 2 to T4 stage respectively. CTCs can be detected in all M1 patients. In the patients with TNM stage I, CTC counts of 1~2 was dominated at the rate of 50%(5/10), while the CTC counts of ≥3 was primary at the rate of 50% (5/10), 54.5% (6/11) and 70%(7/10) in the patients with TNM stage II to IV. It is notable that the patients with TNM stage IV had a 100% CTC positive rate (10/10). The CTC positive rate was higher in patients with high level of Cyfra21-1 than those with low cyfra21-1(94.12% vs. 66.67%).

Conclusion: CTCs is related to the size, invasiveness and the distant metastases of tumor and reflects the activity of tumor cells as well. Additionally CTCs is correlated with the level of tumor marker Cyfra21-1.

A-14

Relationship between *Integrin* gene miRNA-related polymorphisms and the progress of gastric cancer in Chinese Han population

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Background: Considerable evolution in recent studies suggests that integrins play a critical role in the progress of cancer. Many mechanisms have been elucidated that integrins contributed to the solid tumor cells invasion, angiogenesis and extravasation. Besides, miRNA may regulate integrins expression through post-transcriptional control. But the association between single miRNA-related polymorphism of *integrin* and the risk of progress of gastric cancer has not been clarified. So our study aimed to examine whether five polymorphisms, rs1062484 in *ITGA3*, rs17664 in *ITGA6*, rs11902171 in *ITGAv*, rs3809865 in *ITGB3* and rs2675 in *ITGB5,* were associated with the progress of gastric cancer in order to provide data for screening high-risk population in Chinese Han population.

Methods: 327 gastric cancer patients (222 male and 105 female, mean age: 51.13±11.06 years) in Chinese Han population were collected to assess their genotype and allele frequencies of the five promoter polymorphisms by using the high-resolution melting (HRM) method on LightCycler 480 machine and GeneScan software. SPSS 13.0 software was used in statistic analysis.

Results: No significant differences were observed between distributions of the five SNPs and lymphatic metastasis, nerve invasion and clinical stage of gastric cancer. However, significant difference was detected in rs2675 in groups with different depth of invasion (genotype: $\chi2=13.774$, $p=0.032$; $\chi2=11.849$, $p=0.008$). And genotype and allele frequencies of rs1062484 showed significant differences among patients in the appearance of distant metastasis (genotype: $\chi2=3.916$, $p=0.048$; allele: $\chi2=3.904$, $p=0.048$).

Conclusions: In Chinese Han population, *integrin* gene miRNA-related polymorphisms may not contribute to the susceptibility of lymphatic metastasis, nerve invasion and clinical stage of gastric cancer, but may play a role in the invasion and distant metastasis in the progress of gastric cancer.

A-15

Association of two nucleotide variants in *miR-146a* and *miR-499* with Primary liver cancer in Chinese population

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Background: MicroRNAs (miRNAs) have been proved to play important roles in the growth and development of human beings. Single nucleotide polymorphisms (SNPs) within miRNAs could change their production or affinity with target genes, thus lead to malignant diseases. This study is to explore the relationship between polymorphisms in miR-146a (rs2910164 G>C) and miR-499 (rs3746444 T>C) and primary liver cancers in Chinese population.

Methods: 186 Chinese primary liver cancers cases and 483 healthy controls were enrolled to be genotyped by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). The distribution of the genotypes and alleles was first compared between patients and controls, and then in the subgroups of the patients characterized by clinicopathology, furthermore, we also investigated the effect of the two SNPs on liver function and age of onset.

Results: No significant differences were observed between distributions of the two SNPs and susceptibility of primary liver cancer, diverse clinicopathologic features. However, we found that patients with genotype CG of the SNP in miR-146a tended to have earlier onset and better liver function, compared with CC(average age:49.9 vs 54.9,P=0.038,95%CI:0.2778~9.7962; average improved Child-Pugh grade:5.55 vs 6.15, P=0.021, 95%CI:0.0911~ 1.1107),and further analysis showed that patients who had at least one G allele were diagnosed at an earlier age (average age: 49.6 vs 54.9,P= 0.022,95%CI:0.7807~9.7278) and had better liver function (average improved Child-Pugh grade:5.60 vs 6.15 ,P=0.026, 95%CI: 0.0652~1.0206).

Conclusions: Our data suggested lack of association between the two SNPs and primary liver cancer risk, though interestingly, the SNP miR-146a was likely to affect the age of

onset and Child-Pugh grade, which may provide new ideas for research in miRNA.

Table1 . Age of onset and improved Child-Pugh grade with genotypes of miR146a SNP

	Age of onset (n=174)			Improved Child-Pugh Grade (n=102)		
	mean±SD	P-value		mean±SD	P-value	
CG	49.85±14.38	0.038*		5.54±1.02	0.021*	
CC	54.89±14.78		0.022**	6.14±1.35		0.026**
CG+GG	49.64±14.20			5.60±1.04		

* CG vs CC

** (CG+GG) vs CC

A-16

Utility of Serum Biomarkers for Predicting Systemic Progression in Prostate Cancer

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Background: Many men who are diagnosed with prostate cancer have slowly progressing disease, while some rapidly progress and have multiple complications including death. Blood biomarkers that could identify men likely to rapidly progress would be clinically useful. We identified 19 biomarkers from the literature which had potential utility for this role and evaluated their clinical utility for predicting adverse outcomes in a cohort of men with high risk prostate cancer. Men with high risk were selected for this cohort to ensure that we had adequate events within the follow up time.

Methods: A cohort study involving 190 men with high risk for prostate cancer progression following radical prostatectomy was identified from the Mayo Clinic NCI-Prostate SPORE. These men had PSA >20 ng/ml, or Gleason score 8-10, or seminal vesicle involvement, or GPSM score of 10 or more at time of prostatectomy. All of these men had baseline serum samples available and consented to the research use of their specimens. The mean (SD) follow up was 3.3 (1.2SD) years; 86 men had PSA recurrence (>=0.4ng/ml); 15 men had systemic progression, and 7 men died from prostate cancer at the time of analysis. Commercial immunoassays were used for these 19 biomarkers: Angiopoietin 2 (R&D Systems), Chromogranin A (Cisbio), Coagulation Factor III (R&D Systems), Fatty Acid Synthase (FASgen), Growth Differentiation Factor 15(R&D Systems), Interleukin 6(R&D Systems), Insulin-like Growth Factor Binding Protein-2(ALPCO), Ostase(Beckman Coulter), Osteopontin(R&D Systems), Progranulin (R&D Systems), Prostatic Acid Phosphatase (Alpha Diagnostics), PSA (Beckman Coulter), free PSA (Beckman Coulter), [-2] proPSA (Beckman Coulter), hK2 (Mayo), E-Cadherin (R&D Systems), Survivin (R&D Systems), Tissue Inhibitor of Metalloproteinases 1(R&D Systems), and Vascular Endothelial Growth Factor D (R&D Systems). Minimum analytic validation was performed on each of the kits and serum controls were run with each of the assays. Associations of analytes with tumor factors were done using Spearman's rank correlation. The Cox proportional hazards model was used to test associations with follow-up events. Analytes were log transformed (base 2) as needed with hazard ratios (HR) for a doubling and 95% confidence intervals reported.

Results: Eight of these biomarkers were significantly ($p<.05$) associated with high risk characteristics such as Gleason score, tumor volume, and pathological stage. Pathology Gleason Score was predicted by [-2] proPSA, GDF-15, hK2, and Chromogranin A. Tumor volume was predicted by [-2] proPSA, GDF-15, hK2, FASN (neg cor), and Survivin (neg cor). Pathologic stage was predicted by [-2] proPSA, IGFBP-2, hK2, Survivin (neg cor), and VEGF-D. PSA recurrence was predicted by [-2] proPSA (HR 1.31[1.06, 1.61], p=0.01. Only two of these markers, Chromogranin A (HR=1.66[1.04, 2.64], p=0.03) and GDF-15 (HR=2.54[1.50, 4.28], $p<0.001$) were predictive of systemic progression.

Conclusions: Cohort studies are needed to evaluate the predictive utility of serum biomarkers. [-2] proPSA and GDF-15 are promising markers for predicting which men with prostate cancer will have early systemic progression.

A-17

Value of the Prostate Health Index (*phi*)[1] for prostate cancer detection in men undergoing first or repeat biopsy. A multi-center prospective clinical study

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Background: PSA levels correlate poorly with repeat biopsy (Bx) findings. A more specific test would help detect PCa among men with elevated PSA after one or more negative Bx. We evaluated the clinical utility of the Beckman Coulter Prostate Health Index *phi*, [-2]proPSA/free PSA x square root PSA, for PCa detection in men undergoing first or repeat Bx. Hybritech p2PSA measures [-2]proPSA.

Methods: 865 men from 7 sites undergoing prostate Bx, were prospectively enrolled. Inclusion criteria: ≥ 50 years, clinical stage T1c, PSA 2-10 ng/mL, and ≥ 6 cores Bx. 159 had a repeat Bx (34% PCa); 706 had a first time Bx (51% PCa). Serum samples for [-2]proPSA, fPSA, and PSA were obtained prior to Bx and tested on the Beckman Coulter Access 2 Immunoassay analyzer[3]. ROC analyses were performed.

Results: In men having a first Bx, area under curve (AUC) for *phi* was 0.714 vs. 0.553 for PSA ($p<0.01$). In men having a repeat Bx, AUC for *phi* was 0.637 vs. 0.500 for PSA ($p<0.01$). PCa specificity was significantly better for phi than PSA in men undergoing either first-time or repeat Bx. For both tests, PCa specificity was better in men having first-time than repeat Bx, but when sensitivity was set at 60%, *phi* was 64.8% specific for PCa in men having repeat Bx.

	No Prior Biopsy			
% Sensitivity	phi Cutoff	% Specificity	PSA ng/mL Cutoff	% Specificity
85	27.1	43.2	3.4	20.9
60	37.3	71.3	4.6	47.8
45	44.3	84.1	5.4	62.9
	Prior Biopsy			
% Sensitivity	phi Cutoff	% Specificity	PSA ng/mL Cutoff	% Specificity
85	27.0	23.8	3.8	7.6
60	37.0	64.8	5.5	40.0
45	41.9	76.2	6.3	60.0

Conclusion: *phi* has improved specificity relative to PSA for men undergoing first time or repeat Bx. Even though the specificity of *phi* was somewhat decreased for repeat Bx relative to first time Bx, the discriminate value of *phi* was still significantly better than PSA and provides an important role for the new marker.

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A-18

Development and analytical validation of a new prostate-specific antigen (PSA) assay (NADiA® ProsVue™), based on immuno-PCR technology

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Background: PSA assays are valuable for ensuring that men are free of prostate cancer post-prostatectomy. However, conventional assays are not sensitive enough to neither quantitate the minute levels of PSA nor distinguish between values that may be stable or slowly rising. The objective of this study was to develop and validate an investigational ultrasensitive prostate-specific antigen (PSA) assay (NADiA® ProsVue™) with sub-pg/mL sensitivity, suitable for measuring serum PSA levels in prostate cancer patients following radical prostatectomy.

Methods: The assay is based on capturing of serum PSA by one murine monoclonal antibody which is biotinylated and immobilized on streptavidin-coated paramagnetic particles. The detection monoclonal antibody is chemically conjugated to a 59-mer double stranded DNA oligonucleotide (dsDNA). After PSA is bound to the two complementary antibodies, the paramagnetic beads are washed and separated, and then the dsDNA is amplified by polymerase chain reaction (PCR). The progress of PCR

amplification is monitored by SYBR green fluorescence with a Q-PCR instrument. The threshold cycle for generating a specific fluorescence signal is calculated and used for constructing the calibration curve.

Results: Validation experiments were performed according to CLSI Guidelines, when available. Assay dynamic range extended from 0.65 to 100 pg/mL. The limit of detection was 0.27 pg/mL and the limit of quantification was 0.65 pg/mL. Spiking recovery with six serum samples ranged from 86% to 105%, with a mean of 98.4%. Dilution linearity yielded recovery 84% to116% for all samples and all dilutions, with a mean recovery of 100%. There was no high-dose hook effect up to 10,000 pg/mL of added PSA. Precision was assessed in human samples at low (3.79 pg/mL), intermediate (24.1 pg/mL) and high (69.1 pg/mL) mean serum PSA levels. Percent CV at the three levels was 15.2%, 9.4% and 10.6%, respectively. This precision encompasses different days of testing, multiple operators, two reagent lots, multiple instruments and two different testing sites. Equimolarity was verified at various free to complex PSA ratios and recovery ranged from 88.0% to 113.2%, with a mean of 99.5%. The new method was compared with the Siemens ADVIA Centaur® total PSA assay (N=112). The regression equation was NADiA ProsVue = -0.16 ng/mL + 0.99 (ADVIA); Pearson R = 0.94. We further confirmed the reference range of both the NADiA and Siemens methods to be 0-4 ng/mL.

Conclusions: The NADiA ProsVue assay, based on immuno-PCR, is one of the most sensitive and precise analytical methods ever reported for measuring total PSA. The method has excellent analytical characteristics per CLSI testing guidelines and its proposed intended use is to aid in the identification of men at reduced risk for prostate cancer recurrence after prostatectomy. A clinical evaluation for this assay is underway to support regulatory clearance.

A-19

An Eleven Analyte Monoclonal Gammopathy Screening Array Immunoassay - Performance Assessment

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Background: The development of a multi-analyte screening assay for monoclonal intact immunoglobulin, kappa (κ) and lambda (λ) free light chains (FLC) and other specific tumour related proteins, could simplify and fully automate sample testing, whist providing greater clinical utility than the current screening protocols.

The International Myeloma Working Group Screening guidelines, recommend a combination of serum protein electrophoresis (SPE), immunofixation electrophoresis (IFE) and serum free light chain (FLC) ratios for identification of monoclonal related proteins. Gel electrophoresis techniques can require a degree of expert interpretation.

Studies utilising antibodies specific for heavy chain/light chain (HLC) pairs have indicated that HLC ratios provide a sensitive indication of monoclonal intact immunoglobulin production. This combination allows for the development of a screening assay that uses both HLC and FLC ratios and eliminates the requirement for the gel electrophoresis techniques.

Methods: Using a multiplex format developed by Dynex Technologies Inc, a screening assay has been developed to detect eleven tumour markers based on the use of the Binding Site Group Ltd. UK polyclonal antibodies. Assays have been run to establish the following performance characteristics; concordance with confirmed monoclonal gammopathy type, normal blood donor sample (NBDS) classification, within-assay precision and confirmation of total immunoglobulin concentrations by summation of the κ+λHLC concentrations. Assays were run using a modified Dynex DS2™ instrument; total assay time was 2 hours. Diluted serum samples were incubated in wells containing specific sheep antibody-coated beads to: κFLC, λFLC, IgGκ, IgGλ, IgAκ, IgAλ, IgMκ, IgMλ, cystatin C, beta 2-microglobulin and albumin, following a wash step, bound analytes were detected using enzyme conjugated antibodies specific for the respective target analytes. Following a final wash, luminol substrate was added and the resulting image captured and analysed using a Photometrics CCD camera and Array-Pro Analyzer® software.

Results: Concordance was tested using 48 NBDS and 96 sera from patients with a previously confirmed monoclonal gammopathy. The assay correctly confirmed normal FLC and HLC ratios in 48/48 NBDS and abnormal ratios in 95/96 (99%) monoclonal gammopathy samples. The one discordant result was subsequently confirmed to be from a patient with polyclonal hypergammaglobulinemia, but no indication of monoclonality. The mean within-assay precision (percentage coefficient of variation) for the eleven analytes was assessed using 16 replicates of three samples: IgAκ=4.1%, IgAλ=3.9%, IgMκ=6.2%, IgMλ=7.6%, IgGκ=6.3%, IgGλ=4.7%, κFLC=6.4%, λFLC=5.3%, beta-2-microglobulin =5.5%, cystatin-C=6.4% and albumin=5.9%.

Conclusion: These data demonstrate the potential performance of this monoclonal gammopathy screening assay. Eleven results were obtained from each of the144 samples (1584 data points in total). The assay utilises the Dynex DS2 platform which fully automates the assay, the array format simplifies the screening process by combining the assays in one format, making it ideal for routine laboratory use, whist eliminating the need for gel electrophoresis. The inclusion of simultaneous quantitation of cystatin C (for renal function), beta-2-microglobulin and albumin (tumour mass) levels add to the clinical utility of this protocol.

A-20

The Effect of Age-Adjusted PSA ranges on the prostate Health Index (*phi*)[1], a mathematical equation combining PSA, fPSA, and [-2] proPSA, on prostate cancer detection. A multi-center prospective clinical study

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Background and Introduction: Age-adjusted PSA ranges are sometimes used for biopsy decisions to improve the specificity of PSA testing in older men and improve sensitivity in younger men. We assessed the influence of non-age adjusted and age-adjusted PSA ranges on *phi*, a mathematical equation combining PSA, fPSA, and [-2] proPSA to determine the effect on prostate cancer (PCa) detection.

Methods: A multi-center study (7 sites) was conducted, consisting of 430 men with PCa and 462 benign by biopsy, T1c, ≥ 50 years of age, and PSA ranging from 2.0-10.0 ng/mL. The sensitivity, specificity and area under the curve (AUC) from receiver operating characteristic (ROC) analyses for PCa detection of *phi*, (Beckman Coulter Prostate Heath Index) defined as [([-2]proPSA/fPSA) x $PSA^{1/2}$], PSA, and %fPSA were evaluated with and without the following PSA age-adjusted ranges: 50-59 years, 3.5 ng/mL; 60-69, 4.5 ng/mL; and 70-79, 6.5 ng/mL. PSA, fPSA, and [-2]proPSA were all tested on the Beckman Coulter Access 2 Immunoassay Analyzer[3].

Results: As seen in the table below, comparable specificity at various sensitivities were obtained for the unadjusted PSA and the age-adjusted ranges for PSA, %fPSA, and *phi*. Additionally, comparable results were obtained for the AUCs for the unadjusted and PSA age-adjusted for PSA (0.525 vs. 0.514), %fPSA (0.648 vs. 0.645), and *phi* (0.703 vs. 0.718).

	% Specificity					
	Not Adjusted for Age			Adjusted for Age		
% Sensitivity	PSA	%fPSA	*phi*	PSA	%fPSA	*phi*
95	6.5	8.4	16.0	6.5	7.2	15.7
90	11.0	18.0	26.2	11.9	16.4	31.1
85	17.1	27.7	39.0	17.1	27.0	42.3
80	22.3	36.6	45.2	21.2	34.1	48.5

Conclusion: The results of this evaluation do not support the need to use age-adjusted PSA ranges for *phi*. Sensitivity, specificity, and AUC from the ROC analyses are not improved with age-adjusted PSA, simplifying the use of *phi* across all age groups (≥ 50 years).

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A-21

Comparison of Hybritech® and WHO Calibrations for PSA and fPSA used in the Beckman Coulter Prostate Health Index (*phi*) for Prostate Cancer Detection

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Background: Since the mid 1990s, the goal of an equimolarity standard for PSA evolved into adoption of WHO 96/670 as a new mass standard. In 1999, a fPSA mass standard, 96/668, was adopted. Both the Hybritech PSA and fPSA assay calibrations are based on reference preparations of human PSA and fPSA purified from seminal plasma and value assigned by Lowry total protein methods. The objective of this study was to compare the clinical utility of *phi* (a combination of [-2]proPSA, PSA and fPSA) using the Hybritech and WHO calibrations for PSA and fPSA. The [-2]proPSA isoform was measured using the Hybritech p2PSA assay.

Methods: A total of 892 subjects, 430 with and 462 without PCa, from seven medical centers were enrolled. Subjects were ≥50 years, with negative DRE, 2-10 ng/mL PSA and had undergone ≥ 6 core biopsy. PSA, fPSA and p2PSA were tested using the Beckman Coulter Access® Immunoassay Analyzer. The study compared the clinical utility of *phi*** ((p2PSA/fPSA) X $PSA^{1/2}$) using WHO and Hybritech calibrated PSA and fPSA. Comparison between Hybritech and WHO calibrated PSA was performed using Deming analyses. Receiver Operating Characteristics (ROC) analyses were performed for *phi* with Hybritech and WHO PSA and fPSA calibrations. The probability of PCa was determined using the bootstrap technique to repetitively sample the study population 1,000 times and accounted for the 25% prevalence of PCa in the 2-10 ng/mL PSA range.

Results: Deming regression (WHO vs. Hybritech) of 100 serum samples from 0-130 ng/mL PSA using three WHO primary calibrator lots showed strong correlation for PSA with r from 0.9814 to 0.9999. A 4.0 ng/mL Hybritech calibrated PSA equals 3.1 ng/mL based on WHO calibration. ROC analyses of *phi* for the 892 subjects were virtually identical when using either Hybritech or WHO calibrated PSA and fPSA with AUCs of 0.703 and 0.704 (p-value=0.53). Results from the *phi* percent probability of PCa (and Relative Risk), show very similar results when using the Hybritech or WHO calibrations. For *phi* ranges of 0-24.9, 25.0-34.9, 35.0-54.9 and ≥ 55.0, the *phi* probability of PCa for the Hybritech and WHO calibrated PSA and fPSA was 11.0% and 11.3% (RR: 1.0 for both), 18.1% and 18.0% (RR: 1.6 for both), 32.7% and 34.0% (RR: 3.0 for both) and 52.1% and 49.6% (RR: 4.7 and 4.4), respectively. The percent of patients in the four *phi* ranges, for the Hybritech and WHO calibrations, are 24.9% and 26.8%, 32.8% and 31.4%, 29.5% and 28.3% and 12.8% and 13.5%, respectively.

Conclusion: Other than the absolute difference in PSA and fPSA concentrations and calculated *phi* ranges, the clinical performance of Beckman Coulter *phi* is equivalent when using either WHO or Hybritech calibrations for PSA and fPSA. These results apply to the Access Hybritech p2PSA, PSA and fPSA assays on the Access Immunoassay Systems. Values obtained with assays from different manufacturers cannot be used interchangeably.

*Not intended as off-label promotion of any Beckman Coulter product;**Not available in the U.S.

A-23

Association between microRNA genetic variants and susceptibility to colorectal cancer in Chinese population

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Background: MicroRNAs (miRNAs) play important roles in the pathogenesis of tumors. Single nucleotide polymorphism (SNP) within miRNAs can change their phenotype and function. We attempted to analyze the relationship between two SPN loci in miRNA and colorectal cancer (CRC) in Chinese Han population.

Methods: Using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP), we analyzed two miRNA SNP loci, miR-146a (*rs2910164 G>C*) and miR-499 (*rs3746444 T>C*) in 276 CRC patients (65.2% male and 34.8% female) and 373 healthy controls (67.8% male and 32.2% female). The distribution of the genotype and allele of the two SNP loci were compared between patient and control groups, and among subgroups of patients with different clinicopathological features.

Results: The overall genotype distribution of the miR-146a SNP was not significantly different between patients and controls. However, using CG genotype as reference, the distribution of genotype GG was significantly higher in patients than controls (p=0.031) with a significant association with CRC (OR= 1.762; 95% CI =1.050-2.956). Furthermore, patients with a good tumor differentiation showed 100% GG genotype in miR-146a, but patients with a moderate or poor differentiation had only 22.6% and 5% GG phenotype, respectively. A significant association between GG genotype between and CRC differentiation was detected (OR=0.553, 95% CI =0.315-0.971). In contrast, the SNP in miR-499 was not associated with CRC susceptibility or clinicopathological features.

Conclusion: Our data suggested that SNP locus (*rs2910164 G>C*) in miR-146a is not only associated with CRC susceptibility but also histological differentiation, and it may be a useful reference in screening the high-risk population as well as determining tumor differentiation in diagnosed CRC patients.

Table 1 Polymorphisms of SNPs miR-146a (rs2910164*G>C)* and miR-499 (rs3746444*T>C)* in CRC patients and controls.

Polymorphism	Patient n (%)	Control n (%)	χ^2	p*	OR	95% CI
rs2910164G>C(n=200)						
Genotype						
CC	84 (42.0)	142 (38.1)				
CG	82 (41.0)	187 (50.1)	5.413	0.067	N/A	N/A
GG	34 (17.0)	44 (11.8)				
GG vs. CG			4.667	0.031	1.762	1.050-2.956
CC vs. CG			2.462	0.117	1.349	0.928-1.962
Allele						
G	150 (37.5)	275 (36.9)	0.045	0.832	1.028	0.799-1.321
C	250 (62.5)	471 (63.1)				
rs3746444T>C(n=211)						
Genotype						
CC	5 (2.40)	10 (2.70)				
CT	49 (23.2)	81 (21.7)	0.214	0.899	N/A	N/A
TT	157 (74.4)	282 (75.6)				
Allele						
C	59 (14.0)	101(13.5)	0.045	0.833	1.038	0.734-1.467
T	363(86.0)	645(86.5)				

N/A, Not Available; OR, Odd Ratios.

A-24

Detecting circulating tumor cells from a microfluidic chip using a customized imaging platform

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Background: Circulating tumor cells (CTCs) have become increasingly accepted as important independent prognostic factors and as valuable markers in prediction of therapeutic response. Among many advanced isolation methods, the C5 microfluidic chip with a gradient post design has shown advantage in efficiency of CTCs attributable to its dual capture mechanism of using both antibody and size mediated capture.

Methods: Plastic slides were embossed to produce C5 chip consisting of posts arranged in a gradient pattern with decreasing post diameters and gap distances across the chip surface. Decreasing the distances between posts, to a minimum of 12 µm, creates a gap size gradient across the chip allowing smaller blood cells to pass through while capturing larger CTCs within the gaps. Chips were functionalized with an anti-EpCAM monoclonal Ab for specific affinity capture of epithelial cells. High (H1650) and low (MDA-MB-231) EpCAM expressing cancer cells were spiked into buffer and pumped through the chips at a constant flow rate. EPCAM captured cells were then identified by on-chip immunofluorescence using an anti-cytokeratin antibody conjugated to phycoerythrin in combination with a nuclear specific fluorescent stain and imaged on an automated cell imaging platform at 5x magnification. An automated imaging platform has been developed to detect CTCs from a microfluidic chip using advanced algorithms.

Results: Variability of capture rate was modest for replicate measurements, with CV's

of < 15% for within-day, day-to-day, and lot-to-lot precision. EpCAM Ab coated C5 chips captured 97% of spiked H1650 cells into normal blood samples, verifying the advantage of both size and affinity capture. The imaging platform was then evaluated using 8 patients with advanced cancer, 5 colorectal cancer (CRC) and 3 Pancreatic Cancer patients. The system detected an average of 30 CTCs/7.5 ml and 14 CTCs/7.5 ml for the CRC and PaCa patients respectively.

Conclusions: The preliminary evaluation of this C5 CTC imaging platform suggests a high CTC detection rate, both in spiked samples and in patient samples. The system also achieved a high scan throughput that makes this technology practical for both research and clinical use. Further study will be performed using a large sample size to evaluate detection efficiency and repeatability. Circulating tumor cells (CTCs) are frequently present in the blood of cancer patients and can be captured by a variety of mechanisms. Here we described a novel microfluidic chip that can capture CTCs by both cell size and affinity.

A-25

Comparison of Three Commercial Chromogranin A Assays with Reference Laboratory Methods

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Background: Elevated chromogranin A (CgA) concentrations in a patient's serum or plasma may indicate the presence or recurrence of a neuroendocrine tumor, such as a carcinoid or pheochromocytoma. We evaluated the performance characteristics of three commercially available CgA ELISA kits, and compared the results with those from a reference lab. Because a reference method for CgA is not available and each commercial kit employs different antibodies and units, a comparison using patient samples and known diagnoses was warranted prior to conducting a complete validation study.

Methods: A validation plan for method comparison was drafted and approved. Three potentially suitable, commercially available, CgA ELISA kits were identified (CISBIO, ALPCO, and DAKO) and obtained. Patient samples received for CgA sendout testing were split and a portion was retained in frozen storage. The retained samples were later assayed with the ELISA kits according to the manufacturer's instructions. Departmental policies and relevant CLSI guidelines were followed to determine the linearity, precision, and lower limit(s) of detection/blank. Accuracy was determined by comparison of ELISA and reference lab results. Data was analyzed using EP evaluator software. The clinical status for 41 of 47 patients whose samples were tested was available from chart review, allowing an assessment of the sensitivity and specificity of the reference method cutoff points.

Results: All three ELISA kits showed acceptable correlation (R > 0.9) with the reference lab's ECL assay. Each assay had suitable precision, with a CV<15% at low, middle, and high concentrations. However, the ALPCO and CISBIO kits demonstrated unacceptably large bias in the middle range. The limit of blank for all three kits was at or below the manufacturer's claims. However, in 11 of 47 patient samples, the ALPCO kit did not detect CgA where the other two kits were able to provide results. Using the ECL method and reference lab cutoff points, 17 samples were identified as positive. In two of these cases, these were false positives attributable to patient use of proton pump inhibitor medications, which are known to increase CgA in the absence of tumors. Of the remaining 15 positive samples, clinical data was available from 13 of these patients: there were 6 false positives and 7 true positives.

Conclusion: The DAKO ELISA kit compares most favorably with the reference lab ECL method in regard to the characteristics we evaluated. A full validation is planned, including establishment of a clinically suitable cutoff point.

A-26

Turbidimetric immunoassays for IgGκ and IgGλ quantification for the assessment of patients with IgG multiple myeloma

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Quantification of monoclonal IgG immunoglobulins by serum protein electrophoresis (SPE) can be inaccurate in patients with high polyclonal immunoglobulin levels. Furthermore, SPE does not account for the concentration-dependent metabolism of IgG or concentration changes due to changes in haematocrit and plasma volumes. Specific polyclonal antibodies have been produced which recognize conformational epitopes spanning the junction of the heavy and light chains of the immunoglobulin molecule. Here we describe automated, turbidimetric immunoassays for the quantification of IgGκ and IgGλ in serum on the SPA_{PLUS}™, a small, bench-top turbidimeter available from The Binding Site Group Ltd. Determination of the IgGκ/IgGλ ratios can be used as an aid in the diagnosis of IgG monoclonal gammopathies. The main assay characteristics are summarised in the table below.

Assay	IgGκ	IgGλ
Measuring range (g/L)	1.9-40.0	0.92-29.5
Sample dilution	1/20	1/20
Min sample dilution	1/1	1/1
Sensitivity (g/L)	0.094	0.046
Assay time (mins)	5	5
Linearity	y = 0.987x - 0.42g/L r^2 = 0.999	y = 0.959x + 0.55g/L r^2 = 0.997
Intra-assay precision %CV	1.2% (25.18)	0.7% (23.75)
(mean concentration g/L)	1.2% (9.69)	1.4% (5.33)
(n=84)	2.3% (3.18)	2.1% (1.73)
Inter-assay precision %CV	2.0% (25.18)	2.5% (23.75)
(mean concentration g/L)	2.6% (9.69)	2.6% (5.33)
(n=84)	4.1% (3.18)	4.7% (1.73)
Between-day precision %CV	5.7% (25.18)	5.3% (23.75)
(mean concentration g/L)	4.4% (9.69)	5.2% (5.33)
(n=84)	5.3% (3.18)	8.7% (1.73)
Total precision %CV	6.1% (25.18)	5.9% (23.75)
(mean concentration g/L)	5.2% (9.69)	6.0% (5.33)
(n=84)	7.1% (3.18)	10.1% (1.73)

Interference was within ±2.0% when either bilirubin (0.20 g/L), haemoglobin (4.56 g/L) or Chyle (1540 formazine turbidity units) were added to serum samples with known IgGκ and IgGλ concentrations. IgGκ and IgGλ concentrations were measured in 129 normal (blood donor) sera; median IgGκ 6.75 g/L (range 3.84 - 12.07 g/L), median IgGλ 3.90 g/L (range 1.91 - 6.74 g/L), median IgGκ/IgGλ ratio of 1.74 (range 1.12 - 3.21). IgGκ+ IgGλ summation correlated well with total IgG (Binding Site SPA_{PLUS}): y = 0.97x + 0.44 g/L (Passing-Bablok). IgGκ and IgGλ concentrations were measured in 117 IgG (72 IgGκ /45 IgGλ) archived multiple myeloma patient sera. In all cases the IgGκ/IgGλ ratio correctly identified the monoclonal IgG type. The results correlated well with monoclonal IgG measured by SPE densitometry: y = 1.06x - 1.78 g/L (Passing-Bablok). We conclude that serum IgGκ/IgGλ assays provide a rapid, precise method for quantifying IgGκ and IgGλ in serum, and the presence of an abnormal ratio may be useful in identifying patients with IgG multiple myeloma.

A-27

The RareCyte™ system for enumeration of circulating tumor cells that retains all nucleated cells for analyses and doesn't rely on capture of proteins expressed on cells

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Background: Detection and molecular characterization of circulating tumor cells (CTCs) in blood are powerful methods for diagnosing and predicting prognosis and therapeutic response of malignant tumors. Most technologies currently under development are based on capture of cells that express EpCAM or other tumor marker and rely on efficiency of capture and expression of the tumor marker. RareCyte is developing a novel system for CTC detection and enumeration based on density separation and the spread of nucleated cells onto a surface designed for imaging.

Methods: SkBr3 cells were spiked into whole blood from healthy individuals collected in cell preservation tubes (Streck). Clinical samples from cancer patients were obtained by taking left-over blood from CTC analysis by Veridex™. EpCAM, CK, and other relevant antibodies conjugated to DyLight 547, AlexaFluor 488, AlexaFluor647, respectively, were incubated with the blood at room temperature and the mix was added to a tube with a float inserted to spread the cells onto an imaging surface. The tube, float and blood were centrifuged to separate nucleated cells from RBCs and plasma and to deposit the cells onto the imaging layer. Cells on the float were imaged, characterized and counted on an automated platform.

Results: In 14 samples, with 10 to 250 spiked-in tumor cells, an average of 86% of cells were recovered from 3mls of blood using the RareCyte system with linearity from 10 to 250 cells (slope=0.8575; r^2=0.9886, intercept=0.2515). To date, CTCs have been identified in 12 clinical samples from patients of three different cancer types, breast, prostate and colon. CTCs have not been observed in blood from 3 normal

donors, indicating a very low rate of false positives. In one of 14 clinical samples with zero cells identified by the EpCAM capture system, 2 cells were identified using the RareCyte system. These cells were found to have low EpCAM expression and high CK expression. This indicates the potential of the RareCyte system to find CTCs missed by methods that rely on EpCAM capture.

Conclusion: The RareCyte technology is a new platform with advantages over current technology because all nucleated cells can be imaged without EpCAM expression as a prerequisite for capture. Molecular characterization of CTCs can be customized and automated for deployment in clinical laboratories. This ability to customize testing can provide more relevant information about the patient's cancer, guiding their physician in therapy selection.

A-28

Turbidimetric immunoassays for IgMκ and IgMλ quantification for the assessment of patients with IgM monoclonal gammopathies

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Accurate quantification of IgM monoclonal proteins by serum protein electrophoresis (SPE) can be hampered by its failure to migrate from the origin and its heavily-polymerised nature. Immunofixation (IFE) can improve the sensitivity of detection but is non-quantitative. Specific polyclonal antibodies have been produced which recognize conformational epitopes spanning the junction of the heavy and light chains of IgM. Here we describe automated, turbidimetric immunoassays for the quantification of IgMκ and IgMλ in serum on the SPA_{PLUS}™, a small, bench-top turbidimeter available from The Binding Site. Determination of the IgMκ/IgMλ ratios can be used as an aid in the diagnosis of IgM monoclonal gammopathies. The main assay characteristics are summarised in the table below.

Assay	IgMκ	IgMλ
Measuring range (g/L)	0.2-5.0	0.18-4.50
Sample dilution	1/10	1/10
Min sample dilution	1/1	1/1
Sensitivity (g/L)	0.02	0.018
Assay time (mins)	5	5
Linearity	y = 0.992x - 0.144 g/L r^2 = 0.995	y = 0.960x - 0.183 g/L r^2 = 0.987
Intra-assay precision %CV	1.8% (4.13)	1.7% (4.11)
(mean concentration g/L))	1.5% (1.80)	2.0% (0.96)
(n=84)	2.4% (0.34)	2.0% (0.29)
Inter-assay precision %CV	1.8% (4.13)	1.5% (4.11)
(mean)	1.3% (1.80)	0.5% (0.96)
(n=84)	3.3% (0.34)	2.1% (0.29)
Between-day precision %CV	4.6% (4.13)	4.4% (4.11)
(mean)	3.5% (1.80)	3.2% (0.96)
(n=84)	4.9% (0.34)	5.4% (0.29)
Total precision %CV	5.3% (4.13)	5.0% (4.11)
(mean)	4.1% (1.80)	3.8% (0.96)
(n=84)	6.4% (0.34)	6.1% (0.29)

Interference was within ±2.6% when either bilirubin (0.20 g/L), hemoglobin (5.00 g/L) or Chyle (1500 formazine turbidity units) were added to serum samples with known IgMκ and IgMλ concentrations. IgMκ and IgMλ concentrations were measured in 147 normal (blood donor) sera; median IgMκ 0.63 g/L (range 0.19 - 1.63g/L), median IgMλ 0.35 g/L (range 0.12 - 1.01 g/L), median IgMκ/IgMλ ratio of 1.81 (range 1.18 - 2.74). IgMκ+ IgMλ summation correlated well with total IgM (Binding Site SPA_{PLUS}): y= 0.96x + 0.08 g/L (Passing-Bablok). IgMκ and IgMλ concentrations were measured in 47 (25 IgMκ, 22 IgMλ) archived IgM monoclonal protein patient sera. In all cases the IgMκ/IgMλ ratio correctly identified the monoclonal IgM type. The results correlated well with total IgM: y = 1.07x - 0.12 g/L (Passing-Bablok). We conclude that serum IgMκ/IgMλ assays provide a rapid, precise method for quantifying IgMκ and IgMλ in serum, and the presence of an abnormal ratio may be useful in identifying patients with IgM monoclonal gammopathies.

A-29

Elevated CA-125, AFP and β-hCG in Mild and Severe Preeclampsia

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Background: Preeclampsia affects between 0.4% and 2.8% of all pregnancies in developed countries and much more in developing countries, leading to as many as 8,370,000 cases worldwide per year. This study aims to determine whether the levels of Carbohydrate Antigen 125 (CA125), Carcino-embryonic Antigen (CEA), Alpha-feto Protein (AFP) and Beta human Chorionic Gonadotropin (β-hCG) alter in preeclampsia, and, whether these tumour markers are able to differentiate mild preeclampsia from severe preeclampsia.

Methods: This is a hospital based prospective cross-sectional study conducted from May 2009 to August 2010. 60 diagnosed preeclamptic cases were recruited in this study.Preeclampsia was defined as per the Australasian Society Consensus Statement research definition as, a new onset of hypertension after 20 weeks of gestation with Proteinuria. Pre-eclampsia was regarded as serious if severe hypertension was associated with proteinuria or if hypertension was associated with severe proteinuria. The criteria for severe hypertension and severe proteinuria were respectively (1) systolic blood pressure ≥160 mm Hg or diastolic ≥110 mm Hg and (2) proteinuria >5 gm in 24 hours. 17 cases fulfilled the criteria of severe preeclampsia.

Age and Gestational period matched pregnant controls were selected.5 ml of blood was drawn and serum was separated within 1 hour of sample collection for the estimation of CEA, CA125, AFP and β-hCG using chemiluminescent Assay (Beijing Bio-ekon Biotechnology). Mann Whitney-U test was used for the comparison of median between the two groups. ROC curve (Receiver Operating Characteristic) was plotted for the selection of optimal cut-off of tumour markers which could distinguish mild preeclampsia from severe forms.

Results: Though there was no significant median difference in CEA level (0.75 ng/mL Vs 0.7 ng/mL for cases and control respectively), other three tumour markers were significantly elevated in preeclampsia than in normal pregnancy (18.76 IU/mL Vs 9.0 IU/mL; 154.45ng/mL Vs 118.95 ng/mL and 22824.5 mIU/mL Vs 17554 mIU/mL respectively for CA125; AFP and β-hCG). Furthermore, CA125 (16.82 IU/mL Vs 27.94 IU/mL), AFP (149.3 IU/mL Vs 186.6 IU/mL) and β-hCG (22560 mIU/mL Vs 29600 mIU/mL) were also significantly higher in severe preeclampsia in comparison to mild preeclampsia.

As a diagnostic test for severe preeclampsia, AUC was highest for AFP (0.889) followed by CA125 (0.881) and β-hCG (0.732) respectively. The sensitivity and specificity of AFP for differentiation between mild and severe preeclampsia was respectively 92.3% and 82.9% with cut-off point of 166.6 ng/ml. PPV+ and NPV- at this cut-off was respectively 63.15% and 97.14%. Likewise, for CA125, at the cut-off point of 20.7 U/ml, the PPV+ for the differentiation of severe preeclampsia from mild was only 50% but NPV- was 93.75%. Moreover, no tumour markers showed significant correlation with gestational weeks. Only CA125 and AFP showed significant correlation with blood pressure and 24-hr urinary total protein.

Conclusion: CA125, AFP and β-hCG levels were significantly higher in the preeclamptic group in comparison to normal pregnancies and further, higher in severe preeclampsia as compared to mild forms. AFP,among others,best distinguished severe preeclampsia from mild preeclampsia.

A-30

K-ras/BRAF mutations in colorectal tissue: multiplex detection with biochip array technology

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Background: Colorectal cancer (CRC) is the third most common malignant neoplasm world wide, second largest cause of cancer mortality in the Western world but among the most curable when identified early. Therapeutic agents targeting the epidermal growth factor receptor (EGFR) have improved outcomes for patients with advanced colorectal cancer but are only effective in a subset of patients. Mutations in the K-ras gene, are known to disrupt the EGFR pathway, rendering anti-EGFR therapy ineffective and these are found in 30-40% of colorectal tumours. Thus, K-ras mutational status can help predict which patients will benefit from anti-EGFR therapy. Furthermore, BRAF mutations are associated with an additional 12-15% of patients who fail to respond to this treatment.

Relevance: To tailor patient care, it is therefore advantageous to use analytical systems allowing multiplex detection of these mutations. We report the analytical performance

of a biochip array that can simultaneously detect the most frequent mutations in codons 12 and 13 of K-ras and codon 600 of BRAF.

Methodology: DNA from 190 colorectal tissue samples was analysed using the RanplexCRC Array. This assay combines probe hybridisation, ligation, probe-pair amplification and biochip array hybridisation, to generate a sensitive and specific mutation profile of the tissue. Biochips spatially separate multiplex amplicons onto discrete test regions (DTRs), through hybridisation to a tethered capture probe, each representing a specific mutation or wild-type sequence. Chemiluninesecent detection defines hybridisation in each DTR, through analysis with the Evidence Investigator analyser.

Results: From 190 colorectal DNA samples analysed, 93 represented matched normal and tumour tissue. 22% of tumours (21/95) exhibited mutations in K-ras and 7 % (7/95) carried a BRAF mutation. The remaining 71% were confirmed as wild type in these regions for both genes. 3% (3/95) of normal tissue also presented with a K-ras mutation. Five different mutations within K-ras were observed G12D, G12V, G12S, G12C and G13D. Multiple K-ras mutations were found in three samples. For comparison, 25 samples (19 K-ras positive and 6 wild type) were screened for only K-ras mutations using a commercially available assay. 100% correlation with the RanplexCRC Array was observed. The commercial assay also confirmed the presence of K-ras mutations in the 3 "normal" tissue samples but failed to discriminate multiple K-ras mutations.

Conclusion: These findings demonstrate applicability of this biochip assay to simultaneously detect specific K-ras and BRAF mutations in a single reaction in colorectal tissue. Results compare favourably with another commercial kit, which requires multiple uniplex reactions per sample to complete analysis. The RanplexCRC Array can also identify multiple mutations in a sample, which cannot be differentiated by the commercial assay. Furthermore RanplexCRC is open to increased multiplexing. This is important as 40-60% of patients with wild type K-ras (codons 12 and 13) status fail to respond to anti-EGFR therapy. Presence of additional markers therefore might help optimise the selection of candidate patients to receive anti-EGFR treatment.

A-31

Development of a Luminex® Microsphere-Based Assay for the Quantitative Measurement of hCG and hCGβ in Serum

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Background: Human chorionic gonadotropin (hCG) is a dimeric glycoprotein hormone composed of noncovalently associated alpha and beta subunits and normally secreted by placental syncytiotrophoblasts during pregnancy. hCG can also be produced by some malignancies such as trophoblastic and germ cell tumors, both of which may secrete only the free beta subunit (hCGβ). In the United States, commercially available hCG assays determine a total hCG concentration and none provide quantitative measurements of specific hCG variants. The objective of this study is to develop a multiplexed assay to specifically quantify hCG and hCGβ, separately, in serum.

Methods: Two different monoclonal antibodies with specificity for hCG or hCGβ were coupled to two different sets of Luminex microspheres. A third monoclonal antibody with specificity to a common epitope on hCG and hCGβ was labeled with phycoerythrin. WHO Reference Reagents for hCG, hCGβ, nicked hCG (hCGn), and nicked free beta subunit (hCGβn) and WHO International Standards for the free alpha subunit (hCGα), luteinizing hormone (LH), follicle stimulating hormone (FSH), and thyroid stimulating hormone (TSH) were reconstituted in phosphate buffered saline (pH 7.4) containing 1% bovine serum albumin. For all reactions, 100 µL of sample diluted into 20% hCG-free serum was incubated with 30 µL of microspheres for 30 minutes. After washing, 100 µL of PE-labeled reporter antibody was added and incubated for 30 minutes. After a final wash, the median fluorescence intensity of the reporter was determined using a Luminex 100 instrument.

Results: The analytical measuring range for hCG and hCGβ was 1-780 and 12-3,000 pmol/L, respectively. There was no recognition of hCG by the anti-hCGβ labeled microspheres. The anti-hCG labeled microspheres demonstrated 0.5% cross-reactivity with hCGβ. Analytical specificity was assessed by recovery experiments. For the anti-hCG labeled microspheres, there was no interference from hCGα (≤100 IU/L), LH (≤10 IU/L), FSH (≤500 IU/L) or TSH (≤500 mIU/L) but hCG recovery was 111-125% in the presence of 100-500 IU/L LH and 25,000 pmol/L hCGβn. The greatest hCG recoveries were observed with hCGn (137-257% at 250-25,000 pmol/L). For the anti-hCGβ labeled microspheres, there was no interference from hCGα (≤100 IU/L), LH (≤500 IU/L), FSH (≤500 IU/L), or TSH (≤500 mIU/L). hCGn at 250, 2,500, and 25,000 pmol/L produced an hCGβ recovery of 112, 149, and 302%, respectively. The greatest hCG recoveries were observed with hCGβn (145-1,077% at 250-25,000 pmol/L).

Conclusion: The Luminex method described here enables the simultaneous measurement of hCG and hCGβ in serum with high sensitivity. Specificity assessments using samples with hCG variants and related pituitary glycohormones display no to minimal interference, even at concentrations in excess of those expected physiologically. Cross-reactivity with certain hCG variants is noted but is not unexpected as these are derived from intact hCG. In addition to having advantages over traditional methods, this multiplex assay format can serve as a foundation for an even broader panel by including other biomarker-specific microspheres. Further validation is needed to assess the clinical utility of this method for the quantitation of hCG and its variants in individuals with trophoblastic disease and germ cell tumors.

A-32

Remodeling of extracellular matrix in early recurrence of superficial bladder cancer

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Background: Urinary bladder cancer is an insidious disease showing a high rate of recurrence, often in association with more aggressive and invasive tumors. Regular follow-up of patients is thus essential. Matrix metalloproteinases (MMPs) are important mediators of cancer progression and invasion. Also, the balance of MMPs and their specific inhibitors (TIMPs) is considered an important regulation of tumor metastasis and therefore, a disruption of this balance, in favor of gelatinase activity, may result in tumor growth, invasion and metastasis. The study aims to explore the clinical significance of MMP-2, MMP-9, TIMP-2 and the MMP-2/TIMP-2, MMP-9/TIMP-2 ratios in the detection and progression of the early recurrences during the follow-up of superficial bladder tumors.

Methods: We collected urine from 75 patients (59 males, 16 females) with superficial bladder tumors (pTa and pT1), continuously monitored during 16 months. Urinary cytology and quantitative MMP-2, MMP-9, TIMP-2 were measured from the same voided urine sample, collected before the first transurethral resection (TURB) and before each follow-up cystoscopy. The urine sediment was used for cytology and the supernatant for estimation of MMP-2, MMP-9 and TIMP-2 by ELISA. We compared the results with the recurrence status and with the tumor characteristics in the case of disease relapse. Cystoscopy was done for all patients as the reference standard for the identification of bladder cancer. The biopsy of any suspicious lesion was performed for the histopatological examination.

Results: During the follow-up, we performed 131 cystoscopies and 63 of them were positive for 29 patients. Positivity rates and median levels for MMP-2, MMP-9, TIMP-2, MMP-2/TIMP-2 ratio and MMP-9/TIMP-2 ratio showed a significant difference between patients with reccurence and those without reccurence ($p < 0.01$). Median levels of MMP-2, MMP-9 and MMP-2/TIMP-2, MMP-9/TIMP-2 ratios were respectively increased by 4.98-fold, 6.84-fold, 40.2-fold and 54.69-fold, in the group with reccurence as compared with the group without reccurence. On the other hand, in the recurrence group, the TIMP-2 levels were by 7.83-fold lower than those in the group without reccurence ($p < 0.01$). The sensitivity and specificity of the MMP-2/TIMP-2 ratio (98.23% and 87.52%) and MMP-9/TIMP-2 ratio (100% and 93.2%) in the detection of the recurrences during the follow-up of superficial bladder tumors are higher than those of the urinary cytology (68.3% and 91.04%).

Conclusions: The disease recurrence influences the extracellular matrix (ECM) remodeling, by increasing serum levels of MMP-2, MMP-9 and decreasing TIMP-2. Therefore, measuring the MMP-2/TIMP-2 and MMP-9/TIMP-2 ratios in superficial bladder tumor recurrences seems to be a more accurate reflection of the ECM remodeling as than measuring the absolute level of the corresponding proteases. Urinary TIMP-2 level was lower in the group with recurrence as compared with the group without recurrence, indicating that the mechanism of tumor invasion is based on the downregulation of TIMP-2 with abundant activated MMP-2 and MMP-9.

A-33

Exploratory study using comprehensive next-generation sequencing on DNA from FFPE tumor tissue comparing KRAS, EGFR and BRAF gene mutation results with reference laboratory genotyping assays

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Background: Next-Generation Sequencing (NGS) has entered the clinical laboratory, although most current NGS offerings translate existing Sanger sequencing tests for germline mutations to the newer technology. In these instances, average sequence coverage of 30-40-fold over the target is sufficient since sensitivity demands are modest (50% allele frequency detection). For somatic cell (tumor) genetics, average sequence coverage must be much deeper, on the order of 100s-fold, to provide >99% detection power for minor mutant alleles in tumor DNA. Here we report initial results from an exploratory comparison of a comprehensive NGS-based mutation analysis for 189 cancer-related genes and 34 pharmacogenetic markers with standard reference laboratory genotyping assay assignments for EGFR, KRAS and BRAF mutations.

Methods: Specimens were routinely fixed, paraffin-embedded surgical resections, core needle biopsies, or fine-needle aspirates of varied age and tissue type. Each patient sample had been previously tested for a KRAS, EGFR or BRAF mutation by a clinical reference laboratory. DNA extracted from eighty-five specimens using a column-based method was passed through to the assay process. Where possible 200 ng of each sample was used to construct a sequencing library. Targeted sequences (3,230 exons and 37 introns of 223 genes) were hybrid-selected using published methods and sequenced on the Illumina HiSeq2000 platform. Illumina base calls were processed through a newly designed automated analysis pipeline based on a Bayesian approach to generate mutation profiles. NGS results were assessed for agreement with previously reported results.

Results: Exploration of those test results that met our analysis criteria of 100X average coverage showed very high concordance between the two testing modalities. Of the 70 test results, 67 (96%) were found to be in complete agreement. The automated NGS-mutation detection algorithm required refinement to enable automatic detection of a 9-bp EGFR insertion that was reported by the reference laboratory. A KRAS G13D variant was present at 60% frequency but erroneously quality filtered. Refinements to the automated calling algorithm subsequently resulted in agreement on KRAS mutation assignment. Of note among test results, NGS identified a BRAF V600K dinucleotide substitution reported by the reference laboratory as a mononucleotide V600E mutation. In summary, automated mutation calling from NGS data confirmed reference laboratory test results with high concordance, while highlighting one case (BRAF V600K) that likely represents superiority of mutation detection using NGS.

Conclusions: Our results indicate the feasibility of creating a comprehensive, high sensitivity NGS-based mutation detection assay for a large, defined gene set using routinely collected FFPE tissues. This approach is valuable as it allows consolidation of the growing number of single-gene IVDs for clinically important cancer mutations into a single, high quality, comprehensive test. We suggest that clinical-grade next-generation sequencing should be implemented routinely in all oncology clinical trials, and will ultimately become the standard of care for cancer patients.

A-34

Peptide selection and optimization of parameters for a candidate mass spectrometry reference method for measuring prostate specific antigen (PSA) in human plasma.

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Background: PSA immunoassays are widely used, but there are differences among these assays related to antibody specificity and assay standardization. A serum reference method using peptide mass spectrometry (MS) could help standardize and harmonize assays. However, current MS assays are not sensitive enough to measure low blood levels of PSA and there are multiple circulating metabolic forms of PSA. Multiple factors must be considered for the development of a candidate reference method if it is to be used to harmonize existing assays, such as target peptides, reference standards, specificity of antisera used for immuno-extraction, and extraction efficiency and trypsin digestion.

Methods: PSA peptides were selected to provide broad coverage of the molecule to insure that most circulating PSA fragments will be detected. The peptides also needed good MS signals and low probability of post-translational modifications which would alter the charge/mass signal. MRM transitions and LC conditions were optimized for five target candidate peptides (Table 1) and dose response curves were established on API 5000 spectrometer. Isotopically labeled internal standards were synthesized. Potential anti-PSA antisera for extraction were mapped to the ISOBM epitopes and cross-indexed to the antibodies used by commercial PSA immunoassays. Western blotting and silver staining were used to evaluate the efficiency of immuno-extraction and trypsin digestion of PSA.

Results: Five target candidate peptides of PSA were selected for analysis with minimum PSA detection level from 1.2 fmoles (LSE) to 22 fmoles (HSQ). Efficiency of PSA immuno-extraction from human plasma was in the range 98-99%. Efficiency of PSA trypsin digestion was higher than 90% as determined by silver staining and western blotting.

Peptide	AA Sequence PSA sequence #	Native peptide transition	Stable isotope-transition	ISOBM epitope #
LSE_1	*LSEPAELTDAVK* *aa102-113*	636.8^{2+}/943.48	639.9^{2+}/949.5	# 1 aa 86-91
IVG_1	*IVGGWECEK* *aa1-9*	539.54^{2+}/865.35	543.9^{2+}/868.4	# 6 aa3-11
FML_1	*FMLCAGR* *aa171-177*	427.9^{2+}/576.3	432.68^{2+}/586.4	# 3 aa158-163
FLR_1	*FLRPGDDSSHDLMLLR* *aa86-101*	625.05^{3+}/645.4	628.6^{3+}/652.6	# 1 aa86-91
HSQ_1	*HSQPWQVLVASR* *aa10-21*	704.8^{2+}/1055.6	707.6^{2+}/1061.7	# 6 aa3-11

Conclusion: With this strategy the development of a candidate reference method for PSA in serum looks feasible. The utility of this assay for harmonizing existing PSA assays awaits further trials.

A-35

Biomarker Discovery for Early Detection of Hepatocellular Carcinoma in Hepatitis C Infected Patients

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Background: Hepatocellular carcinoma (HCC) is the most common type of primary liver cancer and ranks sixth among cancers as a cause of death worldwide. The projected rise in the new HCC cases in the US is due mainly to latent hepatitis C virus (HCV) infections in the general population with the onset of HCC coming several decades after initial infection. The poor prognosis of HCC is primarily due to the disease being diagnosed at a late stage making successful therapeutic intervention difficult, if not impossible. Early diagnosis is important for the successful treatment by ablation, resection, and/or transplant. Although alpha-Fetoprotein (AFP) is routinely used for screening, it is often normal or indeterminate in early cancer cases. Other screening modalities for early HCC detection are variously inaccurate, expensive or potentially dangerous. Therefore a sensitive and specific facile screening modality for early detection of HCC would provide significant clinical benefit.

Methods: In this study two distinct proteomics methodologies were used to profile and identify potential serum based biomarkers suitable for early HCC detection. All patients choosen are HCV RNA positive but are free of co-infection with HIV and HBV, and a history of low alcohol consumption negating potential confounding covariant risk factors. The HCC status in patients was verified histologically, had AFP>400 ng/mL, or fit AASLD radiographic criteria for HCC. A serum prefractionation strategy using an aptamer-based technology (Bio-Rad) functioned to compress the serum protein dynamic range without affecting the complexity of the serum peptidome. The prefractionated serum was further resolved using 2D-DIGE and the fluorescent signatures captured using GE Typhoon Trio Imager. Precancerous (HCV) and cancerous (HCC) profiles were compared using DeCyder and statistically significant peptide signatures selected for further analysis. The alternative strategy involved serum prefractionation using the Biofluid Analytical Platform to recover the low molecular weight (LMW) serum peptidome (<20kDa) suspected of harboring metabolites and degradation products reflecting HCC, followed by O^{18}/O^{16} stable isotope labeling, a quantitative MS-based proteomics technique that separates individual peptides on the basis of a 4 Dalton m/z change.

Results: The HCV and HCC samples labeled with cy3 and cy5, combined with an internal standard (mixing equal amounts of all HCV and HCC samples labeled with cy2), and separated on 2D-gels revealed 23 differentially expressed protein spots that met selection criteria with a statistical significance of $p<0.05$ at a threshold of 1.5-fold

change. The identified proteins showed >2.0 fold increase/decrease in expression in HCC patient samples relative to HCV patient samples. MALDI-tandem MS and LC-tandem MS were used to identify the proteins. For the LMW peptidome, the ratio of O^{16} (HCV) and O^{18} (HCC) labeled tryptic digestion products were analyzed using nano LC-MS/MS to determine the quantitative changes in peptide abundance and obtain peptide sequence information.

Conclusion: The two proteomic strategies employed have identified multiple serum-based candidate biomarkers with the potential to discriminate between precancerous and HCC and will be evaluated for their capacity to function as biomarkers for early detection of HCC in at risk patient.

A-36

Ouabain Targets Unfolded Protein Response for selective killing HepG2 cells during Glucose Deprivation

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Background: Glucose deprivation is a cell condition that occurs in solid tumors and activates the unfolded protein response (UPR). 2-deoxyglucose (2-DG), a hypoglycemia-mimicking agent, is a synthetic glucose analogue that inhibits glycolysis and blocks cell growth. 2-DG can also cause inhibition of protein glycosylation, thereby altering protein folding within the ER and inducing ER stress. Ouabain is a cardiotonic steroid and specific inhibitor of the Na^+/K^+-ATPase. Previous studies have shown that there might have been some roles of ouabain on apoptosis in human liver carcinoma cells. In addition to this, the relationship between ouabain and UPR is not exactly known. Therefore, we studied the possible effects of ouabain on proliferation, apoptosis and UPR on HepG2 cells. Here, we show that ouabain modulates UPR transcription program and induce cell death in glucose-deprived tumor cells.

Methods: HepG2 cells were cultured overnight in 96-well plates (5×10^3 per well) and then treated with various concentrations of ouabain ranging from 0,75 to 75 nM in the absence or presence of 10 mM 2-DG for 48 hours. We also measured quantitatively expression levels of Grp78, Grp94, CHOP, XBP-1, MTJ-1, Cav-1, HKII, MDR-1, MRP-1, HO-1, Par-4 by RT-PCR. PCR-RFLP was also performed to determine IRE1 activity. Cell number, viability and proliferation of HepG2 cells were monitored by real time cell analyzer system (xCELLigence, Roche, Germany).

Results: Ouabain at all concentrations did not cause cytotoxicity whereas they were very effective under 2-DG stress conditions, as evidenced by real time cell analysis based on electrical impedance signal. 0,75 and 7,5 nM ouabain have the reducing effects on chemoresistance, as suggested by down-regulated MDR-1 expression compared with control. CHOP expression was down-regulated at all concentrations compared with control under glucose deprivation. Although 75 nM ouabain alone induced Grp78 expression, paradoxically, it effectively suppressed 2-DG-induced Grp78 induction, thereby sensitizing HepG2 cells to cytotoxicity. Interestingly, when combined with 2-DG, ouabain decreased both HKII and HO-1 expressions as opposed to 2-DG alone. Par-4 was up-regulated dose-dependently by ouabain alone and it was more pronounced when combined with 2-DG, thereby possibly inducing apoptosis. We also showed that ouabain alone or combined with 2-DG dose-dependently inhibited XBP-1 mRNA splicing.

Conclusions: We revealed that ouabain is not a typical UPR inducer as well as 2-DG and can sensitize HepG2 cells to cytotoxicity or apoptosis under glucose deprivation conditions. Our findings show that disrupting the UPR during glucose deprivation could be an attractive approach for selective cancer cell killing and could provide a chemical basis for developing UPR-targeting drugs against solid tumors. Ouabain use as an adjunct to conventional cancer therapy also warrants vigorous investigation.

A-37

Quantitative and Qualitative Fecal Immunochemical Blood Tests: Prospective Comparison of Specificity and Precision

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Background: Increasingly, fecal immunochemical tests (FITs) are used to detect occult blood in stool as an approach to colorectal cancer (CRC) screening. Various types of FIT are available commercially. While the reliability and program costs of FIT screening for CRC depend, in part, on test precision and specificity, few comparative data are available on these metrics for quantitative and qualitative methods.

Objective: To prospectively assess and compare analytical precision and clinical specificity for quantitative and qualitative FIT methods.

Methods: Polymedco FIT methods (Cortlandt Manor, NY) were selected for this study including the semi-automated quantitative OC-Micro and the qualitative OC-Light. For the quantitative FIT, precision was assessed using manufacturer-provided QC material (intra- and inter-assay) and spiked fecal samples (inter-assay). Linearity and hook-effect studies utilized positive, spiked fecal samples serially diluted with negative fecal samples. Stability was assessed at ambient and refrigerated temperatures using 10 positive patient samples. For the qualitative FIT, two different technologists blindly interpreted subjective results for the precision studies. To assess clinical specificity, 723 asymptomatic patients between the ages of 45-85 with a normal colonoscopy in past 3 years were consented. Each participant collected a single stool from which samples for both types of FITs were evaluated according to manufacturer's instructions. A fecal hemoglobin concentration greater than 100 ng/mL (FDA approved cut-off for CRC screening) was considered positive for the quantitative FIT, and a visible band on the reading strip was deemed positive for the qualitative FIT (manufacturers claimed detection limit = 50 ng/mL Hb).

Results: Precision of the quantitative FIT with QC material ($n = 20$) produced 100% concordance of negative or positive interpretations. Spiked fecal samples produced 22.4%, 21.1% and 17.6% CVs at mean concentrations of 100, 176, and 937 ng/mL hemoglobin, respectively. The cutoff of the qualitative FIT was challenged at 40 ng/mL Hb (20/20 negative results), 47 ng/mL (17/20 negative results), and 60 ng/mL (20/20 positive results). Linearity of the quantitative FIT was demonstrated over the range of 46 to 1019 ng/mL Hb ($y = 2.75x + 17.45$; $r^2 = 0.9887$). A hook effect was observed in extremely high fecal Hb samples (>800 ng/mL); therefore, only a qualitative interpretation should be provided in this range. Manufacturer-claimed stability of 8 days ambient or 14 days refrigerated was verified using positive specimens, and % differences from baseline were <17%. Clinical specificity for the quantitative FIT at the designated cutoff of 100 ng/mL Hb was 97.4% compared to 92.3% for the qualitative FIT ($p < 0.01$).

Conclusion: Precision, analytical sensitivity, and clinical specificity by the quantitative FIT (OC-Micro) were high. As the qualitative FIT (OC-Light) had lower specificity and the potential to yield more false positives, the quantitative FIT may be more suitable for CRC screening.

A-38

Targeted Proteomic Analysis of Predictive and Prognostic Breast Carcinoma Biomarkers by Multiplex Immunoassay of Tissue Homogenates

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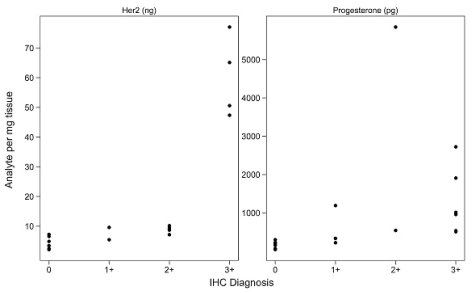
Background: While morphologic examination remains the cornerstone of pathologic tissue diagnosis, determining the quantities of biomarkers in tumor tissue often provides additional prognostic and predictive information no gleaned by morphologic examination alone. Specifically, the concentrations of proteins on the surface (i.e. HER2) and in the nuclei (i.e. estrogen receptor) of breast carcinoma cells can predict patient survival and response to targeted therapies. As recent studies have shown disturbingly frequent molecular misclassification of breast cancer patients by traditional methods, improved diagnostic methods are needed. To examine the feasibility of a targeted proteomic approach for breast carcinoma classification, the concentrations of prognostic and predictive biomarker proteins in homogenates of resected tissue samples were correlated with immunohistochemical methods.

Methods: Protein from fresh/frozen resected breast carcinoma tissue samples was extracted from 3x10µM sections by disruption in a commercial protein extraction buffer. Protein biomarkers were quantitated with Novagen® WideScreen bead-based immunoassays on a Luminex-200 instrument. Results were normalized to homogenate total protein concentration as determined by a standard BCA assay.

Results: In breast carcinoma biopsy samples, we found a bead-based immunoassay for HER2 and Progesterone Receptor (PR) concentration performed on tissue lysates can classify HER2 and PR overexpression, as the IHC and quantitative scores compared well (Figure 1; HER2 IHC-2+ samples were negative by FISH).

Conclusions: These results provide promising data for the application of bead-based immunoassays in the classification of human breast carcinoma as adjuncts, or possibly replacements, for less discerning methods. The entire assay, from pre-analytical specimen preparation to multiplex immunoassay analysis, is amenable to automation, indicating that some currently manual anatomic pathology diagnostic practices could be performed in the automated clinical laboratory. While this current assay utilized unfixed tissue, the same approach is compatible with formalin-fixed, paraffin-embedded tissue (Strathmann et al., AACC 2010, #D-145) and development of this method is ongoing to include other interesting analytes.



A-39

In SILICO Quantitative Evaluation of Biomarkers for the early Detection of Pancreatic Cancer

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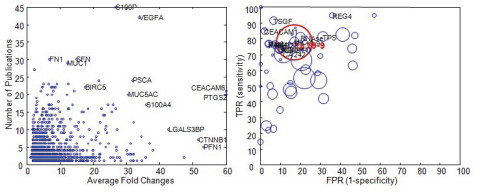
Background: Pancreatic cancer is one of the most deadly cancers with 5-year survival rate of about 5%. Despite the thousands of biomarker candidates reported in the literature, only CA19-9 is cleared by the FDA for clinical use. In this study, we developed an In SILICO approach to evaluate pancreatic cancer biomarkers in the literature using a set of quantitative criteria for selecting biomarkers for further validation.

Methods: PUBMED database was searched for articles using the terms "pancreatic cancer or pancreatic neoplasm or PANIN, or pancreatic adenocarcinoma", combined with "proteomic or biomarker or sensitivity or fold". Information extracted included: sample size, population characteristics, cell lines, assay type, sensitivity, specificity, area under ROC curve, and fold changes in protein or mRNA. A weighted scoring system based on fold changes, number of studies, and cohort characteristics was developed to rank and select biomarkers.

Results: We compiled a biomarker database with over 8000 entries from over 550 studies, including 2309 unique mRNAs and 1716 unique proteins. In the figures, the top ranked candidate biomarkers were plotted according to their average expression fold change and number of publications (left) or their performance (sensitivity, specificity) in clinical samples and the aggregated study sample size (circle diameter) (right). Among the candidates, the ten top-ranked biomarkers showed average expression fold changes of (26), number of cited papers (20), sensitivity (74%), specificity (86%) and samples size of (150), compared to the average fold changes of (7.7), cited papers (3.3), sensitivity (68%), specificity (82%), and sample size of (185) for all candidates.

Conclusion: We developed an In SILICO, quantitative approach to evaluate pancreatic cancer biomarkers in the literature and identified (CA 242, S100p, CEACAM1, SC6, MIC1, MUC5AC, S100A4, TPS, PSCA, and VEGFA) as biomarkers with the highest potential for validation studies to further characterize their utility for the early detection of pancreatic cancer.



A-41

Assessment of an ELISA for Chromogranin A in Serum Absent of the High-Dose Hook Effect Observed in Other CgA Immunoassays

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Background: Chromogranin A (CgA) is a 49 kilodalton protein found in the secretory granules of neuroendocrine cells. CgA functions as a prohormone containing multiple sites for endopeptidases that cleave CgA into several functional peptides including vasostatin I and II, prochromacin, pancreastatin and catestatin. The serum concentration of circulating CgA has been demonstrated to be a useful marker for the detection and monitoring of neuroendocrine tumors. During routine testing of human sera using a CgA ELISA (Alpco Diagnostics, Salem, NH) an apparent, high-dose hook effect was observed in approximately 15% of specimens. This was unexpected given the sequential format of the assay. We hypothesized that a peptide(s) derived from CgA that was present at a high concentration was responsible for the apparent hook effect but Western blot investigations were inconclusive. Here we describe the analytical performance characteristics the CisBio Chromoa™ CgA ELISA (CisBio US Inc., Bedford, MA) that we validated as a potential replacement for the Alpco assay. Of particular interest, was an assessment of the CisBio assay's ability to eliminate the problematic hook effect.

Methods: Serum CgA was measured according to the assay manufacturer's testing protocol. Performance characteristics, including analytical sensitivity, linearity, imprecision, and analyte stability, were performed. The 95th percentile, non-parametric reference interval was established from 150 healthy adult volunteers. Specimens that produced the apparent high-dose hook effect were evaluated by a split-sample comparison study using both assays. The project was approved by the University of Utah's Institutional Review Board.

Results: A limit of detection of 8 ng/mL was calculated by measuring 20 replicates of the zero calibrator and adding three standard deviations to the mean. Linearity was determined by dilution of a highly concentrated CgA serum pool with the assay diluent to create six samples with expected concentrations ranging 14-670 ng/mL. Linear regression generated a slope=1.04, intercept=18.09 and r^2=0.997. Within-run imprecision studies produced CVs of 6.8, 2.8 and 5.9% at concentrations of 32, 93, and 529 ng/mL, respectively (n=20 per concentration). Between-run CVs were 8.6, 6.0 and 5.9% at 47, 337, and 663 ng/mL, respectively (n=15 per concentration). CgA was stable at ambient temperature for a minimum of two days (aliquots from three pools at 0, 4, 8, 16, 24 and 48 hours, 62±1.5, 157±5.3 and 409±14.7 ng/mL), and stable at 4-8 °C for a minimum of 14 days (aliquots from three pools at 0, 2, 4, 7, 10 and 14 days, 60±1.8, 147±6.5, and 386±22.6 ng/mL). An upper non-parametric reference interval limit at the 95th percentile was determined to be 95 ng/mL with no significant differences identified by gender (p=0.49). CgA concentrations increased slightly but significantly with age (r^2=0.083, p=0.0024) but the increase is clinically insignificant. Specimens (n=24) showing the apparent high-dose hook effect utilizing the Alpco ELISA did not exhibit the phenomenon when measured using the CisBio ELISA.

Conclusions: The CisBio Chromoa CgA ELISA demonstrates acceptable performance for quantifying CgA in human serum. The apparent high-dose hook effect exhibited in other CgA assays was not observed using the CisBio CgA ELISA.

A-42

Autoantibody Signatures as Biomarkers to Distinguish Prostate Cancer from Benign Prostatic Hyperplasia in Patients with Elevated Serum PSA

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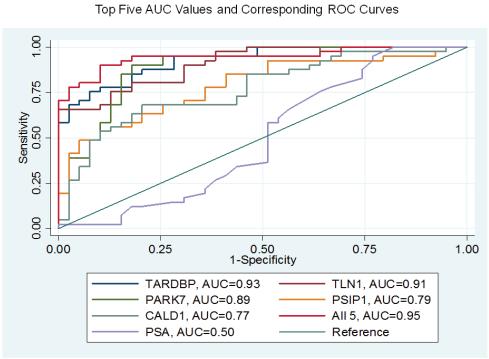
Background: Serum prostate specific antigen (PSA) levels lack the specificity to differentiate prostate cancer from benign prostate hyperplasia (BPH), resulting in unnecessary biopsies. We now report the identification of 5 autoantibody signatures to specific cancer targets which might be able to differentiate prostate cancer from BPH in patients with elevated serum PSA.

Methods: To identify autoantibody signatures as biomarkers, a native antigen capture microarray platform was used. Briefly, well-characterized monoclonal antibodies were arrayed onto nanoparticle slides to capture native antigens from prostate cancer cells. Using the immobilized antigens as bait, autoantibodies from prostate cancer patients and patients with BPH were isolated and probed. From experiments using an initial set of over 500 cancer related antigens, a customized array containing 27 unique antigens was further tested. Prostate cancer patient serum samples (n=41) and BPH patient serum samples with a mean follow-up of 6.56 years (n=39) were obtained. 100ug of IgGs were purified and labeled with a Cy3 dye and incubated on the arrays. The arrays were scanned for flluorescence and the intensity was quantified. Receiver operating characteristic (ROC) curves were produced and the area under the curve (AUC) was determined for the 27 antigens.

Results: Using the native antigen capture microarray platform, we found autoantibody signatures capable of distinguishing between prostate cancer and BPH. The top 5 autoantibody signatures were found to react with TARDBP, TLN1, PARK7, PSIP1, and CALD1. Combining these antigens resulted in an AUC of 0.95 compared to 0.50 for serum level PSA when differentiating between prostate cancer and BPH in our cohort. In addition, the coefficient of variance between duplicate runs for a given sample averaged 14.8%.



Conclusion: Our results showed we were able to identify specific autoantibody signatures that can differentiate prostate cancer from BPH, and may result in the reduction of unnecessary biopsies in patients with elevated serum PSA.

A-43

Proteomic Biomarkers for the Early Detection of Ovarian Cancer Progression

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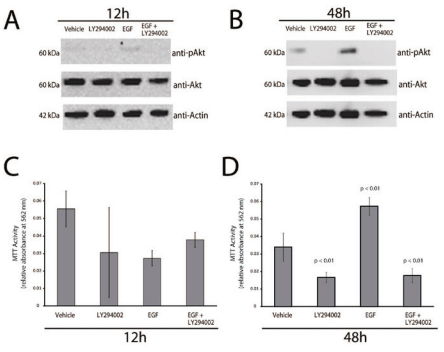
Background: Ovarian carcinoma is a highly metastatic disease rarely detected during early stages of cancer progression, resulting in very poor survival. A cascade of molecular and morphological events results in uncontrolled cancer growth and ultimate metastasis. These events can be monitored via time course studies. The purpose of this work is to analyze these temporal changes in order to identify biomarkers that could be used for the early detection of ovarian cancer progression. In this study, cultured ovarian cancer cells were treated with growth modulators to mimic the cellular/morphological events that occur during cancer cell proliferation and metastasis for both biochemical and high-throughput mass spectrometry/proteomic analyses.

Methods: The human serous OVCAR-3 cell line was originally derived from the malignant ascites of a patient with ovarian adenocarcinoma. Cells were treated with EGF or the growth inhibitor LY294002 for 12 and 48 hours. To assess biochemical changes, immunoblotting for Akt-phosphorylation was performed. For phenotypic analysis, MTT proliferation assays were conducted. Tryptic peptides from cell lysates/cultured media were iTRAQ-labeled for quantitative analysis using the 2D-LC-MALDI-TOF/TOF and LC-ESI-LTQ-OrbiTRAP mass analyzers.

Results: Akt-phosphorylation was upregulated in the presence of EGF at 12h (Fig. A) and potentiated at 48h (Fig. B). Treatment with LY294002 abolished Akt-phosphorylation (Fig. A-B). Proliferation analysis did not show increases in proliferation at 12h under any treatment condition (Fig. C) However, MTT intensity showed a 1.7-fold increase in MTT activity in 48h EGF-treated cells; there was a 2-fold decrease in proliferation in the presence of LY294002 (Fig. D, $p<0.01$ as compared to vehicle). Subsequently, cellular and secretory expression profiles were analyzed by LC-MS/MS to assess protein candidates of ovarian cancer hyperproliferation.

Conclusion: These data demonstrated that the proposed OVCAR-3 model could be used to measure biochemical/proteomic changes over time. Biomarkers associated with these temporal changes may be valuable for the early detection of ovarian cancer progression.



A-44

XRCC1 399 Arg>Gln (28152G>A) variation correlates with deterioration in Quality of Life induced by radiotherapy in prostate cancer patients

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Background: It is estimated that there were over 200,000 new prostate cancer (PCa) cases diagnosed in the U.S. in 2010 and approximately 48% of patients diagnosed with PCa received some form of radiotherapy for their initial treatment. However, some patients develop severe adverse radiotherapeutic effects (AREs), such as severe lower urinary tract irritation, erectile dysfunction, or rectal bleeding, ulceration or dysfunction. Factors associated with AREs have not been well defined, although some genetic and non-genetic variables have been implicated. X-ray repair cross complementing protein 1 (XRCC1) is one of enzymes involved in DNA repair. A previous study has shown that XRCC1 399 Arg>Gln (28152G>A) is associated with increased risk of radiation-induced late injuries in breast cancer patients. The objective of this study is to identify a potential association between XRCC1 399 Arg>Gln (28152G>A) and deterioration in Quality of Life (QoL) due to urinary symptoms caused by radiotherapy in patients with PCa.

Methods: A cohort of 67 PCa patients treated with radiotherapy was observed for one year following the treatment. The QoL due to urinary symptoms was assessed by grading urinary conditions using the following scores recommended by the American Urological Association: 0, delighted; 1, pleased; 2, mostly satisfied; 3, mixed; 4, mostly dissatisfied; 5, unhappy; 6, terrible. It was considered a deterioration in QoL if a patient's baseline score before treatment was less than or equal to 3, but greater than or equal to 4 after treatment. Peripheral blood samples were used to isolate genomic DNA. XRCC1 399 Arg>Gln (28152G>A) genotypes were determined using TaqMan SNP typing assay (Applied Biosystems). The association between deterioration in QoL and XRCC1 399 Arg>Gln (28152G>A) was analyzed using two-sided Fisher's exact test. The differences were considered statistically significant if $p < 0.05$.

Results: In these 67 patients, 41 were wild-type (GG) and 26 were variant (GA + AA) of XRCC1 399 Arg>Gln (28152G>A), and the frequencies of wild-type and variant were 0.60 and 0.40, respectively. In the wild-type group, only one patient experienced deterioration in QoL due to urinary symptom induced by radiotherapy, while in the variant group, 8 patients had deterioration in QoL. The incidences of deterioration in QoL were 0.024 and 0.307 in the wild-type and variant groups, respectively, and the difference between these two groups were statistically significant ($p = 0.0016$).

Conclusion: Our data suggest that the variant genotype of XRCC1 399 Arg>Gln (28152G>A) is associated with a higher incidence of deterioration in QoL due to urinary symptoms in patients with prostate cancer post-radiation treatment. Further studies are needed to discover other genetic variations and non-genetic factors that are associated with the risk of deterioration in QoL induced by radiotherapy in PCa patients.

A-45

Pepsinogen I and II: Applications in Gastric Cancer Patients undergoing Surgery

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Background: Gastric cancer is one the most prevalent malignancies in Northeast Asia. Population screen using serum biomarker Pepsinogen I and II has improved the earlier diagnosis in Japan and South Korea. While southern China has a relatively lower incidence of stomach cancer comparing to Japan and South Korea, the clinical application of pepsinogens (PGs) and its ratio was not reported in patients eligible for surgical procedures.

Methods: A total of 121 patients with gastric caners were consecutively enrolled from September 2009 until January 2010. Pre- and post-surgery (within 7 days of surgery) serum pepsinogen I and II were measured using commercial reagents on Architect i2000SR (Abbott Diagnostics, USA). Serum samples from an equal number of age and gender matched patients undergoing gastric endoscopic check-up, who are otherwise apparently healthy were used as pre-surgical normal controls. Mean age of the cohort is 59.7 yrs (range: 21 to 85, median 63), and gender ratio is 59 male/62 female. All cases were pathologically classified with the majority in adenocarcinomas (55%). Gastric lymphomas were excluded from this study. TNM staging was based on both surgical exploration and pathology evidences. Patients opted for experimental biologic treatment were recorded as exit from this study at the time they commence the therapy.

Results: Presurgical PG I is higher in male patients (82 vs 64 ng/mL, p=0.04), while PG I/II ratio is lower when tumor is larger than 5cm in size (4.5 vs 3.7, p=0.04). The difference in presurgical PGs concentration and ratios are not statistically different in terms of age (50 below and above), tumor sites, pathologic subtypes, degree of differentiation, invasiveness and involvement of lymph nodes. Comparing to control group, PG I at 55ng/mL has the AUC 0.645 (95% CI: 0.572-0.718) for diagnosing the cancer; PG II at 12.6ng/mL has the AUC 0.77 (95% CI: 0.71-0.835). The result is superior to CA724, another stomach cancer biomarker. One hundred ten patients out of 121 went through surgical procedure, and post-surgical PG II level drops more with statistic significance when tumor is lager and TNM stage is late (both $p<0.01$, one-way ANOVA). Event-free survival and total survival at 6 months and 1 year post-surgery favor cases with lower presurgical PG II level and less post-surgical drops ($P<0.05$).

Conclusions: Serum PG levels provide prognostic value at both pre- and post-surgical timepoints among patients with gastric cancer. Less drop of PG II after the surgery may indicate better clinical outcome. Thus, PGs could not only be used as a biomarker to identify the high-risk population for gastric cancer, but also in prognosis and post-surgical risk stratification.

A-46

Comparison of urinary and serum HE4 as a Biomarker for Epithelial Ovarian Cancer

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Background: Survival of ovarian cancer is highly dependent upon stage of disease at diagnosis and therefore there is an urgent need of improved methods for early detection. Human epididymis protein 4 (HE4) is overexpressed in ovarian carcinomas and can be detected in serum with high sensitivity and specificity (*Cancer Res*. 2003; 63:3695-700). Hellstrom et al recently reported HE4 to be a urine marker for ovarian neoplasms (*Cancer Lett*.2010;296:43-8) with improved sensitivity in early disease compared to HE4 in serum. In the present study HE4 was measured in both serum and urine samples from EOC patients and patients with benign disease to investigate if HE4 urine levels showed the same or even better diagnostic performance as serum HE4.

Methods: *Patient material:* Urine and serum samples were analyzed from a prospective study of 449 subjects with pelvic masses: 137 cases with EOC or low malignant potential tumors (30 stage I/II, 74 stage III/IV, 21 LMP and 12 unstaged) and 312 with benign gynecological disease. The clinical diagnosis of the patients was surgically staged according to guidelines by the International Federation of Gynecologic Oncology (FIGO). *Analytical Methods* HE4 was measured with HE4 EIA (Fujirebio Diagnostics, Inc.) and creatinine, used to normalize the HE4 levels, was measured via colorimetric determination by the Jaffe Reaction.

Results: Analysis of receiver operator curve (ROC) for HE4 in urine normalized for creatinine concentration for all cases demonstrated an area under the curve (AUC) of 0.86 (95% CI = 0.82-0.90) with median values for benign and malignant disease of 0.84 (Max/Min: 28.2/0.4) and 3.2 (Max/Min: 142.1/0.0) respectively. The corresponding AUC for serum samples was identical to that of the ratio HE4/urine with median values for benign and malignant disease of 58.2 pM (Max/Min: 1339/21.9) and 281.4 pM (Max/Min: 15146.5/29.4). No significant differences were observed in AUCs for ROC curves with stage I/II cases vs. benign disease for HE4 in serum and urine. Overall the correlation between serum and normalized urine HE4 correlated well ($r = 0.93$) indicating that the glomerular filtration of serum HE4 in large determines the HE4 concentration in urine.

Conclusions: This study demonstrated that HE4 in urine had the same diagnostic performance as serum HE4 for diagnosis of ovarian cancer. In contrast to the study by Hellstrom et al. the present study did not confirm that HE4 in urine would be more sensitive than serum for detection of stage I/II disease. Urine may offer a convenient and less harmful sampling procedure for the patient especially for repeated testing over an extended time period to aid detection of cancer development early as well as monitoring early therapy responses.

A-47

Analytical performance of AccuPSA, a fifth-generation digital immunoassay for prostate specific antigen

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Background: Measuring prostate specific antigen (PSA) in prostate cancer patients following radical prostatectomy (RP) has been limited by the sensitivity of available assays. Because radical prostatectomy removes the tissue responsible for producing PSA, post-RP PSA levels are typically undetectable with current assay methods. However, evidence suggests that more sensitive determination of post-RP PSA status has the potential to improve recurrence prognosis, selection for secondary treatment, and effectiveness of salvage treatment from more timely intervention. We report on the analytical performance of AccuPSA™, an investigational digital immunoassay with two logs greater sensitivity than today's ultrasensitive third-generation PSA assays. Potential utility of the test for precise measurement of PSA status in post-RP patients is also demonstrated.

Methods: Reagents were developed for a paramagnetic bead-based ELISA for use in high-density Single Molecule Arrays (SiMoA™). Individual anti-PSA capture-beads with immunocomplexes and associated enzyme labels (beta-galactosidase) were singulated within the microarrays and interrogated for presence of enzyme label. Wells containing an enzyme immunocomplex convert substrate reporter molecules to a fluorescent product, which becomes concentrated in the small microwell volume. This permits imaging of wells containing single molecules of label with a CCD camera. Poisson statistics predict that each well will contain either one PSA molecule or no PSA molecules when the ratio of bound PSA per bead is much less than one. Raw signal is recorded as "% active wells", which is converted to "average enzymes/bead" to correct for non-Poisson behavior at higher PSA concentrations. The output is related to a standard curve and converted to a PSA concentration of the sample. Analytical performance of the assay was characterized, its accuracy was compared with a commercially available test, and longitudinal serum samples from 30 post-RP patients were analyzed.

Results: Limit of Detection (3SD method) was estimated as 0.000028 ng/mL (0.028 pg/mL) across 20 experiments. Limit of Quantification, LoQ (PSA concentration at 20% measurement variation) was estimated over a six-week period as 0.000035 ng/mL (0.035 pg/mL). Reproducibility was characterized over a 10-day period with a panel of four prepared samples, the lowest of which was near the LoQ. Total CVs (including within run, between run, between day) were 8.8, 8.4, 9.9, and 18.3% at PSA concentrations of 51.5, 5.07, 0.99 and 0.04 pg/mL respectively. Linearity was confirmed across the calibration range (0-100 pg/mL) per CLSI EP6-A, and recovery in the absence and presence of endogenous interferences was within 10% of expected. Accuracy was assessed by comparison to a commercially available equimolar PSA method standardized with WHO reference material. Linear regression statistics across 48 serum samples were SiMoA = 1.01(Centaur) + 0.0025, $R^2 = 0.970$ (standard error 0.53, Centaur range 0.41-13.56 ng/mL). All post-RP samples tested were well above the assay LoQ. PSA nadir values following surgery were strongly predictive of five-year biochemical recurrence-free survival.

Conclusion: The assay demonstrated a robust two-log advance in measurement sensitivity relative to current ultrasensitive third-generation assays, and the analytical performance required for a new enabling tool for highly accurate assessment of post-RP PSA status.

A-48

Survivin mRNA levels in urine as a biomarker for bladder cancer

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Background: Survivin is one of the apoptosis inhibitor proteins and is rarely expressed in adult normal tissues. However, survivin expression has been detected in various tumors. In this study, we evaluated the usefulness of urinary survivin/glyceraldehyde-3-phosphate dehydrogenase (GAPDH) ratio as a marker for bladder tumor.

Methods: Urine samples were obtained from 72 patients with bladder tumor, 36 with urinary tract inflammation as controls. Survivin and GAPDH mRNA expression was measured by quantitative real-time PCR assay in urine cells. The GAPDH housekeeping gene was used for normalization of survivin expression. We also

analyzed survivin protein levels using urine samples and recombinant protein by western blotting.

Results: High expression of survivin was confirmed on the protein level using urine samples of bladder tumor by western blotting. Survivin/GAPDH mRNA ratios of bladder tumor quantified by real-time PCR was significantly higher than those of controls (p=0.001). In pathological stage of bladder tumor, survivin/GAPDH mRNA ratio of pTis was significantly high compared with pTa and pT1 (p<0.001, p=0.001, respectively). Grade3 tumors expressed high level of Survivin/GAPDH mRNA ratio compared with Grade1 and Grade2 tumors (p=0.03). The sensitivity, the specificity and AUC of survivin/GAPDH mRNA ratio was 83.3%, 86.1% and 0.898, respectively.

Conclusion: Measuring survivin/GAPDH mRNA ratio in urine is non-invasive and high sensitive examination. Therefore, survivin/GAPDH mRNA ratio is useful marker for the detection of bladder tumor, especially to detect carcinoma in situ.

A-53

Relationship between % free prostate-specific antigen (%fPSA) and prostate cancer biopsy results

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Backgound: Screening based on prostate-specific antigen (PSA) may detect prostate cancers with variable aggressiveness, which may require prompt treatment or only active surveillance. Some prostate cancer biomarkers are associated with the probability of cancer in the biopsy and with the tumour progression; theirs usefulness for the choice of treatment, however, needs further studies.

Objective: To evaluate the correlation of % free PSA (%fPSA) with the positivity for cancer of prostate biopsy and the tumour grade.

Methods: We did a retrospective analysis of 176 men (mean age ± SD: 68 ± 16 years) who had undergone a prostate biopsy after a PSA-based screening and digital rectal examination, and had both PSA and %fPSA results available; the concentrations of serum PSA and %fPSA had been measured with a chemiluminescent microparticle immunoassay (Abbott Diagnostics Division); the tumour grade was defined as Gleason scores.

Results: The mean serum concentration of PSA and %fPSA were 16 ± 11 ng/ml and 18.7 ± 13, respectively; prostate cancer was detected in 83 subjects (sensitivity: 47%); significantly lower % fPSA was observed in patients with cancer at biopsy compared with patients without cancer (8 ± 7 vs 23 ± 12 respectively; $p < 0.001$); among patients with cancer lower %fPSA was significantly associated with Gleason score > 6, $p < 0.01$ when compared with patients whose biopsy Gleason score was ≥ 6.

Conclusions: Low levels of % fPSA are significantly associated with increased probability of prostate cancer detected with PSA-based screening and with a higher prostate cancer grade. Further analysis is necessary to assess the usefulness of this marker in the decision making about the best treatment of prostate cancer.

A-54

Generation of oocyte-like cells from human hepatoblastoma cell line, HuH-6

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Background: Human hepatoma cell line, HuH6 cells can produce estradiol, but the mechanism remains largely unknown.

Methods: For this study, HuH6 cells were cultured continuously for 2-6 weeks without subculture to attain full confluence and a high cell density.

Results: In specific culture conditions, HuH6 generated spontaneously distinct small round cells with a large nucleus-to-cytoplasm ratio, similar to primordial germ cells (PGCs) in morphology. A subpopulation of the PGCs-like cells were alkaline phosphatase (AP) positive, and expressed *Oct4, Stellar, Vasa and DAZL*, consistent with germ cells.

Interestingly, a small subpopulation of larger germ-like cells assembled with smaller somatic cells suspended in the media (Figure 3A), similar to early ovarian follicles in cell distribution patterns. The germ-like cells in the early follicle-like aggregates were Oct4 positive, whereas the surrounding cells were Oct4 negative, which is consistent with natural early follicles. In addition to oocytes, mammalian ovarian follicles contain granulosa and theca cells, whose cooperation can synthesize estradiol. The concentration of estradiol was increased with the extension of culture time, which indicates the existence and functional activity of granulosa and theca cells in culture. The key genes involved in estrogen biosynthesis, including *steroidogenic acute regulatory protein* (*StAR*), *P450 17α-hydroxylase- 17/20 lyase* (*CYP17*), and *P450 aromatase* were detectable in cultures.

On further culture, the germ-like cells differentiated into oocyte-like large cells which could grow up to about 60 μm and were enclosed in a coat resembling the zona pellucida. Immunohistochemical staining for Vasa confirmed that the large oocyte-like cells were of a germline lineage. Immunohistochemistry staining showed that the oocyte-like cells expressed *synaptonemal complex protein 3 (SCP3),* suggesting that these cells were entering meiosis. The results of RT-PCR showed that oocyte-related markers, including *zona pellucida* (*ZP*, including *ZP1, ZP2,* and *ZP3), Vasa, growth differentiation factor (GDF9),* and meiotic markers *(SCP1and SCP3)* can be detectable in cultures.

Conclusion: The HuH6 cells could give rise to PGCs-like cells, follicle-like structures and oocyte-like cells, thereby demonstrating that these cells actually have the potential of germline cells in vitro. The data may address why the cell line can produce estradiol. Furthermore, the spontaneous germ-line potential of human hepatoblastoma cell line, Huh6, indicated that there are a possible link between tumors and germ cell formation. The findings may provide new insights into tumor biology, diagnosis and therapy.

A-55

Separation and characterization of different isoform populations of PSA from seminal fluid

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Background: Prostate specific antigen (PSA) has been used for the diagnosis of prostate cancer (PCa) and monitoring patient response to treatment for many years. PSA is a single chain glycoprotein with one N-linked glycosylation site contributing approximately 8% of the molecular weight. The modification of proteins by glycosylation occurs in approximately half of all serum proteins and changes in glycosylation have been found to accompany both tumor formation and aggressive clinical behavior and this may, therefore, add an element of specificity to PSA testing.

One of the inherent problems of PSA use in the diagnostic setting is the poor specificity for PCa as patients with benign prostatic hyperplasia (BPH) may exhibit higher levels of PSA. The relative abundance of the specific isoforms may be able to help distinguish BPH from PCa and improve the selectivity and sensitivity of PSA for PCa. A review of the work done on PSA isoforms shows a considerable amount of academic interest with some indication that there may be a clinical utility to measuring specific isoforms of PSA.

Methods: A pool of PSA was isolated from seminal fluid acquired from multiple donors using conventional chromatographic techniques. The material was further purified by specific ion exchange chromatography based on the charge differences attributed to the glycosylation variants. This allowed the specific isoforms of PSA to be isolated. The isoforms were assessed by a normal range of analytical techniques along with IEF and 2D electrophoresis.

Serum samples from patients with PCa or benign prostatic hyperplasia (BPH) were used. PSA was affinity purified, separated and probed with the lectin Ulex europaeus (UEA-1: specific for α1,2 linked fucose).

The UEA-1 lectin was used in a simple, validated enzyme-linked immunosorbant lectin assay (ELLA), to assess the glycosylation and show the changes in fucosylation in both the seminal fluid acquired PSA and patient sample acquired PSA samples. This afforded the comparison of the two sample sets.

Results: Based on the glycosylation analysis of the two sample sets, the results show a similar pattern with the three major isoform populations. We have isolated these populations to provide larger amounts of the specific isoforms and subjected the individual isoforms to glycosylation analysis in order to characterize these changes. This study shows that the PSA isoforms extracted from seminal show comparable glycosylation profiles as the isoforms isolated from patient material.

Conclusion: One of the inherent problems of PSA use in the diagnostic setting is the poor specificity for PCa as patients with BPH may exhibit elevated levels of PSA. The relative abundance of the specific isoforms may be able to help distinguish BPH from PCa and improve the selectivity and sensitivity of PSA and PCa. The isolated PSA isoforms from seminal fluid source provides material that may be used for standards and the subsequent inclusion into diagnostic immunoassay kits for these potentially more specific markers.

A-56

Quantification of serum and urinary free light chains in patients with multiple myeloma : comparison of immunoassay and electrophoresis

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Background: In mutiple myeloma (MM) free light chains (FLC) are involved in the pathogenesis of renal failure, a major cause of morbi-mortality. Since reversibility of renal failure often can be achieved at time of diagnosis, early quantification of FLC contributes to appropriately prevent and manage renal failure. In this study, we have compared for the first time, the quantification of FLC by immunonephelemetry and by electrophoresis in serum and in urine of the patients with MM at presentation or at relapse from remission.

Methods: Fresh paired samples of serum and 24h urine were analyzed in 54 patients with MM at presentation (n=44) or at relapse (n=10). FLC quantification by immunonephelometry (Freelite®, The Binding Site) was realised on Siemens Healthcare Diagnostics BNII Analyser. FLC were expressed as total (K plus L) concentration in serum and as total (K plus L) 24h urinary FLC excretion in urine. Electrophoresis, using sodium dodecyl sulfate-agarose gel electrophoresis (Hydragel Proteinuria®, Sebia) was realised on sample pretreated with b-mercaptoethanol for FLC depolymerization. Total serum FLC (in patients with light chain MM, n=20) and total urinary FLC were quantified by densitometry and were expressed as a fraction of total protidemia and as a fraction of total 24h proteinuria, respectively. The intra-class correlation coefficient (ICC), the Bland-Altman analysis and the Spearman's rank correlation coefficient were performed.

Results: The ICC obtained between immunonephelometry and electrophoresis indicated poor reproducibility for serum FLC values (r =0.11, 95% CI - 0.31_0.53) and for urinary FLC values (r =0.06, 95% CI - 0.20_0.33). The Bland-Altman analysis indicated no significative agreement between these 2 methods for serum FLC values (bias: 5.1 g/24 h, 95% CI 15.1 g/24 h) and for urinary FLC values (4.0 g/24 h, 95% CI 11.8 g/24 h). This was explained by an increasing bias between these 2 methods when increasing serum or urinary FLC values were measured (y = 1.40x - 1704, r = 0.95 and y = 1.56x - 825, r = 0.99, respectively). Thus, in serum and in urine, FLC values were up to 9- and 11-fold higher, respectively, by immunonephelometry than by electrophoresis; urinary FLC values measured by immunonephelometry were up to 7-fold higher than total proteinuria values. Meanwhile, comparison of immunonephelometry and electrophoresis for the quantification of albumin, either in serum or in urine, showed a good reproducibility and a good agreement.

Conclusion: This study shows an overestimation of FLC quantification by immunonephelemetry as compared to electrophoresis both in urine and serum of patients with MM at presentation or at relapse from remission. This overestimation increases linearly with increasing values. Such an effect might result from a poor post-dilution linearity of the immunoassay. Given that the International Myeloma Group recommends the use of the immunoassay for quantification of serum FLC and the use of the 24-h urine electrophoresis for quantification of urinary FLC, it must be emphasised that there is no harmonization of FLC values between these 2 methods. Therefore, serum and urinary FLC values should be interpreted with caution.

A-57

Role of Serum Uric acid in Carcinoma Breast in Libyan Patients

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Background: *Carcinoma of Breast* is one of the most common surgical problems in Libya especially in younger female subjects. Breast cancer is the most dreadful disease in terms of quality of life, though heart disease is a more common cause of mortality here. The present study was designed to evaluate the early diagnostic indicator role of serum uric acid levels in patients suffering from carcinoma breast.

Materials and Methods: 100 patients with a classical presentation of a lump in the breast have been selected from an age group ranging from 16 to 65 years with a mean age of 37 years from the department of surgery, 7th October hospital, Benghazi, Libya (2009 and 2010).43 healthy controls with an age group from 20 to 55 years with a mean age of 35 years were also selected. Out of the 100 cases of lump in the breast, 83 were found to be benign and 17 were malignant irrespective of age. Serum uric acid estimation was done in all the patients after an overnight fast. The biochemical test was performed using an authentic method.

Results: Serum Uric acid level was found to be significantly raised ($p = 0.0270$) in patients with benign breast cancer when compared to age matched controls. In the case of malignant breast cancer, the uric acid levels are observed to be increased but not significant ($p = 0.599$). When the serum levels of uric acid were compared between the benign and malignant, it was observed to be slightly raised in benign rather than in malignant but not a significant rise($p = 0.321$) .

Conclusion: An increase in the serum uric acid levels in carcinoma breast is suggestive of increased adenosine deaminase activity and increased xanthine oxidase activity. However, the values did not rise significantly in malignant breast cancer patients when compared to the controls. This observation in our study suggests a possible role of uric acid as an *antioxidant* in combating the oxidative stress in patients with malignant tumors.

A-59

Non Invasive Tools as Useful Complementary and Diagnostic Markers for HCC

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Background: To investigate the potential role of serum Chromogranin-A (Cg-A) and serum protein induced by vitamin K absence antagonist-prothrombin (PIVKA-II), as a diagnostic, non-invasive marker for HCC. Also, to assess the sensitivity and specificity of serum Cg-A , AFP and PIVKA-II in diagnosis of HCC. As hepatocellular carcinoma (HCC) is the most common primary malignant tumor of the liver. Ultimately, no single diagnostic modality yields diagnostic accuracy consistently over 50% to 60% in detecting lesions less than 1 cm, a time when curative surgery is most likely.

Material and Methods: This study was conducted on seventy two patients attending the Hepatology and Gastroenterology Department, Theodor Bilharz Research Institute (TBRI) divided into two groups 40 patients with HCV and 32 patients with HCC. In addition to twenty apparently healthy individuals served as a control group. They were initially subjected to history; clinical examination, abdominal ultrasonography, CT, liver function tests, HBs Ag, HCV-Ab and liver biopsy. Measurement of serum Cg-A (done by RIA), AFP and PIVKA-II (done by ELISA) had been performed in all groups. **Results:** Our results showed a significant rise in AFP, Cg-A and PIVKA-II levels on both diseased groups compared to control $P<0.001$. Also, they showed a significant rise in HCC group versus the cirrhotic group $P< 0.001$. The results of Cg-A showed a positive correlation with serum bilirubin in HCV gp. $r= 0.421$ and $P< 0.02$, and in HCC gp. $r= 0.450$ and $P< 0.01$. Also it shows a negative correlation with serum albumin level in HCC gp. $r= -0.399$ and $P< 0.01$ with a positive correlation of no significant with serum AFP level in both groups. While serum PIVKA-II level revealed a highly positive correlation with serum AFP in HCC gp. $r= 0.302$ and $P< 0.01$ and positive correlation of no significant value with serum Cg-A level in HCC gp. In addition to that, serum PIVKA-II showed a significant rise of its level in correlation with grade III and IV (Edmondson grading) $P< 0.01$ compared to grade I and grade II, as well as a significant rise of its level in cases with portal vein thrombosis where its P2cm AFP, Cg-A and PIVKA-II showed a rise of their level compared to tumor <2 cm $P<0.05$ the three of them. Our conclusion is: The application of Cg-A and PIVKA II as tumor markers in the diagnosis of HCC is to be considered especially in cases with low levels of AFP and focal hepatic lesions more than 2 cm in size, as determination of Cg-A and PIVKA II serum values represent a complementary diagnostic tool in monitoring chronic liver disease patients for detection of HCC and significantly related to focal lesion's size.

A-60

Urinary HE4 and CA 125 as Biomarkers for Epithelial Ovarian Cancer

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Background: CA 125 and HE4 are serum biomarkers for ovarian cancer. Measurement of urinary CA 125 and HE4 in patients with ovarian cancer has also been reported (Tay SK and Chua EK, 1994; Hellstrom I, et al, 2010). A pilot study was performed to investigate both CA 125 and HE4 for their utility as urinary biomarkers for epithelial ovarian cancer (EOC).

Methods: The levels of CA 125 and HE4 were measured with ARCHITECT CA 125 II (ABBOTT) and HE4 EIA (Fujirebio Diagnostics, Inc.) respectively in urine samples from female subjects (N = 205; 64 with EOC; 141 with benign diseases).

Normalized CA 125 and HE4 were obtained from the measured levels divided by the mg/dL concentration of urine creatinine. The urine samples were obtained from an institutional review board-approved sample bank. Clinical diagnosis of EOC and benign diseases was based on the pathological examination of the subjects.

Results: Table 1

Conclusion: Urinary HE4 and CA 125 appear to be useful biomarkers for epithelial ovarian cancer.

Table 1. Performance of Urinary HE4 and CA 125 for EOC

	HE4	Normalized HE4	CA 125	Normalized CA 125
Median (EOC)	29513 pmol/L	395	6.3 U/mL	0.07
Median (Benign Diseases)	8281 pmol/L	75	1.0 U/mL	0.01
Area under ROC Curve	0.82	0.86	0.75	0.76
Cutoff	13147 pmol/L	100.4	2.94 U/mL	0.0302
Likelihood Ratio	3.0	3.2	2.8	2.6
Sensitivity	73%	78%	70%	66%
Specificity	75%	75%	75%	75%
PPV	57%	59%	56%	55%
NPV	86%	88%	85%	83%
Concordance with Clinical Diagnosis	75%	76%	74%	72%

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Evaluation of serum metallothionein level in patients with spinocellular cancer of head or neck

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Background. Metallothioneins (MT) are low molecular mass, cysteine rich proteins, which have naturally Zn^{2+} in both binding sites. There have been discovering new biological roles of these proteins including those needed in the carcinogenic process. However their use as a predictive marker remains controversial. Several reports disclosed MT expression as a prognostic factor for tumour progression and drug resistance in a variety of malignancies. The aim of this study was to determine metallothionein level in blood of patients with spinocellular carcinoma in head and neck area by using of electrochemical methods.

Methods. *Preparation of blood samples.* The sample was kept at 99 °C for 15 min., then cooled to 4 °C and centrifuged (16 000 *g*, 30 min.). The supernatant obtained was measured. *Electrochemical measurements.* Electrochemical measurements were performed with 747 VA Stand instrument connected to 746 VA Trace Analyzer and 695 Autosampler (Metrohm, Switzerland). The supporting electrolyte (1 mM $[Co(NH_3)_6]Cl_3$ and 1 M ammonium buffer; NH_3(aq) and NH_4Cl, pH 9.6) was changed after five measurements of a sample. The experimental parameters were as follows: initial potential of -0.7 V, end potential of -1.75 V, modulation time 0.057 s, time interval 0.2 s, step potential 2 mV, modulation amplitude -250 mV, E_{ads} = 0 V.

Results. In this study we employed the electrochemical method called Brdicka reaction to determine level of MT in blood samples. Primarily we aimed our attention on automation of the analysis. Autosampler 695 is capable to dose units of µl of a sample and gives us well reproducible results. Therefore we utilized the instruments to measure blood samples of the patients with spinocellular carcinoma to reveal the role of MT as predictive tumour disease marker. The level of MT measured at the patients with the tumour disease was about (2.30 ± 0.32) µM (n = 128). The level of MT at the patients is more than four times higher in comparison with level determined at healthy volunteers (0.51 ± 0.20) µM (n = 58). In addition the differences between localisation of tumour were detected; the highest level of protein of interest are connected to carcinoma of oropharynx ((2.76 ± 1.24) µM, n = 64) and the lowest to carcinoma of parotis ((1.95 ± 0.56) µM, n = 4).

Conclusion. As we report in this study, it is possible to determine the levels of MT in blood samples of patients with cancer automatically, precisely and with low costs. It follows from the results obtained that level of MT enhance in blood of patients with tumour disease compared to control samples.

Acknowledgements. *This work was supported by grants RECAMT GAAV IAA401990701 and GA ČR P102/11/1068.*

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Sarcosine as a potential new marker of prostate cancer

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Background. Sarcosine also known as N-methylglycine, a natural ubiquitous non-protein amino acid has been investigated as a new putative marker of prostate carcinoma. Sarcosine occurs as intermediate and byproduct in glycine synthesis and degradation and acts as an antagonist on the Glycine transporter I, which is the only known biological function of this molecule. Sarcosine was identified as a differential metabolite that was greatly increased during prostate cancer progression to metastasis and could be detected in urine, which is one of the great advantages of this marker. The aim of this study was to suggest a low-cost and simple method utilizable for sarcosine determination in urine and cell lysate. For this purpose ionex liquid chromatography with UV detection, high-performance chromatography with electrochemical detection and capillary electrophoresis with the laser-induced fluorescence detection were tested. Moreover, all techniques were employed for analysis of urine samples from patients as well as healthy volunteers.

Methods. *Analytical measurements.* Capillary electrophoresis with absorbance detection and with the laser-induced fluorescence detection (PACE 5500, Beckman Coulter, USA) was used. High-performance chromatography with electrochemical detection system consisted of two solvent delivery pumps (Model 582 ESA, USA), chromatographic column and a CoulArray electrochemical detector (Model 5600A, ESA). Ionex liquid chromatographic measurements were carried out with Amino Acid Analyser AAA 400 (Ingos, Czech Republic).

Results. Primarily, all techniques were optimized to be able to detect sarcosine in real urine samples. Ionex liquid chromatography with UV detection was the firstly optimized technique. Calibration curve was linear within the tested concentration range from 5 to 1000 µM with equation y=0.0266x-0.2794 R^2=0.9984. Limit of detection of sarcosine was determined as 3S/N and was about 500 nM. Capillary electrophoresis with the laser-induced fluorescence detection was also sensitive to the presence of sarcosine with detection limit as 500 nM. Liquid chromatography with electrochemical detection was the most sensitive technique with detection limit as 1 nM. All techniques were sensitive enough to detect sarcosine in urine of patients with prostate carcinoma (n=10) and also in healthy volunteers (n=25). Both concentrations were re-calculated on the content of creatinine and were 115 ± 10 nM per µM creatinine (patients) and 45 ± 10 nM per µM creatinine (volunteers).

Conclusion. In this study we optimized and tested three various bioanalytical approaches to determine sarcosine. Advantages and disadvantages of these techniques were discussed. Moreover, they were utilized for analysis of urine samples to evaluate role of sarcosine as potential tumour disease marker. Based on our results it can be concluded that this non-protein aminoacid could be of great interest for clinicians.

Acknowledgements. *This work was supported by grants GACR 301/09/P436, IGA MZ 10200-3 and GACR P102/11/1068.*

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Atrophic gastritis and serological markers of gastric mucosa

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Backround: The aim of this study was to evaluate results of serological tests for Pepsinogen I [PGI], Pepsinogen II [PGII], Gastrin 17 [G17] and anti Helicobacter pylori IgG antibodies [HP-IgG] (Gastropanel), to determine the epidemiological characteristics of atrophic gastritis in an adult north italian population.

Methods: We analyzed test results performed by ELISA techniques (Biohit, Helsinki, Finland and Euroclone Milan, Italy) from March 2009 to February 2011 (Cut-off: PGI: 40-100 µg/L; PGII:2.5-10; ratio PI/PII >3; G17: 2.0-7.0; HP-IgG:<32 IU). Data

were retrieved for 1055 patients of Parma performed on 366 men (34.7% mean age ± SD: 51.0±16.3) and 689 women 65.3% (age: 48.4±16.0).

Results: The population was clustered into three major classes. Class 1-*Normal gastric mucosa:* 626 (59,3%), Class 2-*Non atrophic gastritis* (PGII>10µg/L): 321 (30.4 %) and Class 3-A*trophic gastritis* (PGI <40µg/L):108 (10.2%) included 20 (18.5%) *probable widespread atrophic gastritis* (females/males: 15/5; PGI: 26.7, 1.2-33.8 µg/L, PGII: 2.1 1.4-2.5 µg/L, G17: 0.5, 0-6.7 pmol/L; HP-IgG: 1.8, 0.1-75.5 U/L); 26 (24.,1%) probable *atrophic gastritis of the gastric body* (females/males:18/8; PGI: 17.5, 3.5-37.9 µg/L, PGII: 5.9,1.6-10.0 µg/L; G17: 40.0, 7.8-91.2 pmol/L, HP-IgG: 7.1, 0.4-100 U/L) and 62 (57.4% females/males 54/8, mean age ± SD: 43.1.0±15.8) probable alteration of gastric mucosa with PGII and G17 not abnormal: PGI: 34.7, 20.4-39.5µg/L, PGII: 4.0, 2.5-8.0µg/L; G17: 1.0, 0.2-5.6 pmol/L, HP-IgG: 3.2, 0.2-90.9.

Conclusions: We observed a substantial prevalence of women in the number of tests requested. We observe a group (young females /males 54/8) widespread atrophic gastritis like type but more probable related with polyendocrinopathies and female autoimmune tyroiditis or associated with weight and body mass index. We suggest APCA dosage (antibody anti parietal cells of gastric mucosa) with EGDS. The frequent occurrence of PGI levels <40 µg/L (10.2%) lead us to suggest that further investigations (i.e., gastroscopy) are needed to confirm and characterize the histological type of subgroup of atrophy.

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Immunoglobulin ratios are a rapid, sensitive alternative to immunofixation for the identification of monoclonal proteins

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Historically, serum protein electrophoresis (SPE), urine protein electrophoresis (UPE) and immunofixation (IFE) have been used to identify and quantify monoclonal proteins (M-proteins). Whilst this approach is adequate for the identification of intact immunoglobulin multiple myeloma (MM), it is not sensitive enough to detect free light chain MM (LCMM). Therefore, an algorithm which utilises SPE, serum free light chain (FLC) immunoassays and IFE for the identification of M-proteins has been suggested. Assays have now been developed which utilise polyclonal antisera raised against the kappa and lambda light chain types of IgG, IgA and IgM immunoglobulins (HLC). Here we report the use of these assays as an alternative to IFE and propose a screening algorithm which utilises SPE, FLC and HLC. 1495 patient sera were screened using SPE and FLC, Electrophoresis was performed using a SEBIA Hydrasys 2 in accordance with manufacturer's instructions. FLC, IgG, IgA and IgM HLC assays were performed nephelometrically and results compared to historic IFE data. 198/1495 patients had an abnormal SPE or FLC result, of which 106/198 patients were positive by IFE and 81/198 had an abnormal HLC ratio (HLCr). 24/106 IFE positive patients had symptomatic haematological malignancies (14 Multiple Myeloma [MM], 1 Plasmacytoma, 2 Waldenstrom's Macroglobulinemia [WM] and 7 non-Hodgkins lymphoma [NHL]). Abnormal HLCr were identified in 24/24 patients. Moreover, abnormal HLCr identified 7 additional patients that, at the time of testing, were negative by IFE but who subsequently were diagnosed with a haematological malignancy (1 AL Amyloid, 1 IgAλ MM and 1 IgMκ WM and 4 NHL). 82/106 IFE positive patients had monoclonal gammopathy of undetermined significance (MGUS), 50/82 had an abnormal HLCr. 32 patients had 'normal' HLCr, but were all in the low-low/intermediate MGUS risk category. We conclude that HLCr can detect symptomatic haematological malignancies and offer a rapid, quantitiative alternative to IFE. Patients with low risk MGUS may have normal HLCr and further work is required to look at the utility in MGUS identification and risk stratification.

A-65

Nephelometric immunoassay measurements of IgMκ and IgMλ for the assessment of patients with IgM monoclonal gammopathies

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Accurate quantification of IgM monoclonal proteins by serum protein electrophoresis (SPE) can be hampered by the failure of the protein to migrate from the origin and by its heavily polymerised nature. Immunofixation (IFE) can improve the sensitivity of detection but is non-quantitative. Here we describe automated, fully validated nephelometric immunoassays on the Siemens Dade Behring BN™II analyser for the quantification of IgMκ and IgMλ in serum. Determination of the IgMκ/IgMλ ratio can be used as an aid in the diagnosis of IgM monoclonal gammopathies. The main assay characteristics are summarised below:

Assay	IgMκ	IgMλ
Range (g/L)	0.20-6.40	0.18 -5.60
Sample dilution	1/100	1/100
Min. sample dilution	1/5	1/5
Sensitivity (g/L)	0.01	0.009
Assay time (mins)	12	12
Linearity	y =1.008x -0.092 g/L r^2 = 0.999	y = 1.013x -0.086g/L r^2 = 0.998
Intra-assay precision %CV (mean concentration g/L) (n=84)	2.8% (4.85) 3.2% (1.16) 5.6% (0.35)	4.2% (4.24) 1.7% (0.77) 3.5% (0.30)
Inter-assay precision %CV (mean concentration g/L) (n=84)	2.7% (4.85) 2.9% (1.16) 3.3% (0.35)	2.8% (4.24) 1.1% (0.77) 2.5% (0.30)

Interference was within ±9.9% when either bilirubin (0.20 g/L), hemoglobin (5.00 g/L) or Chyle (1500 FTU) were added to serum samples with known IgMκ and IgMλ concentrations. IgMκ and IgMλ concentrations were measured in 120 normal (blood donor) sera; median IgMκ 0.63 g/L (range 0.29-1.82 g/L), median IgMλ 0.42 g/L (range 0.17-0.94 g/L), median IgMκ/IgMλ ratio of 1.59 (range 0.96-2.30). IgMκ+IgMλ summation correlated well with a total IgM assay (Siemens Dade Behring); y= 0.99x - 0.01 g/L. Sera from 60 IFE positive IgM patients (48 IgMκ /12 IgMλ) were assayed. In all cases the IgMκ/IgMλ ratio correctly identified the monoclonal IgM type. We conclude that serum IgMκ/IgMλ assays provide a rapid, precise method for quantifying IgMκ and IgMλ in serum, and the presence of an abnormal ratio may be useful in identifying patients with IgM monoclonal gammopathies.

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The Role of Inflammation in Patients with Intraductal Mucinous Neoplasm of the Pancreas and in those with Pancreatic Adenocarcinoma

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Background. Intraductal papillary mucinous neoplasms (IPMNs) of the pancreas have received much clinical attention in the last decade because they are slow-growing tumours which may be cured surgically in most patients (Oncologist 14:125-36, 2009). Among the causes of the aggressive behaviour of IPMNs, several passive and active strategies appear to be adopted by tumour cells to circumvent antitumour immune defenses. They include altered expression of major histocompatibility complex class I and II antigens and resistance to apoptosis through the Fas receptor pathway coupled with aberrant expression of the ligand. The aim of the present study were to evaluate the circulating concentrations of placental growth factor (PlGF), transforming growth factor-alpha and beta (TGF-α, TGF-β1), tumour necrosis factor receptor 1 (TNF-R1) and matrix metalloproteinase-2 (MMP-2) in IPMNs and pancreatic adenocarcinomas, in comparison with an established serum marker of pancreatic cancer (CA 19-9).

Methods. A total of 69 patients (39 males, 30 females, mean age: 69.8±10.4 years) were enrolled: 23 (33.3%) had IPMNs and 46 (66.7%) had histologically confirmed pancreatic adenocarcinomas. Of the 23 patients with IPMNs, 10 (43.5%) had branch type IPMNs and the remaining 13 (56.5%) had main duct type IPMNs. Finally, 13 blood donors were also studied as healthy controls (7 males, 6 females, p=1.000 vs. all patients; mean age: 57.0±14.6 years, p=0.003 vs. all patients). Blood specimens were obtained in the morning in a fasting state and the serum specimens were stored at -20°C until analysis. PlGF, TGF-α, TGF-β1, TNF-R1 and MMP-2 were determined using commercially available kits (R&D Systems, Minneapolis, MN, USA). The intra-assay CVs were <3.9% and the inter-assay CVs were <7.9%. The reference limits used were: 0.2-26 pg/mL for PlGF, 0.5-32 pg/mL for TGF-α, 18,289-63,416

pg/mL for TGF-β1, 749-1,966 pg/mL for TNF-R1 and 117-410 ng/mL for MMP-2. Furthermore, CA 19-9 was also assayed using an electrochemical luminescence immunoassay (reference limits: 0-37 U/mL).

Results. The only two parameters showing significant differences among the three groups were TNF-R1 (p=0.003) and CA 19-9 (p=0.007). In particular, TNF-R1 concentrations were significantly higher in both patient groups than in healthy subjects (IPMN, p=0.004; pancreatic adenocarcinoma, p=0.001). In contrast, serum CA 19-9 concentrations were significantly higher in patients with pancreatic adenocarcinomas than in those with IPMNs (p=0.044) and healthy subjects (p=0.003). It should be noted that a serum CA 19-9 concentration of 23,688 U/ml was found in one patient with a main duct IPMN and distant metastases; in the other 22 patients, CA 19-9 serum concentrations were 172±269 U/ml (mean±SD) and ranged from 0.53 to 1,151 U/ml. Within the group of patients with pancreatic adenocarcinomas, patients with metastases had serum concentrations of TNF-R1 significantly higher than those without (p=0.034).

Conclusions. Serum TNF-R1 was elevated in patients with IPMNs and in those with pancreatic adenocarcinomas suggesting a high apoptotic activity in both groups of patients studied.

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The use of HE4 as a biomarker for ovarian cancer

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Introduction: Human epididymis protein 4 (HE4) has been reported as a promising marker for detecting ovarian cancer (Hellstrom. Cancer Res 2003;63:3695) and with the highest sensitivity among ovarian biomarkers (Moore. Gynecol Oncol 2008;108:402). Ovarian cancer presents with an adnexal mass and the goal is to determine if that mass is benign or malignant.

Objective: With the paucity of data in Asian subjects we evaluated the use of HE4 and CA-125 in distinguishing benign from malignant causes in patients with an adnexal mass.

Methods: 392 women with pelvic masses confirmed on ultrasonography and scheduled for surgery were recruited into the study from 6 centers in Hong Kong, Japan, Taiwan, Thailand, Korea, and Philippines. Blood was drawn prior to surgery and sent to a central testing site for the analyses of HE4, CA125, and FSH on the Architect i2000SR (Abbott Diagnostics). Histology slides were sent for a second review by a central gynecologic pathologist. The Youden index was used to determine the optimal cut-off points for CA125 and HE4 in separating benign and malignant disease.

Results: Of the subjects enrolled in the study 304 had benign disease and 88 had malignancy, 60 of whom were epithelial ovarian cancer. Overall, the median CA-125 was 20.9 U/mL for benign lesions and 220.2 U/mL for malignancy; the corresponding values for HE4 was 33.9 pmol/L and 114.8 pmol/L. The optimal cut-points for distinguishing benign from malignant samples using the Youden index was: CA125 - 294.1 U/mL premenopause, 30.0 U/mL menopause; HE4 - 45.5 pmol/L pre-menopause, 74.0 pmol/L menopause. From the premenopausal ROC curves, the AUC for CA-125 was 0.72, and 0.8 for HE4. In premenopausal subjects, the diagnostic performance of the test (sensitivity - SENS, negative predictive value - NPV) favored HE4 - 69.4%, 95.3% versus CA125 - 44.4%, 92.5%. However, for specificity (SPEC) and positive predictive value (PPV) CA125 fared better - 96.1%, 61.5% versus HE4 - 87.1%, 43.1%. The AUC from the menopausal ROC curves for CA125 and HE4 were both 0.94. Thus the diagnostic performance (SENS, SPEC, PPV, NPV) of CA125 (92.3%, 91.8%, 92.3%, 91.8%) and HE4 (84.6%, 91.8%, 91.7%, 84.9%) are quite comparable.

Conclusion: HE4 is especially useful in the separation of premenopausal women with benign pelvic masses from those with malignant ovarian cancers. In menopausal subjects both CA125 and HE4 are comparable in detecting ovarian cancers but patients may benefit from the use of both markers in a risk of malignancy algorithm.

Tuesday AM, July 26

Poster Session: 10:00 am - 12:30 pm
Electrolytes/Blood Gas/Metabolites

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Enzymatic Creatinine Assay on Unicel® DxC 600 System : Performance Characteristics

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Objective: The objective of this study was to evaluate the performance of the Beckman Coulter enzymatic method for the measurement of creatinine on the Unicel® DxC 600 clinical chemistry analyzer.

Methodology: The enzymatic creatinine method (CR-E reagent) utilizes a multi-step approach ending with a photometric end-point reaction. The enzyme creatinine amidohydrolase (creatininase) is used to convert creatinine to creatine. Creatine is further broken down through a series of enzymatic reactions with creatinase, sarcosine oxydase and peroxidase to yield a colored chromogen read at 560 nm. The method is traceable to ID-GC/ Mass spectrometry and requires two levels of calibrator for the generation of a multi-point calibration.

Results: Performance characteristics were evaluated according to the French Society of Clinical Chemistry's (SFBC) protocol.

The precision was estimated using 4 serum controls and 1 serum pool with creatinine concentrations of 0.25, 0.60, 1.02, 4.31 and 7.65 mg/dL. For these serums the repeatability was (N=20) 5.1%, 1.9%, 1.5%, 0.6% and 0.7% respectively and the within-run imprecision was (N=20) 6.0%, 4.4%, 2.6%, 2.3% and 2.0% respectively.

We have used an aqueous solution of SRM914 to verify that the upper limit of measurement was 25 mg/dl.

Method comparison using patient plasma samples showed an excellent correlation to other commercially available enzymatic method and to Jaffe methods. The results of Deming's linear regression analysis were as follows (N=151, sample range= 0.29-17.88 mg/dL):

- versus Beckman Coulter IDMS standardized Jaffe method y = 1.03 x, r = 1.00

- versus Roche Diagnostics enzymatic method (Cobas Integra 800) y = 0.98 x + 0.03, r = 1.00

- versus Roche Diagnostics Jaffe compensated method (Cobas Integra 800) y = 1.04 x, r = 1.00

We have tested the interference of hydroxocobalamin, a cyanide antidote, which imparts a strong red coloration to plasma. This phenomenon results in significant interference with a number of chemistry laboratory parameters like creatinine. The testing interference substance was added to a human plasma (creatinine = 1.38 mg/dL) to make 4 testing solutions containing hydroxocobalamin at concentrations of 0 to 103 mg/dL. A positive interference (+ 17%) has been observed for the upper concentration of hydroxocobalamin.

The reagent was shown to be stable open in the reagent compartment for 30 days and for the serum/plasma application the calibration was stable for 14 days.

Conclusion : This enzymatic creatinine assay from Beckman Coulter has demonstrated excellent precision and linearity performance on Unicel® DxC 600. CR-E reagent provides a dependable means of measuring creatinine in serum and plasma.

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Immunoassay for Phosphatidylethanol an Ethanol Biomarker

P. Neilsen, P. Rzepecki. *Echelon Biosciences, Salt Lake City, UT,*

Background: Alcohol abuse and the diseases it cause are a major global health problem_only exceeded by tobacco and hypertension. Phosphatidylethanol (PEth) is a long-lived biomarker for alcohol ingestion, but no chemically active analogs or commercially available antibodies necessary for bioassay development currently exist. Antibody phage display libraries contain a large and diverse repertoire of antigen-recognition clones that have been used to identify recombinant monoclonal antibodies to unusual epitopes.

Methods: We used phosphoramidite coupling methods employed to synthesize acyl-tethered, amine-reactive analogs which were conjugated to proteins and coupled to biotin. These tools were employed to interrogate a human combinatorial antibody library (HuCAL®) at AbD Serotec. Antibody clones were characterized and validated in our lab using standard procedures towards the generation of a sensitive immunoassay for PEth.

Results: Complete synthesis of amine-modified PEth and biotinylated PEth was confirmed by MS and NMR. These analogs were used to identify and test sixteen antibody clones in selective-binding experiments to determine binding of clones to biotinylated PEth and other related biotinylated lipids. Three high-binding clones produced signals between 15 and 30 times stronger for biotin-PEth compared to other biotin-lipids. Binding of these clones was also selectively competed using PEth with less than 10% cross-reactivity to other abundant phospholipids found in erythrocyte membranes including PC, PE, PS, and cholesterol. We also determined whether PEth could be incorporated into washed erythrocytes could be detected; and found that treatment of RBC for 4 hours with 0.5 mg/mL PEth resulted in greater competition from extracted samples than extracts of cells treated for only 2 hours or with 0.1 mg/mL (5x less) PEth.

Conclusions: We have synthesized phosphatidylethanol analogs and discovered unique, high-affinity recombinant bivalent human monoclonal $F(ab)_2$ antibodies that selectively bind this long-lived ethanol metabolite. We have also demonstrated the feasibility of using these antibodies to measure PEth in vitro, and produced a number of tools needed to explore PEth as a potential mediator of ethanol-induced organ damage.

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Evaluation of pleural fluid pH measurement on the Radiometer ABL800 series blood gas analyzers

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Background: Blood gas analyzers typically process whole blood specimens with rapid turnaround times for close monitoring of patient respiratory function and electrolyte status. In addition to this use, analysis of several non-standard specimens has become routine on blood gas analyzers. Pleural fluid pH is important diagnostic marker for management of pleural effusions. Guidelines for treatment of parapneumonic effusions set forth by American College of Chest Physicians recommend drainage of the pleural effusion when the pH is below 7.2.

The goal of the study was to validate a new pleural fluid pH measurement mode on the Radiometer ABL835 and ABL837 blood gas analyzers. Pleural fluids across a broad range of pH were compared between the Roche Omni S and ABL800 series to assess agreement between the instruments.

Methods: Pleural fluid samples were obtained from a total of 21 unique patients. 17 of the samples were stored for up to 1 week at 4 ºC until analysis and 4 samples were pooled, filtered and stored at -70 ºC until use. A total of 57 samples were made by spiking samples with 2% acetic acid to span a pH range of 6.95-7.55. Spiked samples were allowed to stabilize for 5 minutes prior to measurement. Each sample was measured sequentially in duplicate on the Roche Omni S, Radiometer ABL 830 (pleural fluid mode) and Radiometer ABL 837 (pleural fluid mode) within 5 minutes. Results were analyzed in EP evaluator v9.0 (Data Innovations) to generate Deming regression and Bland-Altman difference plots.

Results: Deming regression analysis indicated that the Radiometer ABL800 series had excellent agreement with the Roche Omni S. The Radiometer ABL835 and ABL837 had proportional and constant bias of (slope=1.068, intercept= -0.47) and (slope=1.060, intercept= -0.42) respectively when compared to the Roche Omni S. Both of the ABL800 series showed a slight positive bias with pH values above 7.4 measured in the pleural fluid mode. Standard error of the estimate was 0.015 for both ABL instrument. Of the 57 samples tested, one was discrepant at the medical decision cutoff of 7.2 between the Omni S and ABL800 series. Fisher's Exact test confirmed that the methods are statistically equivalent (P= 1.00) in the classification of fluids using a pH cutoff of 7.2.

Conclusion: The Radiometer ABL800 series can accurately measure the pH of pleural fluid from both pooled and individual patient samples. This study showed excellent analytical and clinical agreement between the measurement of pleural fluid pH using the Omni S reference method and the Radiometer ABL800 series blood gas analyzers.

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Performance Characteristics of a New Lactate Assay for the Synchron LX®20 PRO and UniCel® DxC600/800 Clinical Systems from Beckman Coulter

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Objective: The new Beckman Coulter liquid Lactate assay (LACT) offers clinical laboratories a replacement to the lyophilized assay.

Relevance: LACT reagent, when used in conjunction with Synchron LX20 PRO and UniCel DxC600/800 system(s) and Synchron Multi Calibrator, is intended for the quantitative determination of lactate concentration in human plasma and cerebrospinal fluid (CSF). Lactate measurements that evaluate the acid-base status are used in the diagnosis and treatment of lactic acidosis.

Methodology: This new Lactate assay is based on enzymatic reactions. Lactate oxidase converts lactate to pyruvate and generates hydrogen peroxide. Peroxidase catalyzes a reaction with hydrogen peroxide to form a chromophore. The lactate concentration is directly proportional to the change in absorbance at 560 nanometers.

Validation: Performance characteristics of the reagent were evaluated using Synchron LX20 PRO and UniCel DxC600/800 systems from Beckman Coulter. The plasma/CSF analytical range is linear between 0.3-11.0 mmol/L and had an $R^2 > 0.98$ for all systems tested. Within-run and total imprecision on a UniCel DxC800 were as follows: plasma level 1 mean value was 1.7 mmol/L, the within-run SD was 0.027 mmol/L and the total SD was 0.031 mmol/L; plasma level 2 mean value was 3.9 mmol/L, the within-run SD was 0.056 mmol/L and the total SD was 0.063 mmol/L; plasma level 3 mean value was 6.6 mmol/L, the within-run CV was 1.0% and the total CV was 1.4%; spinal level 1 mean value was 2.1 mmol/L, the within-run SD was 0.049 mmol/L and the total SD was 0.052 mmol/L; spinal level 2 mean value was 4.2 mmol/L, the within-run SD was 0.053 mmol/L and the total SD was 0.062 mmol/L. Interference studies were conducted. Maximum levels with no significant interference (NSI = ≤ 0.21 mmol/L or ≤ 4.8%) were: 30 mg/dL bilirubin (unconjugated), 11.25 mg/dL bilirubin (conjugated), 500 mg/dL lipemia (Intralipid), 500 mg/dL hemolysate, 6 mg/dL ascorbic acid, 12 mg/dL pyruvate and 4000 IU/L lactate dehydrogenase. A methods comparison study was performed against the Beckman Coulter lyophilized Lactate assay. A total of 117 plasma samples, ranging from 0.4 to 10.5 mmol/L were analyzed. Correlation on a UniCel DxC600 was obtained with a slope of 0.92, an intercept of -0.02 mmol/L and a correlation coefficient of 0.998. A total of 119 CSF samples, ranging from 0.6 to 9.6 mmol/L were analyzed. Correlation was obtained with a slope of 0.93, an intercept of -0.07 mmol/L and an R^2 of 0.999.

Conclusion: The new Lactate assay (LACT) from Beckman Coulter demonstrates acceptable precision, linearity and interference performance on Synchron LX20 PRO and UniCel DxC600/800 systems*. *Pending clearance by the United States Food and Drug Administration; not yet available for in vitro diagnostic use. For Investigational Use Only.

A-73

Long-term stability of clinical laboratory data - Serum-sodium as benchmark

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Background. For longitudinal follow-up of individual patients clinicians rely on the long-term stability of the laboratory results they work with. This is also of importance for epidemiological studies in support of public healthcare policy. However, few published data are available in literature on this topic. We investigated the stability of serum-sodium measurements in two university hospital laboratories over a period of 9 and 14 years.

Methods. The serum-sodium patient data retrieved from Ghent University Hospital were obtained by measurement with the Hitachi 917 until November 2006, thereafter, with the Cobas 6000 c501 and Modular (all of Roche Diagnostics) (period: 1/12/2001 - 27/8/2010). The patient results from Brussels University Hospital were obtained with the Vitros 950IRC, which was gradually replaced by three Vitros Fusion 5,1 FS analyzers (both from Ortho Clinical Diagnostics) (period: 2/1/1997 - 20/10/2010). We calculated the 10th-, 50th-, and 90th percentile for the daily patient results, however, omitting those obtained during weekends and holidays. To visualize trends we used the moving average (n = 5) and linked it, where possible, to the available lot information. We calculated representative standard deviations (SDs) of the daily percentiles for several stable periods (n ~168). The percentage patients with a result < 135 mmol/L (onset of mild hyponatraemia) was also calculated for all data, including weekends and holidays.

Results. The long-term averages (SD) were for Brussels 140.5 mmol/L (0.59 mmol/L) and for Ghent 140.0 mmol/L (0.89 mmol/L). In stable periods, representative SDs were for Brussels and Ghent 0.46 mmol/L and 0.47 mmol/L, respectively. Verification of the 50th percentile documented 10 shifts (6 upwards and 4 downwards) for Brussels and 7 (4 upwards and 3 downwards) for Ghent. Detailed investigation of these observations revealed that the shifts in the laboratory of Brussels University Hospital were related to putting into operation the new Vitros, changing slide generations and recalibration of the three analyzers. The laboratory of Ghent University Hospital identified the annual lot changes in ISE compensator and reassignment of the value of the ISE compensator by Roche Diagnostics as cause for the shifts. The percentages of results <135 mmol/L were for Brussels ~12.5% in periods with a 50th-percentile at 139.5 mmol/L and ~4% in periods with a 50th-percentile at 142.5 mmol/L. For Ghent, they fluctuated from ~20% (50th-percentile: 138 mmol/L) to ~7% (50th-percentile: 141.5 mmol/L).

Conclusion. The examined serum-sodium assays as applied in the respective university hospital laboratories were characterized by a good long-term stability, because the maximum deviation from the average for more than 4 weeks was around 2% (~3 mmol/L). However, in view of the 0.7% within-subject biological variation of serum-sodium, 2% may be too high for adequate management of mild hyponatremia. Comparing periods with negative bias to periods with a positive bias showed a threefold increase of the number of patients with sodium concentrations indicating mild hyponatremia (< 135 mmol/L). Therefore, improvement of long-term stability of assays is still desirable and challenges manufacturers to tighten their lot-to-lot variation, which were identified as main cause of the observed biases.

A-74

Analytical evaluation of the Next Generation Uric Acid Assay Application for the Abbott ARCHITECT *c*Systems

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Background: The measurement of serum and urine uric acid (UA) levels can used in relation to the diagnosis of gout and kidney stone diseases.

The Abbott ARCHITECT Next Generation Uric Acid Assay is in development to improve on board reagent stability, reduce calibration frequency and to overcome the interference of ascorbic acid. The uric acid assay utilizes the principle that uric acid is oxidized to allantoin by uricase with the production of hydrogen peroxide (H2O2). The H2O2 reacts with 4-aminoantipyrine and 2,4,6-tribromo-3-hydroxybenzoic acid in the presence of peroxidase to yield a quinoneimine dye. The resulting change in absorbance at 548 nm is proportional to the uric acid concentration in the sample.

We report our findings of the analytical evaluation of this assay by using the CSLI EP-10 and EP-9 protocols. In addition we also report on the effect of ascorbic acid on the Next Generation Uric Acid Assay.

Methodology: The precision of the assay was evaluated according to CSLI EP-10 protocol. For this pooled serum samples we prepared. The correlation with the current on-market Abbott ARCHITECT uric acid assay was carried out according to the CSLI EP-9 protocol. Measurements of uric acid were performed on the Abbott ARCHITECT *c*8000 *c*Systems.

To determine the effect of ascorbic acid on the obtained result, samples we obtained in subject before and after high dose oral supplementation with vitamin C. Serum ascorbic acid concentrations were determined with High Performance Liquid Chromatography.

Results: Based on the EP-10 protocol, the imprecision of the Next Generation Uric Acid Assay was below 3 % for all levels. Based on a intra-individual biological variability of 9.0 % in serum and 24.7 % in urine, this means that this assay meets the imprecision requirements.

Based on the EP-9 protocol, the Next Generation Uric Acid Assay in serum showed a correlation with the current assay of UA(next gen)=0.98UA-0.01, with a 95% confidence interval of 0.00 to 0.01 for the intercept and of 0.96 to 1.00 for the slope, respectively. In urine the correlation was UA(next gen)=1.00UA+0.03, with a 95 % confidence interval of -0.03 to 0.1 for the intercept and of 0.95 to 1.05 for the slope, respectively.

Up to a level of 530 µmole/l ascorbic acid the Next Generation uric acid assay showed recoveries within 4 % compared to the native samples. In contrast, the recovery of the old assay dropped to 80 %.

Conclusion: Based on the CSLI EP-10 protocol, the Abbott Next Generation Uric Acid Assay performs well within the analytical requirements for determination of uric acid in serum and urine. The CSLI EP-9 showed a good correlation of the Next Generation Uric Acid Assay with the current on-market Abbott ARCHITECT Uric Acid Assay.

In addition the Next Generation Assay proved to be robust against ascorbic acid at a level of at least 530 µmol/l.

A-75

Scanning Spectrophotometry for the Detection of Bilirubin and Oxyhemoglobin in Cerebrospinal Fluid

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Background: A subarachnoid hemorrhage (SAH) is caused by spontaneous arterial bleeding into the subarachnoid space, usually from a cerebral aneurysm. While a computerized tomography (CT) scan can detect SAH in 98% of patients that present within 12 hours of symptoms, diagnostic sensitivity decreases to 50% over 7 days. Spectrophotometric analysis of cerebrospinal fluid (CSF) has demonstrated clinical utility at identifying SAH in CT-negative individuals due to its ability to detect the bilirubin formed from the degradation of oxyhemoglobin. The objective of this study was to validate an assay to detect bilirubin and oxyhemoglobin in CSF.

Methods: The absorbance of CSF samples at each wavelength between 350 and 550 nm were recorded using a Beckman DU-800 scanning spectrophotometer, and a cuvette with a 1 cm path length. A baseline was drawn that was tangent to the scan beginning at 350-380 nm and ending at 480-520 nm. The number of absorbance units (AU) measured from the baseline to the height of the scan at 414 and 476 nm was recorded as the net oxyhemoglobin absorbance (NOA) and the net bilirubin absorbance (NBA), respectively. Residual CSF samples sent to the laboratory for routine testing were scanned and the resulting scans evaluated by visual inspection. Those that appeared to lack bilirubin and oxyhemoglobin were pooled. Stock solutions of bilirubin and oxyhemoglobin were added to aliquots of the CSF pool to create samples containing a concentration range of bilirubin (0-0.43 mg/dL), oxyhemoglobin (0-33.8 mg/dL), and combinations of both bilirubin and oxyhemoglobin. Samples were scanned in duplicate and two individuals determined the NBA and NOA from each scan. Results were used to determine bilirubin and oxyhemoglobin detection limits, precision, linearity, and the effect of oxyhemoglobin on the NBA. Published cutoffs for the NBA (>0.007 AU) and NOA (>0.02 AU) were verified using the maximum result of each as determined by two individuals using the CSF pool samples. A total allowable error of 0.001 and 0.01 AU for the NBA and NOA, respectively, was determined to be the quality goal.

Results: Bilirubin produced a NBA >0.007 AU at a concentration of 0.026 mg/dL. Oxyhemoglobin produced a NOA >0.02 AU at a concentration of 0.5 mg/dL. The standard deviation of NBA at 0.005 AU was 0.0001 AU (n=40). The standard deviation of NOA at 0.02 AU was 0.0003 AU (n=40). NBA and NOA were linear from 0-0.176 AU (r=0.99) and 0-1.926 AU (r=0.99), respectively. The NBA decreased from 0.0123 to 0.0060 AU (51.2%) as oxyhemoglobin increased from 0 to 4.2 mg/dL, respectively. The maximum NBA and NOA of 31 CSF samples used for cutoff verification were 0.0005 and 0.0023 AU, respectively.

Conclusions: Scanning spectrophotometry can detect CSF bilirubin and oxyhemoglobin with high analytical sensitivity. The NBA and NOA precision was acceptable given the quality goals. The method was linear over the NBA and NOA ranges likely to be encountered with clinical use. A NBA of 0.007-0.013 AU can be falsely decreased (≤0.007 AU) when the NOA is 0.113-0.230 AU (2.1-4.2 mg/dL).

A-76

Interference Studies of the Next Generation Magnesium Assay Application for the Abbott ARCHITECT® cSystems™

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Background: Plasma and urine magnesium levels are measured in the investigation of magnesium disorders. However, magnesium assays are sensitive to interference from; hemolysis, lipemia and icterus. Endogenous compounds such as glucose, protein, ascorbic acid and inorganic phosphorus have also been reported to interfere with current commercially available assays. Drugs such as L-Dopa are known to interfere with magnesium determination. Furthermore, the effect of preservatives used in urine collection devices on magnesium measurement is not clear and should be evaluated. The Abbott Next Generation Magnesium Assay utilizes an enzymatic reaction using modified isocitrate dehydrogenase to measure magnesium. Increased absorption at 340 nm of the reaction product, NADPH, is proportional to the concentration of magnesium in the sample. We report findings on the effect of common interferents (see table) on magnesium measurement in serum and in urine using the above assay.

Methods: Pooled serum and urine samples were spiked with various interfering substances at different concentrations (see table). Negative control was obtained by substituting deionized water for the interferent. Magnesium concentration was measured in 7 replicates using the above assay on the Abbott ARCHITECT *c8000* Systems. A substance was not considered to interfere if percentage of interference calculated as $[Magnesium_{interferent}\text{-}Magnesium_{water}]/[Magnesium_{water}]*100$ was within 7.5 and 10% for serum and urine respectively.

Results: The effect of interferent substances studied, on the measurement of magnesium at different target levels is shown in the table.

Interferent (highest conc. without interference)	Serum [Mg] mg/dL	Serum interference (%)	Urine [Mg] mg/dL	Urine interference (%)
Ascorbic acid (Serum: 3 mg/dL) (Urine: 200 mg/dL*)	2.04 3.92 6.39	0.33 -0.22 0.08	3.12 10.04	-0.67 1.17
Glucose (Serum: 1200 mg/dL) (Urine: 1000 mg/dL*)	2.08 4.15 6.84	-0.35 -1.19 -2.03	5.63 9.84	-0.44 0.47
Hemoglobin (Serum: 500 mg/dL 1000 mg/dL 2000 mg/dL) (Urine: 250 mg/dL 2000 mg/dL)	1.84 3.98 7.01	6.85 5.67 5.95	1.14 8.79	3.92 6.38
Conjugated bilirubin (Serum: 60 mg/dL*) (Urine: 60 mg/dL)	1.80 3.75 6.18	0.71 0.89 0.68	2.80 10.61	-3.17 -1.42
Unconjugated bilirubin (60 mg/dL*) Conjugated & unconjugated bilirubin (60 mg/dL)	1.95 4.12 6.73 1.90 3.97 6.48	2.80 1.23 2.31 1.09 -0.05 0.14		
Intralipid (1000 mg/dL)	1.85 3.63 6.04	-5.35 -5.45 -5.90		
L-Dopa (5 mg/dL)	1.65 3.81 6.29	-0.75 -1.41 -0.93		
Albumin (50 mg/dL*)			5.69 9.91	-0.60 0.04
Inorganic phosphorus (278.8 mg/dL)			2.81 9.80	-4.04 -2.54
Boric acid (1000 mg/dL*)			2.33 9.00	3.81 1.33
6N HCl (2.5 mL/dL*)			2.57 10.21	2.41 7.26
50% Acetic Acid (6.25 mL/dL*)			2.90 10.20	22.32** 34.30**
6N Nitric Acid (5 mL/dL*)			2.55 10.30	26.32** 20.41**
Sodium Fluoride (400 mg/dL*)			2.59 8.73	-13.36** -6.95

* Only one interferent concentration was tested.

** Significant interference observed at the stated interferent concentration.

Conclusion: The Abbott Next Generation Magnesium Assay is robust against common interferents. In addition, the assay offers the advantage of lack of interference from L-Dopa. Boric acid and hydrochloric acid can be used as suitable urine preservatives; however, acetic acid, nitric acid, and sodium fluoride demonstrate significant interference with this assay.

A-77

Development and Evaluation of a Liquid Chromatography-Mass Spectrometry Method for the Determination of Creatinine in a Urine-Based Standard Reference Material

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Background: The objective of this study is to develop and evaluate an analytical method for the determination of creatinine in human urine for the purpose of assigning a certified value to creatinine in a new Standard Reference Material. Creatinine levels in both serum and urine are important indicators of kidney failure. In addition, very low creatinine levels in urine may be indicators of adulterated specimens when testing for drugs of abuse and levels of other analytes are often normalized to creatinine to adjust for urine sample volume variations. The National Institute of Standards and Technology (NIST) is developing a new Standard Reference Material (SRM) to support accurate creatinine measurements in urine for the clinical community.

Methods: A preliminary experiment was performed to compare two different sample preparation methods prior to liquid chromatography-mass spectrometry (LC-MS) analysis: dilution in acidic solution and multi-mode cation exchange solid-phase

extraction (MCX SPE). A human urine pool was spiked with stable-isotope labeled d_3-creatinine as an internal standard and diluted 1:10 (v:v) with 0.01 M HCl to create a master mix. Half of the master mix was processed using MCX SPE. The original dilution and the eluted SPE samples were diluted an additional 1:10 (v:v) before analysis. Calibrants were prepared by mixing SRM 914a Creatinine and d_3-creatinine in a 0.01 M HCl solution. All samples were subjected to separation by reversed-phase liquid chromatography on a C18 column with an isocratic gradient followed by positive-mode electrospray ionization mass spectrometry detection using single ion monitoring (SIM). The LC-MS parameters were the same as those previously employed to successfully measure creatinine in serum. To ensure that creatine did not interfere with creatinine measurements, SIM mass spectrometry was used to scan for creatine in urine samples to ensure proper separation of these analytes by LC prior to mass spectral detection. The accuracy of this method was evaluated with a recovery study in which known levels of creatinine were spiked into the human urine pool. Creatinine was added to urine at 4 clinically relevant levels within a range of 0.604 mg/g-4.35 mg/g.

Results: Dilution versus SPE sample preparation resulted in the same calculated creatinine values with similar quality LC-MS results. This method displayed linearity over the calibration range 0.5 µg/g-10 µg/g (0.05 mg/dL-1.0 mg/dL) creatinine, with a creatinine:d_3-creatinine ratio (mass:mass) range of 0.06-1.4, which is consistent with dilutions of typically reported urine creatinine levels. Based on measurements from two separate days, the values of creatinine in a urine pool were 0.627 mg/g and 0.604 mg/g, respectively, with within-run % CVs= 0.35 %-0.42 % and total % CV= 2.0 %. The accuracy study resulted in a mean recovery of 104 %.

Conclusion: Due to the comparability of results from the different sample preparations, the simpler dilution method was chosen for further evaluation. The overall LC-MS method displayed linearity over a wide range of concentrations and possesses appropriate precision and accuracy for use in assigning creatinine values to urine-based Standard Reference Materials.

A-78

Re-centrifugation of original specimens significantly increases serum Creatinine and Potassium values

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Background: In clinical laboratories, sometime there is a need to re-centrifuge the original specimens ("clot" tubes) in order to better clarify and clean the serum or plasma for further analysis. Also, in some instances, original tubes are centrifuged for the second time (especially tubes without serum separating gel) to maximize serum/plasma recovery. Recentrifugation is done to ensure there is an adequate volume of serum or plasma for multiple repeating of different tests and/or to run additional tests that are ordered hours after the original analysis was completed. To the best of our knowledge there are no comprehensive studies to show the impact of recentrifugation on the concentrations of different analytes. Thus, we decided to study the effect of recentrifugation on the concentrations of glucose, sodium, potassium, chloride, BUN, creatinine, bicarbonate, calcium, phosphorus, and magnesium.

Methods: A total of 27ml of blood was collected in 9 SST tubes (3ml/tube) from healthy volunteers (n=6, 4 male, 2 females). This study was approved by internal review board at our institution. All specimens were centrifuged at 0hrs, following which they were stored at either RT or 4°C for 2, 4, 8, and 24hrs. Specimens were centrifuged for second time at each time interval and concentrations of the above-mentioned analytes were measured on Roche modular system.

Results: The preliminary results showed that recentrifugation increased the concentrations of glucose, potassium, creatinine, bicarbonate, and phosphorus, to some degree. However, concentrations of potassium and creatinine were significantly increased following recentrifugation. The concentration of creatinine was increased at RT and 4°C (44.4 ± 13.4 and 41.6 ± 8.8, mean ± SD, percent increase from 0 to 24hrs at RT and 4°C, respectively, $p<0.0001$). Also interestingly, concentration of potassium was significantly increased in specimens stored only at 4°C for up to 24 hours (21.7 ± 12.8, mean ± SD, percent increase at 4°C, $p<0.005$). The concentration of bicarbonate was also increased following recentrifugation (11.3 ± 6.4 and 7.9 ± 3.3, mean ± SD, percent increase at RT and 4°C); however not significantly.

Conclusion: Recentrifugation of original specimens increases the concentrations of glucose, potassium, creatinine, bicarbonate, and phosphorus, to some degree. Recentrifugation increases the values of creatinine and potassium, significantly. Therefore, the concentrations obtained for the latter analytes after recentrifugation should be interpreted cautiously.

A-79

Pseudohyponatremia, Pseudohypokalemia and Pseudohypochloremia Due to Lipoprotein X Interference

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Background: Lipoprotein X (Lp-X) is an abnormal lipoprotein that may accumulate in circulation during intra- or extra-hepatic cholestasis. It is characterized by high concentrations of phospholipids (66% by weight) and unesterified cholesterol (22%), as well as by low concentrations of protein (6%), cholesterol esters (3%), and triglycerides (3%). Previous studies have demonstrated that when present at elevated concentrations, Lp-X can produce an electrolyte exclusion effect leading to erroneously low electrolyte concentrations, potentially leading to misdiagnoses and the initiation of inappropriate electrolyte replacement therapy.

In this report we present the case of a 61-year-old female patient with severe prolonged obstructive biliary cholestasis secondary to pancreatic cancer, with high Lp-X concentrations, resulting in pseudohyponatremia, pseudohyperkalemia, and pseudohypochloremia.

Objectives: To identify the interfering lipoprotein producing erroneously low electrolyte concentrations and to evaluate the use of an alternate methodology to accurately quantify the patient's electrolytes.

Methods: Total cholesterol, High Density Lipoprotein (HDL) cholesterol, direct Low Density Lipoprotein (LDL) cholesterol, triglyceride, and electrolyte analysis were performed using the Roche Modular or Cobas 6000 analyzer. Both platforms utilize indirect ion-selective electrode technology for electrolyte analysis. Electrolyte analysis was also performed on the Rapidlab 1250 blood gas analyzer (Siemens), utilizing direct ion-selective electrodes.

Results: At presentation, the patient's total cholesterol was 1,713 mg/dL, triglycerides - 436 mg/dL, HDL - 26 mg/dL, and LDL was undetectable (<10 mg/dL) via our direct-LDL assay. Electrolyte concentrations via indirect ion-selective electrode analysis were: sodium - 108 mmol/L (135-145 mmol/L), potassium - 2.8 mmol/L (3.5-5.0 mmol/L), and chloride - 73 mmol/L (98-107 mmol/L). The patient plasma was clear and transparent, with a low lipemic index. While Lp-X was not quantifiable by our in-house direct LDL method using selective micellary solubilization, the presence of Lp-X was suggested by phospholipid, free cholesterol, and cholesteryl ester analysis, which demonstrated a high concentration of phospholipid (1043 mg/dL; ref. range: 155-275 mg/dL), high free cholesterol (361 mg/dL; ref. range: <80 mg/dL) and a low concentration of cholesteryl ester (58%; ref. range: 60-80% of total cholesterol), values consistent with the constituents of Lp-X. An electrolyte exclusion effect due to a significantly increased cholesterol concentration was suspected when the patient failed to show an appropriate response to electrolyte replacement therapy. The suspect electrolyte values of hospital day 5 (measured using indirect ion-selective electrodes) were: sodium -115 mmol/L, potassium - 3.2 mmol/L, chloride - 85 mmol/L. Repeat analysis of the above specimen on our blood gas analyzer demonstrated sodium, potassium, and chloride concentrations of 127 mmol/L, 3.5 mmol/L, and 100 mmol/L, respectively, confirming the existence of an electrolyte exclusion effect in this specimen.

Conclusions: Lp-X when present at significantly elevated concentrations can produce erroneously low electrolyte results due to the well established electrolyte exclusion effect. The interference in electrolyte analysis attributed to Lp-X decreased in conjunction with the reduction in total cholesterol concentration to a point in which both direct and indirect ion selective technologies produced comparable results.

A-80

Iso 15189 Accreditation Of Routine And Emergency Biochemistry

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Background: Since 2010, a new French legislation (n° 2010-49 dated 2010-01-13) constrains a mandatory accreditation for medical laboratories under ISO 15189. The French Committee of Accreditation (Cofrac), is the independent organism in charge of accreditation. Medical laboratories have 6 years to achieve accreditation of all biology activities. Routine and emergency activities of the laboratory of our hospital are grouped as a unique laboratory since 2007, sharing a common quality management system.

Methods: To anticipate the new legislation, an application was sent to Cofrac in June of 2009 which regarded the whole laboratory quality management system but was limited to biochemistry analyses. General biochemistry laboratory and emergency laboratory (which includes emergency biochemistry) are located in two different

buildings. 29 plasma, 10 urinary and 3 CSF analyses performed on 4 multiparametric analyzers (Beckman Coulter DxC800, AU400, AU640 and AU680) in the 2 locations and 9 blood analyses performed on 2 blood gas analyzers (Radiometer ABL series) in the emergency laboratory were submited to Cofrac.

Our medical laboratory created a quality group leaded by the laboratory quality manager. Members were: head of the laboratory, medical directors, technical supervisors. This group focused on quality management requirements of the ISO 15189 chapter 4. Moreover, medical biologists focused on technical requirements of ISO 15189 chapter 5: training-habilitation of laboratory staff, environmental conditions, laboratory equipment, pre- and post-examination procedures, quality of examination procedures. Additional specific requirements of the French legislation about external quality control and biological validation of results were also taken into account.

Results: In accordance to ISO 15189 chapter 5, performance specifications for each analysis were verified on the 4 multiparametrics and the 2 blood gas analyzers: precision, trueness, measuring range (if pertinent), interferences. Measurement uncertainty was calculated for each analysis. Results for precision and uncertainty were compared to those from the french society of clinical biochemistry and from Ricos specifications; all were found below fixed limits. Comparability of results obtained on the 4 analyzers in the 2 locations (general biochemistry laboratory and emergency laboratory) was contineously verified. Biological reference intervals were also reviewed, and verified for plasma analyses on 20 healthy subjects.

In November of 2010, a 3-day initial audit of 2 Cofrac members (one biochemistry technical auditor and one quality expert) evaluated our practices and their compliance with the ISO 15189 norm. The audit revealed 18 gaps, included 2 critical gaps, to be corrected within 6 months. An action plan was sent within 15 days as required by Cofrac: modifications of the training-habilitation procedure corrected the first critical gap, and for the second one, an improvement of temperature metrologic control was planified.

Conclusion: This is the first experience of an ISO 15189 accreditation of a public hospital laboratory of such size in France. Extension of ISO 15189 accreditation to other activities (microbiology, hematology...) is scheduled before 2016 to be in accordance with the new regulation.

A-81

Liquid-stable enzymatic assay for measurement of potassium on clinical chemistry analyzers

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Potassium is one of the most important electrolytes in the human body. Potassium ion concentration maintains the cellular electrochemical membrane potential and is crucial for heart muscle action and neuronal functions. Monitoring the level of potassium in serum is very important and it is a basic test in all emergency units.

Routinely used methods for the clinical determination of potassium are flame emission spectroscopy (FES) and potentiometry with ion selective electrodes (ISE). The FES, accepted as the reference method, has a high accuracy but a low throughput. It cannot be integrated in a clinical chemistry analyzer. The ISE has a high throughput and can be integrated into a clinical chemistry analyzer, but requires a lot of regular maintenance for reliable performance. Although costs per test of FES/ISE seem to be very low on a running system, investment as well as maintenance costs have to be taken into consideration. A market need for a reliable and accurate photometric test is obvious. There are some colorimetric, turbidimetric as well as enzymatic tests already on the market, but the quality and the ease of use are not always satisfactory. The liquid-stable, enzymatic DiaSys potassium assay is optimized for laboratories with small or midsized clinical analyzers without an ISE. The assay is based on the well-known potassium dependency of pyruvate kinase, which is coupled via lactate dehydrogenase to the conversion of Acetyl-NADH to Acetyl-NAD^+. Pyruvate kinase activity is naturally influenced and interfered by other cationic electrolytes and ammonia, which affects the correctness of the potassium determination. Several additives and recombinant enzymes from specific organisms were used to counteract these influences already described in the literature [Clin Chem (1989), 35/5, 817-820].

For the determination of potassium in human serum samples, the potassium dependent enzymatic rate of pyruvate kinase is measured on a DiaSys respons®920 clinical analyzer. The concentration is calculated from a multipoint calibration. The assay's precision in series is < 2% for samples with approx. 3 mmol/L potassium and < 1% for samples of up to 6 mmol/L potassium. Precision from day-to-day is < 2% within the whole measuring range from 1 to 8 mmol/L potassium. A method comparison to FES (Eppendorf, EFOX 5053) shows a slope of b=1.046 and an intercept of a=-0.163 mmol/L for n=55 serum samples, the correlation coefficient is r= 0.97. The mean bias to FES is 1.1%. There are no significant interferences within ± 4.5% limits from sodium between 100 and 180 mmol/L, ammonia, copper, zinc and iron up to 0.5 mmol/L, calcium up to 10 mmol/L, magnesium up to 8 mmol/l, lipids up to 1200 mg/dL, bilirubin and ascorbic acid up to 30 mg/dL.

Our results demonstrate, that the DiaSys two-component enzymatic potassium assay offers a suitable photometric method for small and medium-sized laboratories. The reagent can be used manually as well as on routine clinical analyzers with a similar performance as ISE and FES.

A-82

Biochemical picture of urinary low molecular weight compounds among Romanian healthy subjects

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Background: The nuclear magnetic resonance spectroscopy method (NMR) is used for the identification and quantification of metabolites present in biological fluids. It has the advantage of being fast, requiring a small amount of sample and providing a complete picture of the composition of biological samples. In general, each research group builds its own database by defining the normal population and the corresponding reference intervals. In the literature there are few studies that have investigated the biochemical changes in urine of healthy subjects due to age, sex, diet characteristics, body mass index or time and storage temperature of urine samples.

In this study we obtained the global profiles of biochemical composition in urine samples from healthy individuals living in Romania and evaluated the gender-related and age-related urinalysis differences by proton nuclear magnetic resonance spectroscopy (^{1}H-NMR). We established the reference range for 18 metabolites as well. The metabolic profiles were compared with ^{1}H-NMR urine profiles of Italian, Greek, British, Swedish healthy subjects and volunteers living at Artic Scientific Base in Svaldbard. Data obtained for the normal population by other analytical techniques (HPLC, GC-MS, LC-MS) was included.

Methods: 104 normal subjects living in Romania were recruited with the following characteristics: absence of metabolic diseases, hypertension, urinary infections, renal impairment and alcohol consumption for 24 hours before sampling. The NMR spectra were recorded on a Bruker Avance DRX 400 MHz spectrometer operating at 9.4 Tesla. Metabolite concentrations were expressed in mmol/mol of creatinine. Statistical analysis was performed with CBStat 5.1.

Results: There are gender-related differences in the excretion of citrate, lactate, 3-hydroxyisovaleric acid, alanine, trimethylamine-N-oxide, glycine and hippurate between males (n=46) and females (n=58) in healthy Romanians. The healthy subjects above 35 years old (n=54) tended to have higher urinary concentrations of trimethylamine-N-oxide, dimethylamine and 3-hydroxyisovaleric acid compared to subjects below 35 years old (n=50). There are significant differences in the urinary excretion of alanine and 3-hydroxyisovaleric acid between Romanian volunteers below (n=61) and above (n=43) 45 years of age. There are significantly decreased differences in the excretion of citrate, hippurate, glycine and trimethylamine-N-oxide and increased excretion of alanine and dimethylamine in Romanian healthy subjects vs. Italian normal group. Average concentrations obtained for lactate, citrate, alanine, trimethylamine-N-oxide and glycine are significantly increased in healthy Romanians compared to the values from the group of volunteers from Svaldbard Scientific Base. In the Romanian volunteers men excreted lower concentrations of trimethylamine-N-oxide than women, whereas the excretion of this metabolite in Greek men was higher. There are significant differences between the excretion of lactate, citrate, dimethylamine, trimethylamine-N-oxide in Romanian Healthy Subjects vs. Greek volunteers above and below 45 years old. The qualitative metabolic picture showed differences between the excretion of dimethylamine and similar profiles for citrate, hippurate and glycine in Romanians vs. British and Swedish healthy groups. Compared with data recorded by other analytical techniques we obtained significant differences for hippurate, glycine and 3-hidroxyisovaleric acid.

Conclusion: There is a strong need for large inter-laboratory and inter-country trials for establishing normal ranges and geographical variations of metabolite concentrations in urine by ^{1}H-NMR method.

A-83

Serum Indices Measurement: Hemolisys, Lipemia and Icterus as hidden source of errors for arterial blood gas analysis

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Background: Several studies have documented the burden and the type of preanalytical errors in different realities worldwide, but most of them have focused on errors related to clinical chemistry, coagulation and hematological testing, while no reliable information has been provided so far on preanalytical errors related to arterial blood gas (ABG) analysis. We investigated serum index in all routine samples referred to our laboratory for ABG analysis over a 1 month period.

Methods: All specimens were systematically analyzed for Serum Indexes (SI) immediately after ABG analysis has been completed, by transferring the blood into secondary tubes, and further centrifugation at 1500xg per 5 min. In vitro hemolysis has then been assessed on Cobas C501 (Roche). We fixed a cut-off value of 60 for Hemolysis Index (HI, i.e, cutoff of visible hemolysis), 30 for Lipemia Index (LI) and 2 for Icterus Index (II) respectively.

Results: Out of a total of 253 ABG specimens received in our laboratory throughout the 1-month study period, we identified 12 samples (5%) with various degree of hemolysis, 33 samples (13%) with lipaemia and 34 (14%) for icterus. A notable difference in haemolysed samples was observed between Emergency and Clinical Departments (7% vs. 4%, p<0.05) (data shown in table 1).

Conclusions: A significant number of specimens referred for ABG analysis are plagued by hemolysis as well as lipaemia and icturus. The former case is due to inappropriate preanalytical conditions (e.g., cumbersome collection). As Our procedure (rapid centrifugation of samples followed by SI assessment on plasma) is a suitable approach to identify unsuitable specimens and limit delivery of unreliable results.

Table 1.

Sample	Serum Index					
	Hemolysis Index (HI)		Lipaemia Index (LI)		Icterus Index (II)	
	<60	>60	<30	>30	<2	>2
All Samples n (%)	241 (95%)	12 (5%)	220 (87%)	33 (13%)	219 (87%)	34 (16%)
Emergency Department n (%)	82 (93%)	6 (7%)	78 (89%)	10 (11%)	78 (89%)	10 (11%)
Clinical Departments n (%)	159 (96%)	6 (4%)	142 (86%)	23 (14%)	141 (85%)	24 (15%)

A-84

Abbott ARCHITECT ICT (ISE) Module Evaluation of NIST SRMs and Sigma Metrics

J. L. Seago, Q. Li, D. Pistone. *Abbott Laboratories, Irving, TX,*

Objective: Characterize NIST Serum based SRMs 909c and 956c for re-standardization of Na/K/Cl serum calibrators for Abbott ARCHITECT Integrated Chip Technology (ICT), solid-state ISE module, because SRM 909b is no longer available.

Relevance: ARCHITECT ICT Serum Calibrators previously traceable to SRM 909b will need to be traceable to one or more of the proposed replacements, NIST SRM 909c and 956c.

Methodology: 5 day Precision study with SRM 909b, 909c and 956c (5 reps twice per day using fresh ampoules/bottles and calibration daily). Three ICT modules, 2, 6 and 9 months old, and concentrated ICT Sample Diluent were used with ARCHITECT c8000 and c16000. Urine results were obtained from SRM 919 (NaCl), SRM 918 (KCl) and Linearity Standards.

Validation: SRMs were evaluated for Precision (EP5-A2) and Accuracy. 'ARCHITECT Results' in the table are the average of 150 determinations over 5 days and three analyzers. Sigma metrics were calculated using the Westgard.com formula {Sigma=[TEa(%)-Bias (%)]/CV(%)} using the precision data, bias based on recovery of SRM target values and TEa from the RiliBÄK.

Results:

SRM 909b	Na+ (L1)	Na+ (L2)	-	K+ (L1)	K+ (L2)	-	Cl- (L1)	Cl- (L2)	-
Certificate of Analysis	126.76 ± 0.92	141.0 ± 1.3	-	3.424 ± 0.026	6.278 ± 0.052	-	89.17 ± 0.57	113.43 ± 0.66	-
ARCHITECT Results	119.48	140.34		3.43	6.25		88.16	119.28	
Pooled Total %C.V.	0.43	0.31		0.53	0.45		0.33	0.31	
Sigma	10	15		15	17		23	35	
SRM 909c	Na+ (L1)	-	-	K+ (L1)	-	-	Cl- (L1)	-	-
Certificate of Analysis	141.6 ± 0.2	-	-	Target values not available from NIST			NIST has no parsific provice values		
ARCHITECT Results	141.07			4.20			104.72		
Pooled Total %C.V.	0.48			0.53			0.40		
Sigma	9			NA			NA		
SRM 956c	Na+ (L1)	Na+ (L2)	Na+ (L3)	K+ (L1)	K+ (L2)	K+ (L3)	Cl- (L1)	Cl- (L2)	Cl- (L3)
Certificate of Analysis	116.8 ± 1.0	137.5 ± 1.6	167.4 ± 1.4	5.375 ± 0.05	3.977 ± 0.03	1.582 ± 0.017	104.5 ± 3.2	121.5 ± 2.5	137.4 ± 1.8
ARCHITECT Results	118.94	137.31	165.37	5.92	3.97	2.1	107.3	123.34	140.04
Pooled Total %C.V.	0.37	0.34	0.40	0.49	0.31	0.76	0.33	0.39	0.39
Sigma	13	14	9	14	25	3	15	16	16

Conclusion: The combined results from three instruments show that the ICT system is stable, precise and accurate with all three SRMs. The measurement uncertainty for Chloride in SRM 956c is too large for anchoring Calibrators. SRM 909c, in 2 mL ampoule size, has no Potassium or Chloride values assigned by NIST and the single Sodium concentration is a "Reference," not a "Certified," value which makes its use questionable. Continued serum Calibrator traceability to NIST may require the use of multiple SRMs. Sigma metrics demonstrate accuracy and precision of the Abbott ICT Module which is well suited for use in the Clinical Chemistry Laboratory providing Na/K/Cl results within 3 minutes from 15µL of Serum, Plasma or Urine and warranted for 20,000 samples (60,000 tests) or three months use.

A-85

Investigation of metabolic disorders associated with type 2 diabetes mellitus by proton magnetic resonance spectroscopy (1H-NMR)

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Background: In spite of the great number of published papers on nuclear magnetic resonance (NMR) urine analysis, and the existence of several groups around the world active in the field, there are only a few published results on metabolites determined in diabetes mellitus (DM).

Objectives: The aim of the first section of the study was to compare the NMR urine profile between healthy subjects and type 2 DM patients, trying to obtain a reliable expression of metabolic control and the degree of the progression of diabetic complications in condition of absence of renal complication of diabetes mellitus. In the next section of the study patients with type 2 DM were evaluated according to age and body mass index and the NMR profile of metabolites concentrations established. In the last section we compared the NMR spectra and the pattern of urinary metabolite excretion in terms of duration of type 2 DM.

Materials and Methods: Serial urine samples of 104 healthy subjects and 121 type 2 diabetes mellitus (DM) patients were examined by proton nuclear magnetic resonance spectroscopy (^{1}H-NMR). The patients had a history of type 2 DM less than 5 years were hospitalized in Diabetes and Metabolic Diseases Department. The NMR spectra were recorded on a Bruker Avance DRX 400 MHz spectrometer, using a 5 mm inverse detection multinuclear probe equipped with gradients on the z-axis. To 0.9 ml urine, 0.1 ml of stock solution of 5 mM sodium 3-(trimethylsilyl)-[2, 2, 3, 3-d_4]-1-propionate (TSP) in D_2O has been added. The ^{1}H-NMR spectra have been recorded with water presaturation. The data were calculated using CBStat version 5.1.The results are evaluated in mmol/mol of creatinine.

Results: A significant difference between the excretion of 3-hydroxyisovaleric acid (0.01<p<0.02), hippurate and creatine [(0.02<p<0.05)], valine (0.002<p<0.01), alanine, gamma-aminobutyrate, glycine, trimethylamine-N-oxide and citric acid [(p<0.001)] at the healthy subjects and type 2 DM patients was found. The values for trimethylamine, pyruvate, dimethylamine, acetic acid and lactic acid are similar in both groups. The type 2 DM patients above 55 years old tended to have higher urinary concentrations of lactic acid (0.01<p<0.02) than patients below 55 years old. We obtained that the concentrations of valine, lactate, pyruvate and trimethylamine in diabetics increased with the increase of body mass index. There are higher urinary concentrations for alanine, 3-hydroxyisovaleric acid, citrate and dimethylamine in newly diagnosed type 2 DM patients, while the values for hippurate increased with

the increase of duration of type 2 DM. The urinary excretions of pyruvate, gamma-aminobuthyrate, glycine and trimethylamine-N-oxide are higher in patients with duration of type 2 DM less than 1 year.

Conclusions: The present study, provided a metabolic trend in urine NMR profiling of type 2 DM patients and underlined the need for larger studies, including extensive interlaboratory trials in order to asses the influence of different factors on the NMR diagnosis of diabetes. Type 2 DM urinary metabolites are interesting in various aspects, such as providing clues for the biochemistry and mechanisms of the disease or potential early diagnostic markers in diabetes renal involvement.

Tuesday AM, July 26

Poster Session: 10:00 am - 12:30 pm
Factors Affecting Test Results

A-86

Utility of Qualitative Result Frequency Tracking in the Clinical Immunology Laboratory

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Background: Consistent performance of clinical laboratory tests can be adversely impacted by changes in a variety of underlying factors. Modern laboratories invigilate against such threats in most, if not all, of their tests by periodic analysis of control materials. Application of multi-rule algorithms to control results increases sensitivity to changes in test performance, yet this approach is still unable to detect some subtle shifts of potential clinical significance. This difficulty can be due, in part, to instability of control materials, different matrix composition and dilution procedures relative to patient samples, and the relative infrequency of control testing. To mitigate these complications, several patient-based quality control strategies have previously been proposed. Such approaches typically employ the numeric value of patient test results as a basis for calculation, and are therefore not necessarily applicable to the monitoring of semi-quantitative or qualitative tests, nor easily utilized to compare the performance of tests which do not report on the same numeric scale--as is the case for many antibody assays. We present a previously undescribed method of patient-based quality control which avoids such drawbacks by monitoring moving averages of qualitative result frequency rates.

Methods: Initial implementation consisted of monthly tallying of results reported within each qualitative reporting range and graphing as a percentage of all results reported within the same period. Improvement of the method introduced exclusion of clinical trial participants (whose result frequencies may differ from the typical testing population), determination of 25-200 day moving averages plotted at the daily level, and the addition of automated flagging to indicate when moving averages of differing periods diverge by more than 2-3 standard deviations from mean separation. Graphs are reviewed on a weekly basis for evidence of significant deviation from previous performance.

Results: A 10-12% increase in equivocal and positive results was observed in an ELISA for antibodies to proteinase 3; investigation revealed that this shift correlated to a lot change which introduced new sources of antigen, conjugate, standards, and controls; rates returned to acceptable levels upon switching to a test from another manufacturer. An ELISA for anti-nuclear antibodies exhibited an 8% drop in negative results after being moved to a new automated platform; rates rebounded by 4% after changes in the wash program, but dropped another 10% upon switching to a lot subsequently recalled by the manufacturer; rates returned to original levels after switching from the recalled lot. A 3% increase in negative results corresponding to lot change was observed in an ELISA for antibodies to deamidated gliadin; discussions with the manufacturer revealed that antigen concentration had been decreased in assay wells, but performance changes were not detectable by their own quality testing.

Conclusion: Moving averages of qualitative patient result frequencies provide a useful quality control tool for the monitoring of semi-quantitative and qualitative tests of medium to high volume. By incorporating large numbers of results, this approach exhibits sensitivity to subtle changes in test performance not detectable by other means.

A-87

Pre-analytical Variables Affecting the DiaSorin Liaison® Vitamin D Assay

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Background: Recent interest in the measurement of serum vitamin D for numerous endocrine conditions has generated its share of controversy from an analytical standpoint. An understated factor in clinical laboratory analysis is the effect of proper storage conditions on the precision and accuracy of the assay. A number of pre-analytical factors common to multi-hospital/multi-site systems can influence test accuracy including: sample handling at site of draw, transportation delays, processing delays, and improper storage at any step. Additional common confounding factors include presence of excessive lipemia or hemolysis.

Methods: We have evaluated the stability of vitamin D in the Diasorin Liaison chemiluminescence assay at 4° and 25° C over one week and the impact of hemolysis and lipemia on test accuracy. This competitive binding assay includes incubation of patient serum and isoluminol derivative-conjugated 25-hydroxy-vitamin D with anti-vitamin D coated microparticles followed by chemiluminescence detection. Patient serum is stored at 4°C awaiting assay. For the stability study, three pools of de-identified patient specimens designated as low (13 ng/mL), medium (28 ng/mL), and high (53 ng/mL) vitamin D were assayed daily in singlet. Pools were utilized to allow repeated serial sampling of specimens throughout the experimental course. Single measurements approximate the reality of clinical testing. To measure the impact of hemolysis on vitamin D concentration, a hemolysate was prepared by treating an ethylenediaminetetraacetic acid plasma specimen to one freeze-thaw cycle. Increasing volumes of the hemolysate (1, 5, 10, 20, and 50 µL) were added to each of five aliquots of each pool. The final hemoglobin concentration was estimated based on the hemolysis index (range 5 - 600 mg/dL; Hb index 4 = 100-200 mg/dL). To study the effect of lipemia, specimens containing average triglyceride concentrations of 1,000 mg/dL, 500 mg/dL or 300 mg/dL were used to prepare a low, medium and high triglyceride pool. Vitamin D was measured in these pools by triplicate, before and after centrifuging the pools in an Airfuge® ultracentrifuge (Beckman, Inc) for 10 minutes. Imprecision between measurements, expressed as the % coefficient of variation (CV) was calculated.

Results: For the stability study, the %CV of all the pools measured from day 1 to day 8, ranged between 9% and 19% for specimens stored at room temperature or at 4°C. Hemolysis caused underestimation of specimens with hemolysis indexes greater than five but only in the high concentration vitamin D pool (63 ng/mL). For the lipemia study, the %CV for all the pools ranged between 4% and 12%. The CV between-run on QC samples for the assay are 8% and 15% at 16ng/mL and 53 ng/mL, respectively.

Conclusions: Overall, vitamin D measured by the Liaison is stable at least eight days at either 4 or 25 ºC and is not affected by the presence of moderate hemolysis and lipemia. In specimens with severe hemolysis, and high concentrations of vitamin D, an underestimation of vitamin D may occur. However, the clinical relevance of this effect was not evaluated.

A-88

Roadmap for harmonization of clinical laboratory measurement procedures

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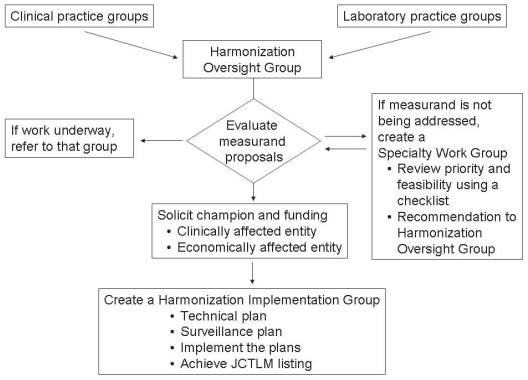
Background: The objective was to develop a process to improve harmonization of laboratory results for measurands that do not have reference measurement procedures. Results from clinical laboratory measurement procedures must be equivalent to enable effective use of clinical guidelines for patient management. A limitation for such measurands has been inadequate definition of the measurand, inadequate attention to the commutability of reference materials, and lack of a systematic approach for harmonization.

Methods: The AACC convened an international conference in 2010 to improve harmonization of laboratory results for measurands that do not have reference measurement procedures. Laboratory, clinical, metrology and regulatory organizations were invited to send representatives. The 90 participants examined issues and potential processes, and agreed on an achievable path forward.

Results: An infrastructure (see figure) consisting of a harmonization oversight group (HOG), specialty work groups (SWG), and harmonization implementation groups (HIG) will be created and may be housed by an existing organization. The HOG will coordinate all activity and will solicit and receive input from clinical practice and laboratory organizations, in-vitro diagnostics industry, journal editors, research organizations, and government or regulatory agencies. Structured checklists will be used to prioritize measurands and secure funding based on input and analysis by a SWG formed to address a specific measurand. A HIG will manage the technical implementation of a harmonization process for a specific measurand. Technical

procedures will be developed that include individual clinical sample panels, commutable reference materials and manufacturers' internal calibration controls. The harmonization procedures should be in accordance with the JCTLM quality system and lead to JCTLM listing. Thus, manufacturers will be able to implement harmonization in conformance to regulatory requirements.

Conclusion: The AACC is committed to supporting further development of the infrastructure and technical operations needed to support harmonization for these types of measurands.



A-89

Accuracy of data generated through an External Quality Assessment programme for analytical parameters from a chemistry control material

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Background: Internal control materials are used routinely for the daily monitoring of analytical performance. They are provided generally in two formats; precision (with approximate target values) to which the laboratories assign their own targets and ranges and then assayed (with assigned target values already defined). The provision of predefined target values reduces the workload for a laboratory and can also minimise bias that could remain undetected by the use of precision material. These benefits are only achieved if the target values are accurate and reliably assigned.

Relevance: We report here the accuracy of target values, assigned to internal quality control materials, by an external quality assessment scheme with a large population of participating laboratories. This was determined by comparing target values assigned to the same material by reference methods. This is relevant for laboratory internal control as it indicates that the values assigned to the assayed material are close to the true values. Such assayed quality control materials can facilitate reliable bias assessments.

Methodology: Chemistry control materials (HN1530 and HE1532), covering a range of concentrations, were used. Their target values were previously assigned through generation of all method consensus means, by the External Quality Assessment (EQA) programme 'Randox International Quality Assessment Scheme' (ISO/IEC Guide 43-1:1997 accredited). The same controls concurrently had target values, with known uncertainties, assigned by reference laboratories utilizing assigned reference material and methods (isotope dilution mass spectrometry, atomic absorption and flame photometry).

The 5000 participants registered on the clinical chemistry programme provided a large number of results, after removal of outliers, to generate the consensus means. Target values for 11 chemistry parameters (calcium, chloride, cholesterol, creatinine, lithium, magnesium, potassium, sodium, total protein, triglycerides and uric acid) assigned through the EQA and reference methods, were compared and the average % deviation calculated.

Results: From the 11 parameters assessed, 3 parameters (chloride, cholesterol and sodium) showed an average %deviation, between the EQA and reference method determinations of less than 0.7%, 6 parameters (creatinine, lithium, magnesium, potassium, total protein and uric acid) showed % deviations of less than 1.9% and 2 parameters (calcium and triglycerides) of less than 2.5%. The maximum individual %deviation observed was 6% for calcium, the other parameters tested ranged from 0% to 3.6%. It was observed that the majority of the all method means generated, fell within the uncertainty ranges quoted for the reference values.

Conclusion: The data for the 11 chemistry parameters indicates that the internal quality control target values, derived from the reported EQA programme, are comparable to values produced by reference methods. This is relevant as a means for confirming accuracy of assigned internal quality control values, leading to error, time and cost reduction when compared with precision control material.

A-91

Monitoring absolute bias and harmonization by PT-EQA - five year experience from the Netherlands

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Background: Comparability of results among laboratories is a major mission for medical laboratories. The monitoring of harmonization and standardization is a standard part of the Proficiency Test programmes of the Dutch EQA Organizer SKML. In the general chemistry programme EQA-samples are commutable, fresh frozen human sera (CLSI C37-A2 for lipids), targeted with definitive/ reference methods by JCTLM endorsed reference laboratories. The EQA-design used guarantees that samples can be seen as trueness verifiers, allowing to evaluate absolute bias. In the annual cycle of the Dutch EQA programme 24 samples, covering the clinically relevant range, are assayed by the 220 participating laboratories at biweekly intervals.

Methods: To monitor harmonization trends overall interlaboratory CVs were used as study parameter. Median interlaboratory CVs of the programmes in 2005 and 2010 were compared to demonstrate evolution of harmonization in 2005-2010. In addition and for each analyte, absolute biases were calculated from the deviations of the individual values to the target values per method group and overall, both for the 2005 and 2010 EQA-programmes. The median absolute bias per individual lab was checked against the allowable bias criterion, and the percentage of labs that pass the allowable bias criterion is monitored.

Results: Median interlab CVs of the monovalent electrolytes Na^+, K^+, Cl^- range between 0.9-1.4% and did not improve between 2005 and 2010. The exception was Li^+ for which the median interlab CV improved from 13.5% in 2005 to 5% in 2010. Median interlab CVs of Ca^{2+}, iron, phosphate, urea, creatinine and glucose dropped from 6% to 3%, with major improvements for creatinine and glucose. Median interlab CV of Mg^{2+} detoriated, with a median interlab CV of 4.0 % in 2005 and 5.4% in 2010. For enzymes (ASAT, ALAT, γ-GT, ALP, CK, LD, α-amylase) the *overall* median interlab CV decreased from 42% in 2005 to 28% in 2010, whereas for the completely IFCC-standardized enzyme method groups median interlab CVs narrowed to 4.3-8.2% in 2010. In the group of lipids and apolipoproteins median interlab CVs were halved in 2010 as compared to 2005, coming down to <3% for cholesterol and TG, and to <7% for HDLc, LDLc, apo A1 and apo B. In a rest group of analytes -encompassing osmolality, lactate, total protein, albumin, bilirubin- median interlab CVs were constant for osmolality, lactate and total protein between 2005 and 2010 but increased for albumin and bilirubin, the latter due to the restandardization in 2010.

In general, the improvement in interlab CVs over the past five years was paralleled by a reduction in absolute bias and an increase in the % of labs that pass the allowable bias criterion (>70-99.2%).

Conclusion: The SKML EQA programme, using commutable, targeted multi-level samples, proves to be a powerful instrument to establish absolute bias and the status of harmonization. The interlab CV's in 2010 were in general better than those in 2005, median interlab CVs becoming < 5% for electrolytes and substrates, and < 10% for enzymes, lipids and others.The percentage of labs meeting the allowable bias criteria has improved and ranges between 70-99.2% in 2010.

A-92

The potential diagnostic pitfalls in capillary electrophoresis: A clinical study with review of the literature

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Capillary electrophoresis (CE) is being increasingly used in clinical laboratories due to its high throughput, quickness, and technical convenience. However, several problems with CE have been also reported. Here we describe 3 unusual cases for which CE with Minicap (Sebia, Lysse, France) was done in our laboratory. Case

no.1; August 2010, a 56-yr-old Korean man was transferred to our hospital due to sudden left side weakness. Brain CT with contrast (Iopamidol) enhancement revealed cerebral infarctions. Right after contrast injection, CE was done for routine protein analyses. Despite the unremarkable results in quantitative assay of immunoglobulin and free light chain (LC), CE showed a suspicious peak in both serum and urine on alpha2 regions, which were not immunosubtracted in immunotyping. After 6 days, the complete disappearance of those peaks was confirmed in both kinds of specimens. Concluded to contrast interference, the patient was discharged after 10 days for outpatient follow up. Case no. 2; August 2010, a 50-year-old male was admitted for an altered mentality. Brain CT with iopamidol showed acute cerebral infarctions. About 1 week later, urine CE presented a small but discrete peak that was not subtracted in immunotyping. Six days later, we identified the complete disappearance of this small peak in the urine specimen, just like case no.1. Case no. 3; A 61-year-old Korean female with nephritic syndrome was admitted to our hospital on 3 September 2010, because of aggravated back pain. Serum CE shows no definite peak, however, lambda FLC was markedly increased and the kappa/lambda free LC ratio was drastically reversed in both serum and urine. Moreover, there was a discrete peak on alpha2 region in urine CE and confirmed to the lambda LC in immunotyping. Following radiologic findings presented multiple punched-out bone lesions and BM aspirations showed 20.2% plasma cells in all nucleated cells. Conventional gel electrophoresis revealed clear band of restriction in the gamma region in both kinds of specimens, and immunofixiation electrophoresis identified it as lambda LC. Finally, the patient was diagnosed with plasma cell neoplasm.

In the literature review, there were two previous cases that describe pseudoparaproteinemia by contrast interference in CE. To our knowledge, our cases make the third sets of reports. Iopamidol involved in 3 cases and all of these peaks lied within alpha-2 or beta region. Important point to differentiate from real monoclonal peak was that false peaks by contrast materials are not immunofixated and immunosubtracted. However, we could not find a comparable case study with our case no.3. Although the reason is not fully known, a limitation of the UV detection method in CE can be recognized as one of causes to decrease the sensitivity. Moreover, the presence of interfering substances is considered as be challenges to overcome. Through these 3 cases, we decided to perform gel electrophoresis as well as CE on questionable samples for monoclonal components and instructed clinicians not to collect blood for CE shortly after contrast injection. We suggest that multiple methodologic modalities should be combined in diagnosing and monitoring plasma cell neoplasm and related disorders.

A-93

Methotrexate Interference in Urine pH Using the Clinitek Status Dipstick Analyzer

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Background: Patients undergoing methotrexate therapy require frequent monitoring of urine pH to prevent crystallization of methotrexate metabolites in the kidneys which can lead to renal failure. The standard methotrexate treatment protocol requires that urinary pH be maintained between 7.0 and 8.0, which is accomplished via administration of bicarbonate. We recently noted that pH values obtained using the Clinitek Status (Siemens Healthcare Diagnostics, Inc., Deerfield, IL) are often clinically discordant from those obtained using pH meters (Accumet AR15 and XL15, Fisher Scientific, Pittsburgh, PA), which are used in our practice to measure the pH of highly colored urines that could interfere with Clinitek readings. The discordance between Clinitek and pH meter values can make it difficult for care providers to make decisions regarding the appropriate bicarbonate dose required to achieve urinary alkinization.

Methods: To evaluate potential methotrexate metabolite interference in the Clinitek Status dipstick method, 116 urine samples from four patients receiving methotrexate treatment were tested for pH using the Clinitek Status dipstick analyzer and an Accumet AR15 or XL15 pH meter. To determine if differences in pH are unique to patients receiving methotrexate, we compared pH measurements made using the Clinitek Status and Accumet AR15 pH meter on 50 samples with pH values between 6 and 9 from 50 patients not receiving methotrexate. In both patient populations we defined acceptable bias as ≤0.5 pH units. Clinical concordance was defined as results from both methods within or outside of the 7.0 - 8.0 target pH range. Since the original clinical protocols were developed using the dipstick method, the Clinitek Status was treated as the reference method.

Results: For methotrexate patients, the mean bias (SD) was -0.71 ± 0.37 pH units, while the mean bias (SD) for non-methotrexate patients was -0.37 ± 0.30 pH units. Of the 116 methotrexate specimens tested, 27 (23.3%) were within 0.5 pH units and 31 (26.7%) were concordant within the range of 7.0 - 8.0. Of the 50 non-methotrexate specimens tested, 33 (66.0%) were within 0.5 pH units and 25 (50.0%) were concordant within the range of 7.0 - 8.0.

Conclusion: The pH meter demonstrated a systematic negative bias on both methotrexate and non-methotrexate patient urine samples. The negative bias is exaggerated in urine from patients receiving methotrexate, most likely due to the bright yellow color of the sample caused by methotrexate metabolites that could interfere with the dipstick reading. Despite this apparent bias, clinicians may still prefer using a dipstick to monitor urinary pH in patients receiving methotrexate, since the clinical protocols were developed using a dipstick. Laboratories that use a pH meter to confirm the pH of highly colored urine samples should be aware of the bias between dipstick and pH meter results, which could cause confusion to providers using the methotrexate protocol with both pH meter and dipstick results.

A-94

Effect of Iron Status, Age, and Gender on a Transferrin/TIBC Conversion Factor

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Background: Total iron-binding capacity (TIBC) and serum iron are commonly used to calculate percent saturation of transferrin (TRF), a measurement of overall iron status. TIBC has traditionally been measured indirectly from UIBC and serum iron, and laboratories have been replacing TIBC assays with direct and more robust TRF assays. In order to keep percent saturation reference ranges consistent, TRF results are typically multiplied by a factor to convert back to TIBC before calculating saturation. However, this conversion factor is not standardized between laboratories and it is uncertain whether a single factor is applicable to all patient populations. The purpose of this study was therefore to examine the TRF to TIBC conversion factor over a wide population and determine how it may be influenced by iron status, age, and gender.

Methods: A retrospective review was performed on 1271 patients (658M/613F) who underwent TRF, TIBC, serum iron, and ferritin testing at Mayo Clinic, Rochester from 2006 to 2011. Patients were classified into iron-depleted (ferritin<11 mcg/L in females and <24 mcg/L in males, n=157), normal (ferritin 11-307 mcg/L in females and 24-336 mcg/L in males, n=850), iron-overloaded (ferritin 307-999 mcg/L in females and 336-999 mcg/L in males, n=196), or highly overloaded (ferritin>1000 mcg/L, n=68) subgroups based on their ferritin results. The TRF to TIBC conversion factor was determined for the entire population and for each subgroup by linear regression analysis of TRF and TIBC results. Percent saturation was calculated by dividing serum iron by TIBC, TRF, or TRF multiplied by the population, subgroup, or previously published conversion factors. Correlation between TIBC-calculated saturation and TRF-calculated saturation was determined by linear regression. Concordance between percent saturation calculations was defined as similarly categorizing results as low (<15%), normal (15-40%), or high (>40%).

Results: The TRF to TIBC conversion factor calculated from the entire population was 1.11. Ferritin subgroup analysis showed that the factor was significantly affected by iron status (p=0.005), with a factor of 1.04 in the iron-depleted, 1.11 in the normal, 1.12 in the iron-overloaded, and 1.20 in the highly overloaded groups. While the factor was not influenced by patient age, it was significantly affected by gender (p=0.02). The factor was 1.09 for all males, ranging from 1.07 in iron-depleted males to 1.22 in very overloaded, and 1.13 for all females, ranging from 0.97 in iron-depleted females to 1.16 in very overloaded. Linear regression analysis of TIBC-calculated versus TRF-calculated percent saturation showed reasonable correlation (slope 1.36, r^2 0.97), and this correlation improved when a conversion factor was applied to TRF (slopes 0.95 - 1.22). TRF-calculated percent saturation had an 85% concordance with TIBC-calculated saturation. This concordance improved to 92-95% if the conversion factors were applied.

Conclusions: The TRF to TIBC conversion factor is significantly influenced by gender and iron status. Laboratories should therefore establish factors independently to best represent their patient populations.

A-95

Effects of temperature and light on the stability of bilirubin in serum

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Background: Blood samples collected in remote areas are sometimes assessed in central laboratories with delay and exposed to light at various temperatures for different periods of time during shipping and storage. Although it is known that bilirubin is photo-sensitive, detailed effects of both temperature and artificial light have not been well studied. The objective of this study is to determine the effects of

temperature and artificial light on bilirubin stability in serum samples.

Methods: Serum samples with elevated total bilirubin were selected for this study. Total and direct bilirubin were analyzed on a DxC 800 Chemistry Analyzer (Beckman Coulter) using a timed endpoint diazo method. To determine the influence of temperature on the stability of bilirubin, after the baseline measurement of 38 samples, each was aliquoted into eight tubes and four of them were stored at 3°C, while another four at 22°C, protected from light. Total bilirubin was analyzed after 2, 4, 8, and 24 h respectively. To study the impact of light exposure on the stability of bilirubin, additional 20 samples were analyzed similarly. However, all samples were exposed to artificial light for 2, 4, 8, 24, and 48 hours at 22°C, and then total and direct bilirubin were measured. The differences between the baselines and all subsequent measurements were evaluated using analysis of variance (ANOVA) with a *post hoc* Dunnett's test and $p < 0.05$ was considered statistically significant. The allowable total error for bilirubin was less than 20%.

Results: The average baseline total bilirubin concentration was 9.6 ± 8.1 mg/dL (mean ± SD) and the average concentrations after 2, 4, 8, and 24 h were 9.6 ± 8.2, 9.0 ± 7.4, 9.0 ± 7.5, and 8.8 ± 7.5 mg/dL at 3°C and 9.5 ± 8.1. 9.0 ± 7.4, 9.6 ± 8.1, and 9.5 ± 8.0 mg/dL at 22°C. There was no statistically significant difference between any of the study groups and the baseline ($p>0.05$, $n = 38$). In the second set of samples, the average baseline total and direct bilirubin concentrations were 10.2 ± 1.7 mg/dL and 5.0 ± 1.9 mg/dL, respectively. After 2, 4, 8, and 24 h light exposure at 22°C, the average total bilirubin concentrations were 10.1 ± 1.8, 10 ± 1.8, 10.0 ± 1.8, and 9.3 ± 2.0 mg/dL and direct bilirubin concentrations were 4.9 ± 1.8, 4.9 ± 1.9, 4.8 ± 1.8, and 4.2 ± 1.6 mg/dL. No statistically significant difference was found between any of the study groups and the baseline ($p > 0.05$, $n = 20$). As we extended the light exposure to 48 h, total and direct bilirubin concentration decreased to 8.4 ± 2.3 mg/dL ($p = 0.01$) and 3.5 ± 1.5 mg/dL ($p = 0.01$) compared to the baselines.

Conclusions: Bilirubin is relative stable in refrigerator and at room temperature for at least 24 h in a dark environment. Artificial light exposure can cause gradual decrease in both total and direct bilirubin, but the differences are neither statistically nor clinically significant for at least 24 h.

A-96

Effects of collection tubes and storage temperatures on measurement of L-arginine, symmetric dimethylarginine and asymmetric dimethylarginine by an LC-MS/MS method

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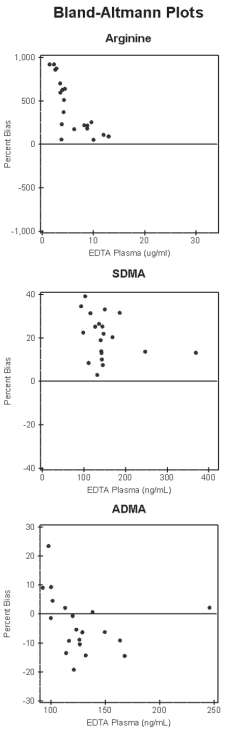
Background: Symmetric dimethylarginine (SDMA) has been identified as a biomarker for renal insufficiency, while asymmetric dimethylarginine (ADMA) has emerged as a promising biomarker of endothelial dysfunction in cardiovascular disease. EDTA plasma has been the preferred specimen type while serum separator tubes are frequently used in clinical labs. Objective To compare serum separator tubes (SST) and EDTA plasma tubes for the measurement of L-arginine, SDMA and ADMA, and to determine the stability of these analytes at different temperatures.

Methods: For the tube-type comparison, left-over SST and EDTA tubes for the same patient (n=20) were extracted and measured using an internally developed and validated liquid chromatography tandem mass spectrometry method (LC-MS/MS). For the stability study, left-over EDTA specimens (n=10) were obtained and an aliquot of each tube was frozen at -80°C. Then five tubes were stored at 2-8°C, while the remaining five tubes were stored at room temperature (RT) and sample aliquots were removed and frozen at -80°C after 2h, 6h, and 96h. All samples were thawed and analyzed in a single batch using the LC-MS/MS assay.

Results: Comparing with EDTA tubes, results from SST tubes had insignificant difference for ADMA, but significantly higher values for L-arginine and SDMA. In addition, L-arginine correlated poorly (r=0.2180), while SDMA had a good correlation (r=0.9816) between these tube types. SDMA was stable for 96h at RT and 2-8°C, while L-arginine was only stable for 6h at both temperatures and ADMA was stable for 6h at RT, and 96h at 2-8°C.

Conclusion: SST and EDTA tubes can be used interchangeably for measuring ADMA, but not for L-arginine or SDMA. In EDTA plasma, SDMA is stable under tested conditions, while L-arginine and ADMA are both stable for 6h at RT, and 6h and 96h at 2-8°C, respectively.



A-97

Effects of variant hemoglobin traits on measurements of HbA_{1c} by 3 cation exchange methods

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Background: Hemoglobin A_{1c} (HbA_{1c}) is the most important marker for long-term assessment of the glycemic state in patients with diabetes mellitus and is directly related to the risk of diabetes complications. An international expert committee has officially endorsed the use of Hb A_{1c} to diagnose diabetes. The recommended cutoff for diagnosis and goals for therapy are set at specific HbA_{1c} target values. It is essential that routine HbA1c methods provide comparable results. The accuracy of HbA_{1c} methods can be adversely affected by the presence of hemoglobin (Hb) variants. Here we investigated the accuracy of HbA_{1c} measurements in the presence of HbC, HbD, HbE and HbS traits.

Methods: Whole blood samples were collected in EDTA tubes for HbA_{1c} analysis. Hb variants were identified by inspection of chromatograms obtained with a Bio-Rad Variant analyzer. Hb A_{1c} analysis was performed using 3 different ion-exchange HPLC based methods (Bio-Rad Variant II Turbo 2.0, Tosoh G7 Variant Mode and Tosoh G8 Variant Mode). The Primus boronate affinity HPLC method (Primus ultra[2]) was used as the comparison method since it has previously been shown to be unaffected by the presence of most Hb variants. An overall test of coincidence of 2 least-squares linear regression lines was performed using SAS software (SAS Institute) to determine whether the presence of Hb variant traits caused a statistically significant difference ($P < 0.01$) in results relative to the comparison method. Deming regression analysis was performed to determine whether the presence of these variant traits produced a clinically significant effect on HbA_{1c} result. After correcting for calibration bias by comparing results from the homozygous HbA group, method bias attributable to the presence of Hb variants was evaluated with the use of ± 7% relative bias at 6% and 9% HbA_{1c} as evaluation limits (ie, 0.42% at 6% and 0.63% at 9% HbA_{1c}). The use of a 7% limit for bias is consistent with CAP proficiency testing grading limits projected for 2011.

Results: We observed statistically and clinically significant differences attributable to the presence of HbE trait for both G7 and G8 methods (large negative biases) but not for the Variant II Turbo 2.0. Samples containing HbS trait showed negative biases of 8% at 9% HbA_{1c} for both the Tosoh G7 and G8 methods. There were no clinically

significant differences attributable to the presence of HbD trait for any of the three methods but there was statistically significant difference attributable to the presence of HbD trait for the Tosoh G7 and G8 methods. Although there was a bias of ~8% with HbC trait at 6% on the G8, the difference was only borderline statistically significant (p=0.0540) possibly due to the low number of samples with HbC trait.

Conclusion: Some current HbA_{1c} methods show clinically significant interferences with samples containing HbE and HbS trait. Laboratories should be aware of the limitations of their methods with respect to these interferences. Ongoing efforts should be directed towards further improving the accuracy of HbA_{1c} measurements for samples containing Hb variants.

A-98

Quantitation of Creatinine in Various Peritoneal Dialysate Solutions

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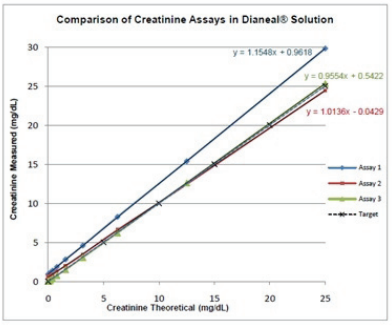
Background: Glucose at high concentrations is known to interfere with the Jaffé creatinine assay. Peritoneal dialysis (PD) solutions utilize high concentrations of glucose as the osmotic agent. Therefore, a creatinine assay unaffected by or that can be corrected for glucose interference is required.

Methods: Accuracy and linearity were evaluated in three different PD solutions (Extraneal with 7.5 % icodextrin, Dianeal with 4.25% dextrose, and Physioneal with 3.86% dextrose) spiked with creatinine at levels of 0.01 to 25 mg/dL. Quantitation of creatinine was performed using the Olympus AU400e Chemistry Analyzer as follows: Assay 1 - Kinetic Modification of the Jaffé Method, Beckman Coulter (formerly Olympus Diagnostics); Assay 2 - Jaffé Method, Roche Diagnostics; Assay 3 - Enzymatic Creatinine, Randox Laboratories, Inc. The selected assay was then validated for precision (repeatability and intermediate), accuracy, linearity, specificity, analytical range, LLOQ, and LOD.

Results: Comparison of the three assays resulted in the selection of the Enzymatic Creatinine assay. An example of results shown in the graph below compares the three assays in the Dianeal PD Solution. While all assays are linear, the enzymatic assay had the best accuracy. The enzymatic assay was validated for use in each of the three PD solutions. Precision (repeatability) was acceptable (CV ≤ 4.0% for values > 0.50 mg/dL). Intermediate precision percent recoveries ranged from 86.4% to 114.5% for values > 1 mg/dL and from 86.2% to 107.6% for values ≤ 1 mg/dL. The assay is accurate at concentrations ranging from 0.20 - 25 mg/dL and linear from 0.00 - 25 mg/dL. The LLOQ is 0.20 mg/dL and the LOD is 0.10 mg/dL. The assay is specific for creatinine in the dialysate solutions evaluated.

Conclusion: The Randox Enzymatic Creatinine Assay is the method of choice for the quantitation of creatinine in peritoneal dialysate solutions with high concentrations of dextrose or icodextrin.



A-99

The Difference In The Interference Of Fetal Hemoglobin Between Cation-Exchange Hplc And Turbidimetric Inhibition Immunoassay For Hba1c Testing

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Background: Elevated fetal hemoglobin (HbF) can be found in patients with various conditions such as hereditary persistence of fetal hemoglobin, β-thalassemia, δβ-thalassemia, and many other diseases. Studies have shown that significantly increased HbF interferes with certain HbA_{1c} assays, but the degrees of interference vary in different methods. The objective of this study is to determine if there is a bias in HbA_{1c} values in patients with elevated HbF between a cation-exchange (CE)-HPLC and an immunoassay and if the bias correlates with HbF and HbA_{1c} levels.

Methods: Thirty-four EDTA whole blood samples with elevated HbF were tested with Bio-Rad Variant II TURBO Link CE-HPLC and Siemens Dimension turbidimetric inhibition immunoassay (TINIA) HbA_{1c} methods. HbF was quantified by Bio-Rad Classic Variant CE-HPLC with Beta-thalassemia Short Program. The differences in HbA_{1c} results between these two methods were compared using paired, two-tailed Student's t-Test and the differences were considered statistically significant if the *p* values were less than 0.05. Linear regression analysis was used to determine the association between the inter-method HbA_{1c} bias and HbF as well as HbA_{1c} levels.

Results: The HbF levels in these 34 samples ranged from 7.8% to 35.7% with an average of 23.5% ± 7.3% (mean ± SD). The average HbA_{1c} values with CE-HPLC and TINIA were 7.4% ± 2.2% and 6.4% ± 1.9% (mean ± SD) with an average bias of 1.0% (p = 0.0005). Linear regression analysis showed a proportional relationship between the bias of HbA1c and the levels of HbF: y (HbA_{1c} bias) = 0.06x (HbF) - 0.36 (R = 0.3, SEE = 1.4). Linear regression analysis also showed a proportional relationship between the %bias of HbA_{1c} and HbA_{1c} levels with CE-HPLC: y (%Bias) = 3.5x (HbA_{1c}, CE-HPLC) -14.8 (R = 0.5, SEE = 15.3).

Conclusions: There is a significant bias in HbA_{1c} levels between Bio-Rad Variant II TURBO Link CE-HPLC and Siemens Dimension TINIA HbA_{1c} assays in patients with elevated HbF. The bias correlates with HbF values and the %bias correlates with HbA_{1c} values at small (R = 0.3) and medium (R = 0.5) correlation levels, respectively.

A-100

Does blood sample transportation affect the quality of laboratory results?

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Background: Financial constraints impose progressive consolidation of clinical laboratory facilities; many big laboratories are meeting their territorial diagnostic needs by a network of blood collection points connected to the main facilities through a route transportation system. Both poor quality storage and/or transportation are known causes of preanalytical variation, particularly inadequate temperature (T) conditions. This work aims to evaluate the influence of T storage conditions on thyroid and fertility hormone blood tests.

Methods: The collection of blood samples was performed on 17 healthy adults in a far laboratory facility, following standard procedures and with the aid of a tissue transilluminator device in order to avoid venous stasis. Blood was collected into two identical sets of 5 mL SST II Advance® vacuum tubes with clot activator and acrylic gel separator (BD Vacuntainer®). All tubes and needles were of the same lot. After allowing complete blood clotting at room T, the tubes were centrifuged at 2000g for 10 min. Then one tube set was placed in a T controlled refrigerator (+5°C) in the laboratory. The second set was introduced in a transport box (Coleman®) where low T was secured by four pre-cooled ice reusable dry gel packs with carbopol gel inside (2 x 200mL and 2 x 500mL). A calibrated T recorder (Trix-8 Temperature Recorder®, LogTag recorders, Adarve®, Brazil) was inserted in order to verify T course during transportation. T registration was done every 5 min during 8 hours. The box was carried by car from the above far laboratory facility to our laboratory together with other sample containing boxes. T data were analyzed with LogTag Analyser®. At the end of 8 hours (transportation time), all thyroid and fertility hormones tests were performed in the same instrument Roche/Hitachi Modular Analytics E 170® (Roche Diagnostics, Brazil), calibrated according to the manufacturer's specifications and using proprietary reagents. Test panel included: free T4 (FT4), thyroid-stimulating hormone (TSH), follicle-stimulating hormone (FSH) and luteinizing hormone (LH). The significance of the differences between samples (considering as reference the values of the samples stored in the far laboratory refrigerator), was assessed by paired Student's t-test after checking for normality. Not-normally distributed FSH and LH results were assessed by Wilcoxon ranked-pairs test. Statistical significance for both tests was set at $P < 0.05$.

Results: Significant differences were observed for TSH, FSH and LH. Records from transport box interior showed T max. 10.8°C / 51.4°F and min. 6.3°C / 43.3°F. The external T range during transportation was: 23.5°C / 74.3°F to 38°C / 100.4°F. T of laboratory refrigerator ranged from 5.0°C / 41.0°F to 5.8°C / 42.4°F during 8 hours.

Conclusion: The differences observed among results are likely due to influence of external T conditions during transportation. More attention should be given to this pre-analytical aspect, by improving transportation means and conditions, in order to provide clinicians with reliable laboratory data.

A-101

Investigation of hemolysis impact on plasma ammonia measurement

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Background: Hemolysis is the most common interference to clinical chemistry tests. Methods of evaluating the impact of hemolysis are by spiking hemolysate into plasma/serum or by aspirating blood samples through a needle. These two methods have been shown to give significantly different conclusions on the hemolysis impact on ammonium assay.

Objective: To investigate the hemolysis impact on measured plasma ammonia levels using either hemolysate or syringe method. **Methods:** Hemolysate was prepared from EDTA whole blood (5mL) by an hypotonic lysis with water and an overnight freeze (-20°C). After serial dilution with saline, the resulting hemolysate solutions were spiked into EDTA plasma aliquots. The specimens were measured for hemolysis index (HI) and ammonia. The syringe method involved aspirating EDTA whole blood through a needle in increasing cycles in different aliquots. The samples were centrifuged and the supernatant analyzed for HI and ammonia. To determine if hemolysate aging resulted in ammonia production, the hemolysate was split into two samples before freezing. One sample was serially diluted, spiked into plasma samples, then analyzed, while the second sample was frozen overnight then thawed, serially diluted, spiked into plasma samples and analyzed.

Results: Using the hemolysate method, the measured ammonia concentrations at 29 micromol/L and 53 micromol/L showed >10% change with HI of 27.6 and 116.5, respectively. Using the syringe method, the measured ammonia at 34 micromol/L and 59 micromol/L had >10% change with HI of 219.7 and 814.2, respectively. Further investigation demonstrated that the unfrozen hemolysate sample gave similar results to the syringe method, while the overnight frozen sample gave results in agreement with the hemolysate method (Figure 1).

Conclusions: Plasma ammonia measurement was not significantly interfered by hemolysis at HI of 220 or below in fresh plasma samples. Overnight freezing of whole EDTA blood during hemolysate preparation produced significant amounts of ammonia.

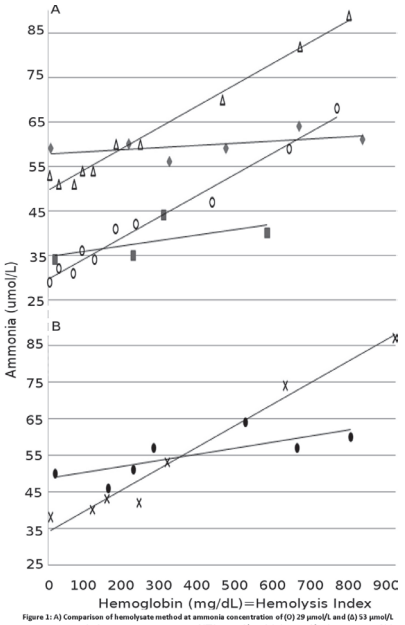

Figure 1: A) Comparison of hemolysate method at ammonia concentration of (○) 29 µmol/L and (△) 53 µmol/L versus syringe method at ammonia concentration of (■) 34 µmol/L and (◆) 59 µmol/L. B) Comparison of (X) frozen hemolysate versus (●) unfrozen hemolysate in affecting ammonia measurement.

A-102

Is Acidification of Calcium Urine A Necessary Preanalytical Procedure?

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Background: Acidification of urine prevents cations such as calcium from precipitating as salt, hence urinary calcium measurement involves preanalytical acidification. Safety issue has always been a concern when handling acid by our laboratory staff and patients. In this study, we determined the effect of acidification on calcium measurement.

Materials and Methods: Random spot urinary specimens from 47 patients were collected in bottles without acid. These samples were split into two 1 ml aliquots, with 1 aliquot acidified with 6mol/L hydrochloric acid (10-15 µls) to pH <2 and equilibrated at room temperature for 1 hour. Both urine samples were analysed for calcium as baseline. After keeping for 24 hours at room temperature, the non acidified sample was split into two aliquots, with one aliquot acidified. Both urine aliquots (non acidified and acidified) as well as baseline acidified aliquot were analysed for calcium.

The urinary calcium were assayed using Arsenazo III endpoint assay on Siemens Advia 2400 Chemistry system.

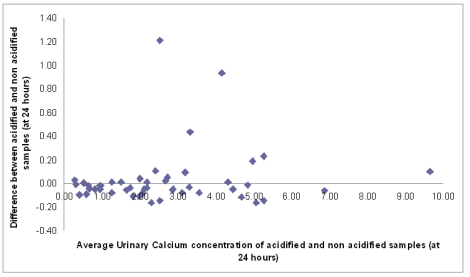
Results: The tested urinary calcium ranged 0.31mmol/L-9.5mmol/L, with median of 2.3mmol/L.

Our study shows that there was no statistical difference in urinary calcium concentration between control and acidified samples, whether acidification was done at baseline 0 hour or after 24 hours (p = 1.0).

Using our assay analytical imprecision of < 5%, 70.7% of the samples had <5% difference in calcium concentration between acidified and non acidified aliquots. Using biological within-subject variation of 27.5% as quoted by Dr Ricos and colleagues, 97.5% of the samples had < 27.5% difference.

There was no significant difference in urinary calcium concentration in control or acidified samples after 24 hours of storage.

Conclusions: Our study showed that preanalytical acidification of urine for calcium measurement is not necessary for calcium measurement on both random and 24 hour urinary specimens.



A-103

Assesment And Comparison Of Hepatitis C Virus (Hcv) Viral Loads Assays In Patients With Genotypes 2, 3, & 4 In An At Risk Population

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Background: Hepatitis C Virus (HCV) infects 170 million people worldwide and is the leading cause of cirrhosis and liver cancer in the United States. Studies have demonstrated the efficacy of using the viral load to monitor and guide current standard of care (PEG Interferon and Ribavirin) therapy. Although the Roche instrument provides the only currently FDA approved assay for the quantification of viral RNA, studies have shown that it significantly underperforms in genotypes 2 and 4. This claim has special significance in Montefiore due to its genetically diverse immigrant population, which lends to an increased prevalence of genotype 4 (2% vs. 0.3% in NY State).

Methods: To assess and compare two methodologies of Hepatitis C viral load testing in the highly diverse population serviced by Montefiore Medical Center. A retrospective analysis of Montefiore's databases was conducted to evaluate its Hepatitis C patient population. 143 HCV positive patient samples were collected (62 genotype 2, 52 genotype 3, and 29 genotype 4) and tested simultaneously with both assays. A commercial panel (Acrometrix) of genotypes 1, 2, 3, and 4 was used to confirm patient results. Lastly, two lots of 2.5 log IU/ml and 5 log IU/ml (Abbott laboratory) were used to evaluate the precision of five samples and reproducibility of three runs spread across one week on each assay.

Results: Overall, the results from the two assays in this study demonstrated a positive bias between the Roche Taqman HCV assay and the Abbott RealTime HCV assay of 0.2 log IU/mL (p<.001). These findings are in keeping with published literature. It is interesting to note that bias observed with genotype 2 and 3 patient samples (0.34 log IU/mL and 0.12 log IU/mL respectively) differed from the bias observed with genotype 4 patient samples (0.03 log IU/mL). This bias was confirmed in the commercial panels (0.53, 0.31, 0.59, and .69 log IU/ml for genotypes 1, 2, 3 and 4

respectively). There was a high correlation on the patient samples between the two systems, with few outliers in the Bland Altman plot, and an R^2 value of 0.98. It was also found that the Abbott assay had greater precision and reproducibility.

Conclusion:: Overall there appears to be great concordance between the Abbott RealTime HCV and Roche Taqman HCV assays in the Montefiore patient population. While some statistically significant differences were observed between the two assays they were not clinically significant. This data would imply that patients on therapy could be monitored with either assay described here. This allows for clinical labs to move from one platform to another without worrying about an impact on patient care and for patients to move locations without worrying about finding a laboratory with the same instrument. The differences in the bias observed with the Roche Taqman HCV assay in genotypes 2 and 3 patients, versus genotype 4 patients are noteworthy and, while, not as pronounced, may support the findings in published literature that the Roche Taqman HCV assay values are lower for genotype 4 than for other genotypes.

A-104

Muscle damage and inflammatory biomarkers reference intervals from physically active population

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Background: Physical exercise has been associated with muscular damage and acute phase inflammatory response characterized by free radical production, elevation of cytokines and other inflammatory proteins. Biochemical reference intervals (RI) for physically active population are lacking. The aim of this study was to establish RI for muscle damage and inflammatory biomarkers from physically active population.

Methods: The reference population included male volunteers (n=146), with an average age of 18 ± 1 years. They participated for four months in a regular and strictly controlled exercise program which consisted predominantly of aerobic activities for three hours daily (five days per week, with two days of rest). Eight mL of blood were collected in tubes with separator gel to obtain serum for biochemical analysis (Vaccuete® Greiner Bio-One). Samples were collected under standardized conditions, after 12 h of fasting, in the morning. The samples were centrifuged at 1.800 x g for 15 minutes under refrigeration (4ºC). The creatine kinase (CK), CK-MB activity, myoglobin, high sensitivity C-reactive protein (h-CRP), and complement C3 concentrations were analyzed in the same day with Randox reagents and calibrators in an RX Daytona™ analyzer. The Randox internal quality control was performed in parallel with the tests. The RI were established in according with International Federation of Clinical Chemistry rules using RefVal program 4.1 beta. The outliers were removed by Horn's algorithm. We calculated the non-parametric 2.5th and 97.5th percentiles, with their 90% confidence intervals (CI), by Bootstrap methodology.

Results: The muscle damage biomarkers and h-CRP (Table 1) showed upper limits higher values when compared to traditional RI from a healthy non-exercised population.

Table 1. Reference intervals and confidence intervals for muscle damage and inflammatory biomarkers in physically active population

Analyte (Method)	Coefficient of analytical variation (CV_A)	Quality specification based on biology	Reference interval	CI 2.5th	CI 97.5th
Myoglobin (ng/mL) (Immunoturbidimetric)	2.0%	7.0%	56 - 133	54 - 58	126 - 162
CK-NAC (U/L) (DGKC)	1.7%	5.7%	123 - 1032	93 - 140	894 - 1166
CK-MB (U/L) (DGKC)	3.8%	4.9%	8 - 29	7 - 9	27- 31
h-CRP (mg/L) (Immunoturbidimetric)	3.2%	13.1%	0.3 – 15.0	0.3 - 0.3	7 – 30.2
Complement C3 (g/L) (Immunoturbidimetric)	3.3%	3.9%	0.93 – 1.52	0.91 – 0.94	1.44 – 1.55

Conclusion: In the practice of sports medicine it is common to use blood biomarkers to prevent muscular damage and to adjust training/rest periods. However, it is crucial to establish specific RI in a trained population to monitor the training stress.

A-105

Eosinophiluria is common among patients after ileal conduit surgery

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Background: Acute interstitial nephritis (AIN), most often associated with prior exposure to specific drugs, is important to recognize since the condition often resolves with the cessation of the offending agent. The most frequent laboratory test used to screen for AIN is a quantitative measure of urinary eosinophils. Since technicians in our lab observed that urine samples obtained from patients with urinary tract reconstructive surgeries appeared to have an unusually high number of eosinophils which could confound use of this test, the current study was performed to systematically examine the eosinophiluria among these patients.

Methods: Waste urine samples were identified from three separate cohorts of patients for this study (n=20 each): consecutive urine samples with the source listed as stoma indicating previous ileal conduit surgery (Stoma cohort); consecutive urine samples clinically submitted for eosinophil quantification (Non-stoma cohort); and randomly selected urine samples with a minimum of 1-3 white cells per high-powered field (Control cohort). Urinary eosinophils were identified using the Hansel stain as per the standard lab protocol, slides were blinded, and the mean of independent readings by 4 separate technologists was used to quantify the percentage of eosinophils.

Results: Eosinophils were commonly present in the urine of patients with ileal conduit surgery, composing an average 19% (range 0.25 - 63%) of the urinary white cells, despite the absence of any clinical suspicion of AIN. By comparison, among patients with clinically ordered eosinophiluria testing only 3.5% (range 0 - 37%) of urinary white cells were eosinophils, and in a random sample of patients with pyuria 4.6% (range 0 - 23%) of white cells were eosinophils. Importantly, 18/20 of the stoma patients had >5% eosinophils, meeting criteria for a positive test.

Conclusions: Patients with previous ileal conduit surgery have markedly elevated levels of eosinophils in their urine that far exceeds a control group of patients with pyuria, and the third group of patients with clinically ordered eosinophiluria testing. These results suggest that urinary testing for eosinophils is not a useful screen for AIN among patients with previous ileal conduit surgery.

A-107

Inflammatory marker stability in Li-Heparin plasma under various storage conditions

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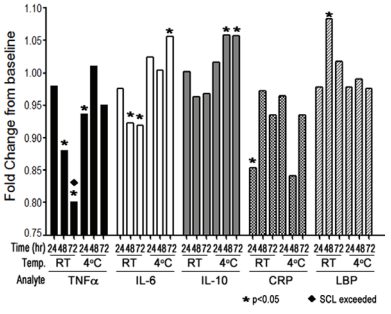
Introduction: Cytokines like tumor necrosis factor alpha (TNFα), and interleukins (IL)-6 and -10 along with inflammatory markers, such as C-reactive protein (CRP), and lipopolysaccharide-binding protein (LBP), have growing clinical and diagnostic utility for patients in a variety of disease states. However, the stability of these analytes for routine storage is largely unknown.

Objective: To determine the stability of TNFα, IL-6, IL-10, CRP, and LBP in human plasma stored under various common laboratory conditions.

Methods: Immediately following routine testing and within 3 hours of initial collection, 23 left-over Li-heparin plasma samples from 14 hospitalized patients were collected and frozen at -80ºC. The samples were combined into a sample pool, and aliquoted into single-use tubes for testing the following conditions in triplicate: storage at 4ºC or room temperature (RT) for 0, 6, 12, 24, 48, and 72 hours. A separate patient pool was subjected to between one and three freeze-thaw cycles. Following these treatments, the concentrations of IL-6, IL-10, TNFα, CRP, and LBP were measured for each sample, using the Siemens Immulite 1000 analyzer. Statistical change limit (SCL) was calculated as 2.8 times the standard deviation, and p-values were determined by Student's t-test.

Results: Results are summarized in the bar chart. Notably, only TNFα fell outside the SCL after 72 hr at RT. Though not shown, samples stored at 4ºC or RT for up to 24hr did not change outside the SCL from baseline. Additionally, none of the analytes demonstrated a change which exceeded the SCL with up to three freeze-thaw cycles.

Conclusions: TNFα, IL-6, IL-10, CRP, and LBP are stable in Li-Heparin plasma for up to 72 hours at 4ºC, and all but TNFα, which fell below the SCL before 72 hours, are stable at RT for 3 days. All analyte concentrations were similar after multiple freeze-thaws.



A-108

Standardization of a cystatin C particle-enhanced turbidimetric assay (PETIA) and comparison to nephelometric assay Results

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Background: Cystatin C is an alternative blood-borne biomarker used to assess glomerular filtration rate (GFR). Potential advantages over creatinine include less dependence on gender, age and muscle mass. Lack of standardization between cystatin C assays has been one barrier to more widespread use. In this study, we compared a new cystatin C particle-enhanced turbidimetric assay (PETIA) available from Gentian AS to the more commonly used particle-enhanced nephelometric assay (PENIA) available from Siemens. Standards for the PETIA, but not the PENAI, are traceable to the new ERM-DA471/IFCC international reference material.

Methods: Three different patient cohorts at the Mayo Clinic were studied. (1) Clinical convenience samples (n=100) were used to compare analytic performance between the PETIA performed on a Cobas c501 analyzer and PENIA performed on a BNII nephelometer. (2) Samples from 105 patients undergoing iothalamate urinary clearance testing (direct glomerular filtration rate measurement) were used to validate existing equations that estimate GFR using cystatin C. (3) Banked samples (stored at -70ºC since 2000, n=67) were re-analyzed to compare PENIA cystatin C values obtained in 2010 to PENIA results obtained before freezing in 2000.

Results: The PETIA cystatin C assay was linear between 0.33 and 5.97 mg/L with a lower limit of quantification of 0.35 mg/L and corresponding CVs < 2 %. PETIA cystatin C results were consistently 25-28% higher across all levels than those obtained with the PENIA assay. Further, cystatin C levels using the PENIA were 19% higher when reanalyzed in 2010 compared to when they were initially bio-banked in 2000. While an equation developed using the PETIA-determined cystatin C values estimated GFR comparably to existing creatinine-based equations, a cystatin C equation developed using PENIA results from 2000 performed relatively poorly, unless the current PENIA results were corrected for this apparent 19% assay drift.

Conclusion: Cystatin C can be precisely measured in blood using PETIA and a routine chemistry autoanalyzer. Results can be used with previously developed equations then used to accurately estimate GFR. There appears to have been significant drift of the widely-used PENIA over the past decade that produces bias when GFR is estimated using these cystatin C values. Therefore, efforts to standardize cystatin C assays are important if this analyte is to be routinely used for GFR estimation.

A-109

Effect of Hemolysis on the Cardiac Troponin I and Creatinine Assays on the Siemens Dimension Vista® Analyzer

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Background: Cardiac troponin (cTn) and creatinine concentrations are critical to support the emergency department in the diagnosis of acute myocardial infarction and management of stroke. Pre-analytical conditions that affect the accuracy of results jeopardize patient care. Investigators have reported that as high as 8.8% of the specimens collected in the emergency department are hemolyzed and that hemolysis can cause a negative interference in cTn concentration, but no current studies on the impact of hemolysis on cTn I or creatinine concentration on the Siemens Dimension Vista® using lysed erythrocytes (hemolysate) have been published. Here, we evaluate the effect of hemolysis on the Siemens Dimension Vista cTnI and creatinine assays. Hemolysis effect on the Beckman Synchron DxC® creatinine assay was also studied.

Methods: Discarded serum specimens were used to create low, borderline and high pools for cTnI and creatinine. Target average concentration for the low, medium and high pools were < 0.04 ng/mL, 0.04-0.20 ng/mL, and >0.2 ng/mL for cTnI, and 0.3-0.8 mg/dL, 1.2-1.5 mg/dL, and 4.5-5.5 mg/dL for creatinine. The cTnI medium pool and the creatinine low pool were intentionally created to overlap the clinical decision limits for ruling out myocardial infarction or examine renal function in stroke patients. A hemolysate was obtained by treating an ethylenediaminetetraacetic acid plasma specimen to one freeze-thaw cycle. Increasing volumes of the hemolysate (1, 5, 10, 20, and 50 uL) were added to each of five aliquots of each pool. The final Hb concentration was estimated based on the hemolysis index (range 5 - 600 mg/dL; Hb index 4 = 100-200 mg/dL). cTnI and creatinine concentrations were measured in triplicate. Siemens Dimension Vista cTnI assay is a homogeneous luminescent oxygen channeling immunoassay. The analytical measurable range (AMR) is 0.015-40 ng/ml. The Siemens Dimension Vista and the Beckman Synchron DxC creatinine assays use the modified kinetic Jaffe reaction to measure a red chromophore at 510-520 nm. The AMR for the Siemens Dimension Vista and the Beckman Synchron DxC are 0.2-20 mg/dL and 0.1-25 mg/dL respectively. The average concentration for each aliquot was subtracted from the baseline (no added hemolysate). Recovery was calculated as a percentage of the baseline concentration. Recovery of 100±5% and 100±10% was considered acceptable for cTnI and creatinine respectively.

Results: Moderate hemolysis produces a negative interference in the cTnI and creatinine assays in a concentration-dependent manner. For the Siemens Dimension Vista, % recovery for cTnI for the medium pool was <95% at Hb concentrations > 100 mg/dL, and <90% at Hb >150 mg/dL. Recovery for creatinine for the low and medium pools was < 90% at Hb concentrations > 200 mg/dL. The Beckman creatinine % recovery was acceptable for all the pools in the presence of up to 600 mg/dL of Hb.

Conclusions: We have demonstrated that hemolysis may compromise the diagnosis of acute myocardial infarction and the management of stroke. Moderate hemolysis decreased cTnI and creatinine concentrations at the medical decision limits. Clinicians should be cautioned on the interpretation of cTnI and creatinine concentrations in specimens presenting moderate hemolysis.

A-110

Effect of Hemolysis on Bilirubin Measurements Using the Siemens Advia 2400, Beckman DxC and Siemens Dimension

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Background: In response to concerns raised by our pediatricians, we undertook this study to determine the impact of hemolysis on bilirubin measurement. Bilirubin was measured by either vanadate oxidation (Siemens Advia 2400) or diazo (Beckman DxC, Siemens Dimension) methods. Manufacturers indicated maximum hemoglobin (Hb) levels tested in interference studies were 100-1000 mg/dL for total bilirubin (Tbili) and 100-750 mg/dL for direct bilirubin (Dbili).

Methods: Using a stock hemolysate (Hb of 19,300 mg/dL by Sysmex HE3), nine working solutions made by 1:2 serial dilutions with saline were spiked (1:4 by volume) into each of eight bilirubin pools (final Hb levels: 15-3,900 mg/dL). Bilirubin pools diluted with saline instead of hemolysate were designated blanks (final concentrations: Tbili, <0.1-25.3 mg/dL; Dbili, <0.1-16.4 mg/dL). Each bilirubin pool or blank was divided into three aliquots, tested in duplicate and results averaged. Interference was considered significant if bilirubin values changed by $\geq \pm 10\%$ of the bilirubin blank.

Results: Tbili: Advia: Significant negative biases were observed at the lowest Tbili levels only (14-97% at Tbili 0.5 mg/dL with Hb 122-3,900 mg/dL; 15-32% at Tbili 1.7 mg/dL with Hb 1,950-3,900 mg/dL; 12% at Tbili 4.9 mg/dL with Hb 3,900 mg/dL). DxC: Positive biases were observed at most Tbili levels (17-2333% at Tbili 0.5 mg/dL with Hb 15-3,900 mg/dL; 10-687% at Tbili 2.0 mg/dL with Hb 244-3,900 mg/dL; 11-242% at Tbili 5.3-7.6 mg/dL with Hb 488-3,900 mg/dL; 11-100% at Tbili levels 12.5-19.8 mg/dL with Hb 975-3,900 mg/dL). Dimension: Negative biases were observed at lower Tbili levels (>60% at Tbili 4.8-7.0 mg/dL with Hb 1,950-3,900 mg/dL; 74% at Tbili 11.6 mg/dL with Hb 3,900 mg/dL); positive biases were observed at higher Tbili levels (10-20% at Tbili 14.9 mg/dL with Hb 975-3,900 mg/dL; 13-18% at Tbili 18.8 mg/dL with Hb 1,950-3,900 mg/dL).

Dbili: Advia: Negative biases were observed at all Dbili levels, mostly beginning at Hb >488 mg/dL. DxC: Positive biases were observed at lower Dbili levels (25-550% at Dbili 0.2 mg/dL with Hb of 61-3,900 mg/dL; 13-88% at Dbili 0.8 mg/dL with Hb 244-1,950 mg/dL). Negative biases were observed at all other Dbili levels mostly beginning at Hb >244 mg/dL. The DxC suppressed several results at the highest bilirubin and hemolysis levels. Dimension: Negative biases were observed at most Dbili levels (19-88% at Dbili 1.0 mg/dL with Hb 30-975 mg/dL, results suppressed with Hb >975 mg/dL; 11-97% at Dbili 3.3-6.5 mg/dL with Hb 61-3,900 mg/dL; 11-82% at Dbili 9.2-14.0 mg/dL with Hb 122-3,900 mg/dL).

Conclusions: The Bhutani nomogram categorizes kernicterus risk based on hour-of-life Tbili levels. With the Advia, increasing hemolysis had minimal clinically relevant impact on Tbili. Using the DxC, positive interference with increasing hemolysis could falsely place newborns into higher risk zones, especially at lower Tbili levels. With increasing hemolysis on the Dimension, negative interferences at intermediate Tbili levels and positive interference at high Tbili levels could place newborns into inappropriate risk zones. On all platforms, hemolysis interference on Dbili could result in incorrect assumptions regarding the newborn's ability to conjugate bilirubin.

A-111

Comparison of the BD Vacutainer Rapid Serum Tube with BD Hemogard Closure with the BD Vacutainer SST Tube for Routine Chemistry Analytes on Two Instrument Platforms

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Objective: The clinical performance of the BD Vacutainer® Rapid Serum Blood Collection Tube with BD Hemogard™ Closure (BD RST) was evaluated in comparison with the BD Vacutainer® SST™ Blood Collection Tube (BD SST™) on two instrument platforms. Testing was performed for 19 routine chemistry analytes on the Roche Modular in one study and for 16 routine chemistry analytes on the Siemens Dimension® RxL in a second study.

Background: The BD RST is a new blood collection tube that contains a thrombin-based clot activator and a gel barrier. The thrombin promotes rapid clotting of the blood, allowing centrifugation five minutes after collection and providing a serum specimen that can be sampled directly from the primary tube. The reduced clot time of the BD RST can decrease sample processing time and enhance laboratory efficiency.

Methods: Fresh blood specimens were collected from adult subjects with a range of diagnoses and disease states by routine phlebotomy or in-place venous catheter/line draw into BD RST (evaluation) and BD SST (control) tubes. After clotting, the tubes were centrifuged to provide separated serum specimens. Serum was tested for the routine chemistry analytes within two hours after centrifugation. The data were analyzed to determine mean biases (confidence limits) of results from the BD RST compared to the control tube for each analyte. The maximum allowable difference (bias) in test results was predetermined for each analyte. Deming regression was also performed.

Results: All routine chemistry analyte results were determined to be within the maximum allowable clinical difference criteria at all medical decision levels, except calcium (Ca) and gamma-glutamyltransferase (GGT) on the Siemens Dimension RxL and carbon dioxide (CO2) on the Roche Modular. The mean systematic biases for Ca, GGT and CO2 were within the clinical criteria; however, the confidence limit overlapped the clinical criteria. Ca had a positive bias with a confidence limit that extended over 0.3 mg/dL at the critical value of 12.84 mg/dL; GGT had a negative bias with a confidence limit that extended over -10% at the lower end of the analyte reference range. CO2 demonstrated a positive bias with a confidence limit extending over 10% at the critical value of 11 mmol/L. After review of the data, these results were considered clinically acceptable.

Conclusions: Overall, clinically equivalent or clinically acceptable performance was demonstrated for the BD RST when compared with the BD SST for all chemistry analytes tested on each instrument. The reduction in the clot time of the BD RST may help laboratories to meet today's demands for more rapid reporting of test results and improved throughput.

A-112

Biological variation (as critical difference) of blood glucose as a tool to improve diabetic patient safety

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Background: Diabetes care requires continuous medical support and patient collaboration in order to prevent/reduce the risk of long-term complications. Over the past 13 years the diagnostic approach to diabetes has undergone many changes, as for the criteria based on blood glucose assay. Patient data are usually reported by laboratories by numerically expressing glucose concentration. It is well known that a laboratory result represents a point casually set within the confidence interval of a measure, according to the laboratory and personal sources of variation. The impact of biological variability in the monitoring of diabetic patients is seldom considered as a source of error by caring physicians. Aim of this work was to estimate the Reference Change Value (RCV) and check its impact on therapeutic management in type 2 diabetic patients followed by Public Health Care Program for Adult with Hypertension and/or Diabetes - a Project for Expansion and Consolidation of Family Health (PSF) in Natércia municipality of the Minas Gerais state - Brazil, where 100% of population is covered by assistance.

Methods: RCV and Critical Difference (CD) in 100 type 2 diabetic patients followed by PSF were calculated and the impact on therapeutic management was deduced. After 8 hours fasting blood 5 laboratory measurements were done for each patient in order to estimate RCV and CD(quarterly from January 2009 to April 2010), after subtracting the analytical variation (as %CV). We compared the measurement of glucose levels obtained at diagnosis (first determination) with each of the subsequent four as recommended by FRASER, and RCV and CD calculated. For CD significance, results > 38.4% ($p < 0.05$) were considered.

Results: 59 patients showed CD <38.4% as compared with the first determination. Of the 41 patients with CD > 38.4%, 18 had a prescription for change in the lifestyle and 23 had a prescription for both change in the lifestyle and oral hypoglycemic agents. Our results showed that 41 of the 100 diabetic patient followed could improve their metabolic control simply by providing CD to caring physicians together with interpretation means. Brazilian Society of Diabetes claims that poorly controlled diabetes rates are at 89.6% for type 1 disease and 73.2% for type 2.

Conclusion: The type 2 diabetes patients followed by PSF showed less poor controlled case than reported by Brazilian Society of Diabetes. We suggest that clinical and laboratory monitoring of diabetes can significantly improve when tools like RCV and CD are implemented in the routine procedures and caring physicians are able to interpret this information correctly.

A-115

Performance of phlebotomists in public and private laboratories in Korle Bu

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Background: Most mistakes in laboratory tests are the result of lack of standardization during pre analytical phase, particularly in patient identification, collection and sample processing. The aim was to evaluate the performance of 40 phlebotomists (20 from public and 20 from private laboratories) in the town of Korle bu in Ghana.

Methods: The activities of each phlebotomist were observed during the blood collection procedure and six parameters were evaluated. 1. Tourniquet application technique. 2. Tourniquet application time. 3. Request to the patient to clench the fist repeatedly. 4. Friction procedure of the forearm by the phlebotomist, during the cleaning of the venipunture site, to induce the venostasis.. 5. Correct sequence of vacuum tubes collection according to CLSI/NCCL H3 - A5 document. 6. Suitable blood mixing in tubes containing addictives or clot activators according to manufacturer.

Results: 15(75%) of the phlebotomists in the public laboratories had the tourniquet application technique right, while 18(90%) of the phlebotomists in the private laboratories had the application technique right. 6 (30%) of the phlebotomists in the public laboratories had the tourniquet application time within acceptable limits, while 8 (40%) of the phlebotomists in the private laboratories had the tourniquet application time within acceptable limits. 16 (80%) of the phlebotomists in the public laboratories asked for fist clenching repeatedly, while 20(100%) of the phlebotomists in the private laboratories asked for fist clenching repeatedly. 14 (70%) of the phlebotomists in the public laboratories induced venostasis, while 20 (100%) of the phlebotomists in the private laboratories induced venostasis. 20 (100%) of phlebotomists in the public laboratories did not used the sequence of vacuum tubes collection, while 16 (80%) of the phlebotomists in the private laboratories did not used the sequence of vacuum tubes collection. 20 (100%) of the of the phlebotomist in the public hospital did not perform a suitable blood mixing in tubes containing addictives or clot activators according to the manufacturers, while 14(70%) of phlebotomists in the private laboratories did not perform a suitable blood mixing in tubes containing addictives or clot activators according to the manufacturers.

Conclusion: The main causes of errors during blood collection in the laboratories evaluated were related to inefficient training programs. The performance of the phlebotomists was unsatisfactory when the CLSI/NCCLS documents were considered. The implementation of an efficient training programs to all phlebotomists evaluated probably will minimize pre analytical errors and cause improvements in all laboratory process.

A-116

Influence of five different manufactures kinds of K2 (or K3)-EDTA tubes on Erythrocyte Sedimentation Rate (ESR)

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Background: The erythrocyte sedimentation rate (ESR) is a nonspecific marker of disease and is often used by clinicians in assisting diagnosis and follow-up of a variety of infectious and inflammatory disorders. Nevertheless, no information is available on the influence of different Vacuum Tubes on ERS analysis. The aim of the present investigation is to compare ESR results obtained on blood specimens collected with five different types of K2 or K3 Ethylenediaminetetraacetic acid (EDTA) vacuum tubes.

Methods: Blood samples from 21 consecutive healthy volunteers were collected by a single and phlebotomist, by venipuncture with 20 G straight needles into five different EDTA-tubes: Tube I: 3.0mL Venosafe ® with 5.9 mg K2EDTA (Terumo Europe, Leuven, Belgium); Tube II: 3.0mL Vacutainer ® with 5.4mg K2EDTA (BD Vacuntainer, Becton Dickinson Diagnostics, Plymouth, United Kingdom); Tube III: 4mL Vacuette® K2EDTA (concentration of K2EDTA unavailable, Greiner Bio-One GmbH, Kremsmünster, Austria); Tube IV: 4.5mL Labor Import® (concentration of K3EDTA unavailable, Shandong Weigao Group Medical Polymer, Weihai, China); Tube V: 1.2mL S-Monovette® with 1.6mg K3EDTA (Sarstedt, Nümbrecht, Germany). The sequence of tubes was randomized but K2EDTA was always collected before than K3EDTA to eliminated possible contamination. All samples were assayed for ESR on the TEST 1 THL® (ALIFAX, Padova, Italy). Calibrations were performed according to the instructions provided by the manufacturer. Analytical imprecision, expressed as inter-assay coefficient of variation (CV) and calculated according to internal quality control is 0.8-2.2%. Data were analysed with the non-parametric Friedman test.

Results: Results of testing are expressed as geometric mean ± standard error of the mean (SEM), as follows: Tube I (9.8±2.2 mm/h), Tube II (8.7±2.4 mm/h), Tube III (7.9±2.3 mm/h), Tube IV (6.4±2.2 mm/h), Tube V (5.2±1.7 mm/h). A statistically significant difference was observed among tubes observed by Friedman test ($P < 0.001$).

Conclusions: The results of this investigation clearly attest that the preanalytical variability might also affect ESR testing, in that the type of the tube as well as the type and concentration of EDTA can dramatically influence test results, in some circumstances reaching clinical significance and approaching the critical difference of this parameter (3.1 mm/h) as established by Penev et al. Reference. Penev MN et al Study on long-term biological variability of erythrocyte sedimentation rate. Scand J Clin Lab Invest 1996;56:285-8.

A-118

Comparison of Three Immunoassays for Measurement of Anti double stranded DNA Levels

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Background: Following the discontinuation of the Enzyme Linked Immunosorbent Assay (ELISA) kit (The Binding Site) for Anti double stranded DNA (Anti ds DNA), an equivalent kit was sought.

Objective: This study purpose was to make qualitative and quantitative comparisons between The Binding Site ELISA with two other immunoassays: Inova ELISA and AtheNA ANA II Plus kit.

Methods: Thirty-one patient serum samples covering the analytical measurement range were tested for Anti ds DNA using The Binding Site ELISA kit, Inova ELISA kit, and AtheNA ANA II Plus kit. Acceptability criteria within the EP Evaluator [Release 8-Alternate (Quantitative) Method Comparison] were defined as: Slope within 1.00 ± 0.05, absolute value of the intercept is <5.00% of X mean, and correlation coefficient is ≥0.95.

Results: Quantitatively (reporting units IU/mL), neither the Inova ELISA kit nor the AtheNA kit were equivalent with The Binding Site ELISA. Data were also evaluated qualitatively for Anti ds DNA results for the following: sensitivity (Sens), specificity (Spec), positive predictive value (PPV), and negative predictive value (NPV). The AtheNA kit had an agreement at ≥85% in all four qualitative assessment categories with The Binding Site ELISA; whereas, the Inova ELISA yielded ≥85% agreement in only two categories (Spec and PPV). An additional study corroborated The Binding Site ELISA and the AtheNA ANA II Plus kit as qualitatively equivalent.

(n=31 samples)	The Binding Site ELISA vs.	
Assessment	AtheNA ANA II Plus	Inova ELISA
Sens	100%	70%
Spec	86%	100%
PPV	85%	100%
NPV	100%	33%

Conclusion: The discontinued The Binding Site manufactured ELISA kit for quantitative measurement of Anti ds DNA levels does not correlate with either the Inova ELISA or AtheNA ANA II Plus kit. However, the AtheNA kit correlated acceptably by four qualitative criteria (Sens, Spec, PPV, NPV) with The Binding Site ELISA for the determination of positive/negative status of Anti ds DNA levels.

A-119

Study On The Possible Interference Of In Vivo Carbamylated Haemoglobin On The Determination Of Hba1c In Healthy Individuals, Diabetic And/Or Uraemic Patients

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Background: carbamylated haemoglobin (carbHb) is the most common biochemical modification of haemoglobin (Hb), appearing in hyperuraemic patients due to the in vivo reaction of urea with haemoglobin. The aim of this study was to check the possible interference of blood urea levels on the determination of HbA1c by different methods.

Methods: we used two HPLC analysers and one immunoassay for the determination of HbA1c. Total numbers of individuals n=832, extracted randomly from our laboratory database over 30 days SIEMENS Data warehouse for data extraction from our L.I.S. (ServoLab 1.00.10), and we divided into four groups based on the presence of diabetes (HbA1c > 6%) and/or renal failure [glomerular filtration rate (GFR) < 60 ml/min (MDRD)]. The Mann-Whitney U test was carried out to establish whether there were significant differences between the non-diabetic patient groups with and without renal failure (groups 0 and 2), and to compare the diabetic patient groups with and without renal failure (groups 1 and 3), on the three analysers, in order to establish the behaviour of the three analysers as well as the possible interference of high urea levels in the determination of HbA1c in RF patients

Results: the HbA1c results showed similar behaviour in the three methods, as regards the presence or absence of RF (Fig. 1). In the comparison between groups 0 and 2, a statistically significant increase was observed in the mean HbA1c concentration ($p<0.05$); with respect to the comparison between groups 1 and 3, we observed a statistically significant mean decrease in the HbA1c concentration ($p<0.05$).

Conclusion: In our study, both chromatography methods and the immunometric method evaluated behaved similarly in the presence or absence of RF, indicating that serum urea concentrations do not interfere, and therefore there is no interference from the formation of carbamylated haemoglobin in the determination of HbA1c.We can conclude that, in the case of non-diabetic patients, there is overestimation of HbA1c concentrations in patients with RF and higher urea levels, with the opposite occurring in the DM group, where the HbA1c values in the group with RF (and higher urea values) are underestimated. This decrease in the HbA1c estimation (by the 3 methods) should be taken into account in those diabetic patients who do not have RF but who later develop it.

A-120

Electro conductivity and bacteria account of distilled water affect results of internal quality control in clinical chemistry test

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Background: Qualify of distilled water directly affects accuracy of laboratory test and this therefore is one of the important factors for internal qualify control. However experimental procedure, instruments, reagents used in experiment, calibration or qualify control are on the top list when internal control result out of range, fact is people never consider about water qualify in most case when there is a problem or problems. We have studied role in monitoring two important factors and how they affect final testing results.

Method: We monitor electrical conductivity of water daily, culture and count up

colonies of bacteria weekly, and simultaneously check sodium(Na^+) and chloride(Cl^-) ions, alkaline phosphatase(ALP) in qualify control reagents.

Results: When electrical conductivity is equal to 5 cm µs/cm, coefficient of variation (CV) for Na+, Cl- and ALP are ± 2.8%,± 3.1% and ±3.0% of their target value respectively;while when electrical conductivity reach to 8µs/ cm, CV of the above factors become to ±4.8%, 5..2% and 5.5% of target value. Further even the electrical conductivity stay at 5µs/cm, when bacteria colonies ≥ 15cuf/ml, CV of the above factors go up ±5.7%,±6.8% and± 8.9% of their target value respectively. The higher value of electrical conductivity or the more of bacterial colonies;the higher CV of the target value of those factors we monitoring . The correlation coefficient of electrical conductivity of water to the above factors is 0.9318

Conclusion: It's vital for us to get accurate and reliable testing results to do routinely maintenance on the distilled water preparattion system, to monitoring electro conductivity, to culture and count bacteria colonies. Especially important to cut down bacteria number for accuracy and reliability of final testing results.

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A-121

Evaluation of a new Uric Acid application with enhanced robustness compared to the current routine method on Abbott ARCHITECT Clinical Chemistry Systems

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Background: The measurement of the concentration of uric acid (UA) in heparine plasma and serum is a basic analysis in laboratory routine. In general, UA assays are very sensitive to bilirubin and hemoglobin interferences.

Methods: The new generation assay Uric Acid (Abbott Laboratories, IL 60064, USA, List No. 3P39-21) was evaluated using heparine plasma and serum as sample source. This assay was compared to the well established Uric Acid assay (Abbott Laboratories, IL 60064, USA, List No. 7D76-21) on a c8000 Architect System. The imprecision was tested by using control material (Lyphocheck Assayed Chemistry Control Level 1 and 2, Bio-Rad, Munich, Germany) twice a day for a period of five workingdays in duplicates.

Results: The CV was 1.2 % (mean concentration 256 µmol/L) and 0.89% (550 µmol/L). According to CLSI guidelines the total imprecision has been calculated with 1.0 %. The limit of analytical detection was 2 µmol/L. The equations of the regressions were calculated by y = 0.979x+0.33 (r=0.99) for heparine plasma and y = 0.999x+0.10 (r=0.99) for serum. The linearity was checked from 107 to 1136 µmol/L with recovery rates between 95 and 103%. In spiked samples hemolysis had no impact for up to 10g/L and the assay was not affected by bilirubin and triglycerides for concentrations of up to 1129 µmol/L and 25 mmol/L respectively. To examine any interferences of ascorbic acid heparine plasma was spiked to a final concentration of 50 mg/L, which caused no disturbance. In urine samples the total imprecision was 1,32 %. The recovery rate of linearity investigation was between 97 and 102% (tested range: 6-1719 µmol/L). In urine the ascorbic acid interference was tested up to 500 mg/L. Although, there was a slight decrease in UA concentrations the recovery rate was always higher than 95%.

Conclusion: The evaluated uric acid assay is a reliable test for clinical routine in heparine plasma, serum and urine. This assay is unaffected to interferences with hemoglobin, bilirubin and lipids up to concentrations, which are common in the daily clinical routine. The assay is suitable for measuring the uric concentration of uric acid with a low imprecision and over a wide range.

A-123

Evaluation of 7-Day Stability of Selected Chemistry Analytes in BD Vacutainer® SST™ and BD Vacutainer® PST™ Tubes

J. Chance, J. Drew, L. Khumush. *BD Diagnostics, Franklin Lakes, NJ,*

Background: Evaluation of analyte stability in serum or plasma stored in primary gel tubes is required to establish acceptable time frames in which samples stored in these tubes can be tested (or re-tested) to provide accurate results. However, there is limited information available on the long-term stability of chemistry analytes in these tubes.

Objective: To evaluate the stability of selected chemistry analytes in serum and heparin plasma stored in opened and unopened gel separator tubes over 7 days at 2-8°C.

Methods: Two BD Vacutainer® SST™ Tubes (BD SST) and two BD Vacutainer® PST™ Tubes (BD PST) were collected from each of 40 apparently healthy adult subjects. After tube inversions, tubes were allowed to clot/stand for a minimum of 30 minutes and a maximum of 2 hours. Tubes were then centrifuged for 10 minutes at 1300 x g. For one tube of each tube type, the closure was removed and the sample was tested at initial time (within 4 hours of centrifugation). After testing, a new plastic closure was applied. These two tubes and the other two unopened tubes were then stored upright at 2-8°C for 7 days. All four tubes were then removed from the refrigerator and allowed to stand at room temperature for a minimum of 1 hour. Tubes were gently inverted, closures removed, and an aliquot from each tube removed and centrifuged at high g-force to clean up the sample. These samples were then transferred to new secondary plastic containers, and analyte testing was repeated on these samples. Thus, for each subject, two samples were tested at initial time and four samples tested after 7 days storage. Testing at both timepoints comprised a routine chemistry panel including immunoglobulins (28 analytes) on the Roche Integra® 800, and selected immunoassays on the Siemens ADVIA Centaur® (Cortisol, Free T3, Total T3, TSH). Data were analyzed by ANOVA to determine mean biases between samples tested at 7 days vs. initial time, including both "unopened" (tube tested at 7 days not previously opened/tested) and "opened" (tube tested at 7 days same tube opened/tested at initial time) comparisons. The clinical significance of changes over time was assessed by comparison of mean biases to a pre-defined clinical acceptance limit (CAL) for each analyte.

Results: Most analytes were stable over 7 days in both BD SST and BD PST tubes, as evidenced by the mean bias of the comparison being within the CAL. For BD SST, a negative bias was observed for HDL. For BD PST, negative biases were observed for HDL, glucose and CO2, while positive biases were observed for calcium, LD, phosphorus, potassium and Total T3. For some analytes in BD PST, the magnitude of the change over time was greater for the "opened" comparison in which the tube tested at 7 days was the same tube that had been opened and sampled/tested at initial time.

Conclusion: Overall, analyte stability was improved in serum stored in BD SST tubes vs. in plasma stored in BD PST tubes.

Tuesday AM, July 26

Poster Session: 10:00 am - 12:30 pm
Management

A-124

Do the boxes for carrying clinical laboratory samples warrant temperature stability?

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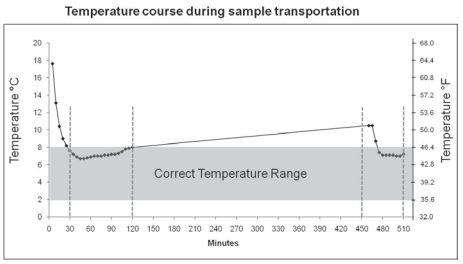
Background: Among the pre-analytical causes making laboratory results unreliable, poor quality storage and/or transportation are cited. Inadequate temperature (T) conditions of specimens during transportation may engender unsatisfactory results. Aim of this investigation was to evaluate T conditions of samples carried in transport boxes.

Methods: To maintain the correct T range (from 2°C to 8°C / 35.6°F to 46.4°F) four ice reusable dry gel packs with carbopol gel inside (2 x 200mL and 2 x 500mL) were correctly placed inside the Coleman® transport box with no biological specimens. Moreover a calibrated T recorder (Trix-8 Temperature Recorder®, LogTag recorders, Adarve®, Brazil) was inserted in order to verify T stability. T registration was done every 5 min during 8 hours. The box was carried by car from a private facility to our laboratory together with other sample containing boxes. T data were analyzed with LogTag Analyser®.

Results: See T graph:



0 - 30 min: T in open box before ice introduction; the box is suited to receive the samples after about ½ hour. T maintains stable only for 90 min (from 30 to 120min). Moreover during approximately 64% of the transportation time (from 125 to 450 min) T stability was unsatisfactory. It is noteworthy that 20 min after arrival to destination and regaining conditions of controlled room T, the box T came back within range (from 2°C to 8°C).

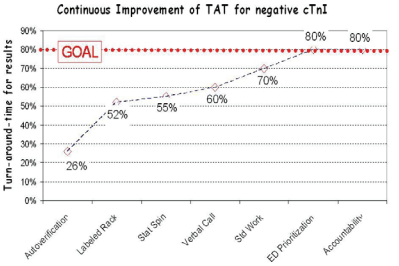
Conclusion: This study showed that usual boxes for sample transportation do not warrant T stability probably due to the intrinsic box structure which is easily influenced by external T. Future studies should verify the impact of inside-box temperature variations on labile analytes, i.e. hormones.

A-125

Improving Emergency Department Patient Care by Decreasing Cardiac Troponin Results Turn-Around-Time

J. Zajechowski, V. Luzzi, E. Herberholz, R. Benitez, J. Carey, C. S. Feldkamp. *Henry Ford Hospital, Detroit, MI,*

Background and **Aim:** Lean principles have been applied in our clinical laboratories to decrease the time between test request and result delivery (TAT) by eliminating non-value-added activities, and standardizing processes. Our laboratory supports a 950 bed hospital and is the core laboratory for a multi-center health system. Using Lean principles, we aim at meeting TAT targets for cardiac Troponin I (cTnI), improving patient care, and reducing cost and patient length of stay in the Emergency Department (ED). **Methods:** To achieve the aim, we employed multiple Lean principles and the Plan-Do-Check-Act (PCDA) cycle. The Lean tools used were work simplification, visual display, and level load. Specifically, the following changes were implemented: (1) auto-validation, (2) labeled exclusive rack for ED specimens to increase visibility, (3) added a faster centrifuge, (4) verbal announcement of ED cTnI, (5) standardized work, (6) prioritized ED specimens using colored label, and (7) increased technologists' accountability. We measured TAT for negative cTnI's (<0.2ng/dL) at 30', 45' and 60'after each change and ED length of stay of patients evaluated for chest pain. TAT was defined as the time a specimen arrived in the laboratory to the time the result was reported in the laboratory information system. **Results:** TAT goal for negative cTnI was 80% of negative results reported in <30min. After multiple PDCA cycles, we achieved the TAT goal. 80 % of negative cTnI results are reported within 30'. The improvement observed with each PDCA cycle was gradual (Figure) and the overall TAT improved ~ 3 fold since we first introduced Lean tools into our processes. TAT improvement contributed to the 18% decrease in the patient's length of stay in the ED during their process improvement initiative. **Conclusions:** Use of Lean tools improved TAT for negative cTnI results. Continual monitoring sustains improvements and identifies new opportunities.



A-126

Creating Lean Culture through Coordinated Systematic Training

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Background: A specimen procurement division (collection, transportation, processing, and client services) at a large medical center is becoming a learning organization through implementing Lean. This has involved use of outside experts, benchmarking against labs further in the process, and collaborating with a University-based Industrial Engineering department. Additionally, surveys and interviews conducted with staff at all levels highlighted the following problems in implementation among both staff and management.

1. Uncertain commitment to Lean.
2. Insufficient ability to work in teams.
3. Incomplete or absent knowledge of Lean methods and ideologies.

Objective: Create a systematic training process to communicate the department's mission, and teach effective team skills and Lean methods to staff and management. The overall goal is to develop a training system to help transform the culture.

Methodology: A curriculum of 7 interactive modules was created using Adobe Captivate, totaling about two hours of online content. The modules cover the following topics:

1. Departmental mission and vision and role of staff in the lab's success.
2. Standard lab workflow emphasizing the cultural and technical duties required of each bench.
3. Standard work and the crucial role played by standard operating procedures.
4. Effective teamwork, using Patrick Lencioni's "Five Dysfunctions of a Team" model.
5. Lean concepts/ tools including waste, 5S, value stream mapping, work flow diagrams, root cause analysis, the A3, and continuous process improvement.
6. The causes of error and the importance of quality error reporting in a "Just Culture" and designing a high quality system.
7. Technical instruction on the division's online error reporting system.

For each module, pre and post quizzes are generated from a 100-question bank which is divided into topic-specific pools. At the curriculum's conclusion, question pools are combined to create a final exam, which serves as a summative assessment of each trainee's understanding. The curriculum also includes learning activities that provide the trainee with standardized opportunities to apply their knowledge. The activities address key cultural issues such as trust, communication, and safety as well as technical competencies such as process analysis using Lean tools. At the curriculum's conclusion, the trainer and trainee evaluate the trainee's competency, using a quantitative scale (0="Untrained" to 5="Can mentor and set standards"). The modules, quizzes, activity procedures, checklists, and final exam are provided online. Quizzes are administered and documented using the

SumTotal learning management system. At the conclusion of the training, trainees are asked to fill out course and trainer evaluations and participate in a structured interview regarding the effectiveness of the course.

Results:The training program has been received well by both training staff and new hires and the overall understanding of Lean initiatives is improving. Initial findings show training evaluation scores increased by 62%, teamwork scores increased by 23%. The program establishes consistency in training and has made documentation more reliable.

Conclusion: Clearly defining the desired work culture and then teaching it to all staff is essential to transformational change. Creating a standard training program can establish consistency in developing a Lean culture.

A-127

Laboratory audit facilitates improved serological test ordering in the diagnosis of celiac disease

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Background: Suboptimal laboratory test ordering is common in clinical practice. Laboratories can play important roles to facilitate physician test ordering. Current guidelines for celiac disease include recommendations to measure anti-tissue transglutaminase (tTG) to screen for celiac disease in children and adults. We recently completed a laboratory audit to improve the serological test ordering in the diagnosis of celiac disease in our clinical service.

Methods: The investigation followed an audit cycle. The specimen number, age, serological test ordered and ordering physician were extracted from the Laboratory Information System over three months at McMaster University Medical Centre before (2008) and after (2010) the implementation of corrective actions identified by the audit. Test ordering patterns were compared to the requirements of guidelines. The corrective actions included: updating serological test ordering policy, changing test menus, group interaction with physicians and publication of a newsletter.

Results: Specimen number during three months before and after corrective actions was comparable (633 vs. 656). Total test numbers were decreased (1105 vs. 763). After the corrective actions, ordering of tTG alone was increased from 23.7% to 79.40%. Non hospital physicians' ordering was reduced from 54.0% to 2.2%. Test ordering for children by physicians in different specialties met the requirements of guidelines. Test ordering patterns for adults were significantly improved (Table1).

Conclusions: The audit identified that overall the test ordering did not meet the recommendations of the clinical guidelines. Changes to laboratory test ordering by the use of comprehensive strategies greatly improved the compliance to the clinical guidelines.

A-129

Use of Biological Variation for Establishing and Harmonizing Performance Limits for Clinical Chemistry PT Schemes

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Background: Proficiency Testing (PT) providers use predetermined performance goals, or allowable limits of performance (ALP), for the assessment of analytical quality of clinical laboratories. The different providers establish ALPs based on achievable performance, but there is significant variation between the ALPs established for the different tests and no consensus for linking PT performance to medical needs. In 1999, the main outcome of the Stockholm Consensus Meeting for Global Quality Specifications in Laboratory Medicine was agreement to evaluate the effect of analytical performance on clinical outcomes, followed by clinical decisions including data based on biological variation (BV) and PT performance goals. There are still few studies to support clinical outcomes. However, BV data is available for many analytes. We use QMP-LS scheme data to compare BV-based ALPs with ALPs of various PT providers to determine feasibility of using them for establishing harmonized ALPs that link performance to medical needs.

Methods: QMP-LS participant data for albumin, bicarbonate, chloride, glucose, phosphate, potassium, sodium, protein (total), urea and urate were evaluated. Six human serum samples were distributed to 187 laboratories and the results evaluated. Results were analyzed statistically using the ISO 13528:2005 robust algorithm to calculate the adjusted mean and standard deviation and z-scores. Three BV based limits, Optimum (BV-OP)*, desirable (BV-DES)† and minimum (BV-MIN)‡ were established for each analyte as described by Fraser[1] and Ricos[2]. ALPs from QMP-LS, and CLIA were compared.

Results: Calculated BVs and satisfactory participant result percentages are provided. Except sodium, >95% labs met the BV-MIN ALP and >85% the BV-DES ALP. percentages

	BV-OP* (%)	Satis-factory (%)	BV-DES† (%)	Satis-factory (%)	BV-MIN‡ (%)	Satis-factory (%)
Albumin (n=1049)	3.3	66.8	5.3	86.6	8.0	97.4
Bicarbonate (n=896)	4.8	61.9	7.9	89.7	11.8	98.1
Chloride (n=1097)	1.3	62.3	2.0	87.0	3.0	95.6
Glucose (n=1107)	5.9	96.5	9.6	99.4	14.4	99.9
Phosphate (n=960)	8.7	97.7	14.1	100.0	21.2	100.0
Potassium (n=1115)	4.9	99.0	8.0	100.0	12.1	100.0
Sodium (n=1107)	0.8	56.4	1.2	64.9	1.8	84.5
Total Protein (n=1031)	2.9	72.6	4.7	90.4	7.0	98.4
Urate (n=986)	10.7	99.4	16.6	100.0	24.8	100.0
Urea (n=1094)	13.4	97.2	21.4	98.9	32.1	99.7

* BV-OP = $[0.125(CV_i^2+CV_g^2)^{1/2}] +z\ (0.25CV_i)$
† BV-DES = $[0.250(CV_i^2+CV_g^2)^{1/2}] +z\ (0.50CV_i)$
‡ BV-MIN = $[0.375(CV_i^2+CV_g^2)^{1/2}] +z\ (0.75CV_i)$
CV_i: Intra-individual BV
CV_g: Between-individual BV
z = 2.58 (99% CI)

Conclusion: BV-based ALPs can successfully form harmonized ALPs for global performance goals for PT. Stratification of performance using BV-Min, BV-DES and BV-OP ALPs may identify analytical methods needing improvement and assist participants in setting objective, analytical quality goals and monitoring analytical performance to meet medical needs.

A-130

Data Mining of Serial Patient TSH Results to Derive Biologic Variation

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Background: Most estimates of biologic variation (s_b) are based on periodically acquiring and storing specimens from reference subjects, followed by analysis within a single analytic run. We demonstrate for TSH, only previously obtained paired patient results and summary QC data need to be statistically analyzed to provide reliable estimates of s_b.

Methods: A laboratory data repository provided all of the TSH results measured over a three year period at University of Alberta Hospital in Edmonton. These TSH measurements (on mostly inpatients) were made on the Beckman Coulter DxI (Brea, CA). After removing outlying patient results under 0.3 and over 5.0 mIU/L, 1856 different patients had 4155 TSH ordered at least twice within 48 hours (median TSH = 1.7, average TSH = 1.98 mIU/L). We tabulated the pairs of intra-patient TSH that were separated by 0- 1 ,1- 2, 2-3, . . 47-48 and 48-49 hours. The standard deviations of duplicates (SDD) of the paired PT's were calculated for each time interval. The graph of SDD vs. time interval was linear; the y intercept provided by the linear regression equation represents the sum of s_b and short term analytic variation (s_a): $y_0 =(s_a^2 +s_b^2)^{1/2}$. The short term analytic variation (s_a) was determined with the same type of consecutive pairs SDD analysis of 2 levels of Bio-Rad quality control specimens analyzed 5 to 7 times daily over 12 months.

Results: The QC derived s_a was 0.10 at the TSH median of 1.7 mIU/L. The regression equation of the intra-patient SDD vs. time yields a y intercept of 0.52 mIU/L. Substitution of the QC derived s_a results in a s_b of $(0.52 \times 0.52 - 0.1 \times 0.1)^{1/2} = 0.51$ mIU/L. When expressed as a relative error, the s_b is 30%, higher than the 19% estimate in Ricos' compilation of biologic variation. Reasons for these differences include high prevalence of non-thyroidal illness in hospitalized patients, acute effects of therapy and disease affecting the hypothalamic-pituitary-thyroid axis.

Conclusion: As would be expected, the comparison of the biologic to analytic variation indicates that the Beckman Coulter DxI is extremely suitable for monitoring TSH in inpatient settings. We are extending this analysis to TSH measurements in the outpatient (referral laboratory) environment. Such derivations of biologic variation should be extended to other endocrinology tests and immunoassay systems.

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Development of an Online Tool for Exception Handling in Specimen Processing

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Background: Exception handling refers to the handling of specimens which do not meet laboratory acceptance criteria for routing, processing, and analysis. Robust exception handling requires accurate communication and tracking. To improve exception handling in two Specimen Processing (SP) areas of a large academic medical center, we evaluated the current QA paper-based system then developed an online tool specific to lab needs. The goals were to: 1) increase QA form submission, 2) increase efficiency of QA form submission, and 3) use the database generated by the online QA form for quality improvement.

Methods: Retrospective review of lab information system (LIS) records and paper QA forms was performed at two hospitals in August 2010. The LIS was queried for the tests canceled, the accession number, and the cancel code used. Paper QA forms were reviewed and assigned a cancel code independently. Next, all LIS cancel codes were assigned a category from 1-5 signifying a likelihood for patient harm (1 highest potential, 5 lowest potential). Lastly, the LIS and the QA databases were correlated by accession number to determine 1) the percent of canceled cases generating a QA form, 2) the accuracy of the LIS cancel code relative to the code assigned by the QA form, and 3) the percent of QA forms submitted for each level of potential patient harm.

Results: In August, 2010, 196,482 tests were ordered generating 70,227 unique accession numbers of which 7,818 (11%) were canceled. SP performed 2,468 (32%) cancelations, and 436 cancelations (18%) generated a QA form. Of the 191 cancel codes in the LIS, 59 were used by SP. The number of times the QA and LIS cancel codes matched was 221 (9%). Only 41% of the cancel codes with the highest potential of patient harm had an associated QA form submitted. The online QA form was developed using literature review and laboratory staff input. The online form generates 1 unique record per exception handling event. Employees choose a phrase describing the exception, and the correct LIS error code is displayed. The record is forwarded to a lead or supervisor depending on the potential for patient harm. The database is searchable with different levels of access depending on job classification. Pilot studies testing the online QA tool showed increased QA form submission (18% to 38%) and increased QA submission (38% to 100%) for codes associated with the greatest risk for patient harm. Employees participating in the pilot elected to continue using the online tool rather than return to the paper QA form at the end of the pilot.

Conclusion: Exception handling is a central activity in specimen processing. Cumbersome systems for tracking exceptions lead to low compliance in QA activities and mask quality problems. An online tool can improve speed, compliance, reproducibility, and transparency in exception handling.

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Improved Turn Around Time for Send Out Testing of Neuron Specific Enolase to Meet American Academy of Neurology Practice Parameter for Prognosis in Post Anoxic Coma

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Background: The American Academy of Neurology (AAN) published practice parameters that recommend multi-factorial assessment and testing to predict poor outcomes of comatose patients after cardio-pulmonary resuscitation. These assessments and tests include pupillary light response, corneal reflex, motor response to pain, myoclonus status epilepticus, somato-sensory evoke potential (SSEP) studies and Neuron Specific Enolase (NSE) testing. In our practice, 5 of the 6 indicators can be assessed within a 72 hour window after post-anoxic resuscitation. NSE, a non-FDA approved test performed off-site had a variable turn around time (TAT). Prolonged NSE test resulting caused delays in clinical decision making. Therefore, a multi-disciplinary team was formed to evaluate the process and make recommendations for decreasing TAT. Decreased TAT will improve patient clinical management and decrease length of stay in Intensive Care Units (ICU).

Objective: To decrease the TAT to <= 72hrs from time of specimen collection to time of electronic medical record (EMR) test resulting.

Methods: We formed a multi-disciplinary process improvement team which included all departments that were involved in NSE ordering, sample collection, sample processing, transport, analysis and test resulting. The team members included a physician, nursing staff, and personnel from the following work units: laboratory central processing, reference lab, core lab, and lab quality assurance. Lean principles were used to record each discrete process in the work flow from test ordering to test resulting in the EMR. We determined the TATs using service management reports from the laboratory information systems of our internal and reference laboratories. Team members investigated obstacles for their assigned step in the process and proposed improvements for optimizing that TAT interval. We then identified steps in the overall workflow with the greatest opportunity for improved TAT. Process improvements were implemented and TAT tracked to quantify effects on TAT.

Results: The average TAT prior to implementing process improvements was 87.3 hours, with a range of 36.0-166.0 hours. Of the 19 samples, 6 (31.6%) were reported in >96 hours. The ANN practice parameter criterion was met 36.8% of the time. The average TAT after implementing the process improvements was 52.6 hours, with a range of 24.7-146.9 hours. Only 3 of the 45 (6.7%) samples were reported in >96 hours. The ANN practice parameter criterion was met 75.6% of the time.

Conclusion: Process improvements significantly improved TAT for NSE Testing in post-anoxic comatose patients. By monitoring the NSE Testing TAT as one of our Quality Program indicators, we have been able to maintain the decreased TAT. This provides the physician with access to test results in a timely manner and facilitates clinical management thereby decreasing the length of stay in the ICU.

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Using sigma-metrics in the evaluation of test analytical performance

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Background: Understanding test analytical performance is important for quality monitoring. CLSI (guideline C24-A3) proposes applying sigma-metrics for evaluating test performance as it integrates test quality specification, imprecision and accuracy. We used sigma-metric principles to establish effective quality monitoring for routine chemistry tests in our laboratory. Some of our findings are presented here.

Methods: We included 27 routine chemistry tests performed on the Roche Modular system using Roche reagents. Total allowable error (TE) was defined based on biological variation (Bio-TE; Ricos, et al, $p<0.05$) and on CAP proficiency testing surveys (PT-TE). Cumulated test imprecision data for each level of quality control (QC; Bio-Rad Assayed Chemistry) were extracted from the Bio-Rad Unity™ QC Report. Test accuracy (bias) was obtained by comparing our observed mean with the peer group mean. Sigma-metric of test was calculated using the formula published in CLSI guideline C24-A3.

Results: Bio-TE was tighter than PT-TE in 72.7 % tests. In half of the tests, the difference between Bio-TE and PT-TE exceeded 50%. When evaluated against Bio-TE or PT-TE respectively, 40.7% and 63.0% of tests showed sigma-metrics ≥5 with low level QCs, whereas 55.6% and 74.1% tests showed sigma-metrics ≥ 5 with high level (Table1). Moreover, the sigma-metrics of serum sodium, chloride and bicarbonate were less than 4 at both QC levels when evaluated against any of the above test quality specifications.

Conclusions: We have used test sigma performance to establish achievable analytical goals and to improve test analytical performance.

Table 1. Sigma performance of routine chemistry tests on Roche modular.

			Bio-TE	
			Sigma-metrics <5	Sigma-metrics ≥5
Low level of QC	PT-TE	Sigma-metrics <5	Albumin, AST, bicarbonate, chloride, phosphorus, sodium, urea	ALT, bilirubin direct, bilirubin total, GGT
		Sigma-metrics ≥5	ALP, calcium, cholesterol, cholesterol HDL, creatinine, glucose, magnesium, potassium, total protein	Amylase, pancreatic amylase, CK, LDH, lipase, triglycerides, urate
High level of QC	PT-TE	Sigma-metrics <5	Albumin, bicarbonate, calcium, chloride, sodium	GGT, urea
		Sigma-metrics ≥5	ALP, cholesterol, cholesterol HDL, creatinine, glucose, magnesium, total protein	ALT, amylase, pancreatic amylase, AST, bilirubin direct, bilirubin total, CK, LDH, lipase, phosphorus, potassium, triglycerides, urate

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Lean in Virology delivers a predictable service; on time every time

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Introduction: Lean improvements are not new to our organisation (the Imperial College Healthcare NHS Trust, UK). We here demonstrate how the incorporation of Lean into the Virology department supports the delivery of a predictable service; on time very time. Lean is a set of tools that deliver demonstrable improvements in service provision through evidence based methodology by eliminating non-value adding activities. We describe our successful efforts to improve viral serology turnaround time (TAT) by improving pre-analytic processes, using existing resources and the adoption of principles of Lean production. Our goal was to report 80% of serology tests in less than 2 hour and to no longer distinguish between urgent and routine testing. High volume, HIV testing was chosen to compare TAT.

Methods: Data was captured on current process capability and performance (November 2010). A Rapid Improvement Event (RIE) was delivered by Cepheid Healthcare Consultants which consisted of 2 days Lean theory training followed by 3 days practical implementation in Virology (January 2011). Value Stream Mapping (VSM) was performed pre- and post-Lean implementation and the magnitude of change captured. During the RIE, a communication plan was established which was implemented (24th January 2011). Using the current state VSM as a baseline, the process was re-engineered and metrics for measurement established. The new process was implemented as a pilot for two weeks.

Results: Pre- and post-RIE turnaround times were compared. Median processing time (from receipt to result release) was reduced from 69 hours to 90 minutes (>95% reduction in turnaround time), and the laboratory surpassed the goal of reporting 80% of viral serology tests in less than 2 hour. Staff feedback was that after an initial phase of scepticism was that the changes had resulted in a dramatic improvement in staff morale and working environment. Samples flowed, the work was completed and staff freed to perform other duties. The pilot was adopted into routine use.

Conclusion: The adoption of Lean in practices in Virology has resulted in dramatic improvement in the speed and quality of service delivered to Imperial Trust. The service now delivers predictable TATs; on time, every time. The phone does not ring any more because users know that the results will be where needed, when needed by them. Staff freed up were directed to other tasks such as supporting the quality manager and inventory management. There has been a decrease in overtime and the results were so compelling that the Lean process will be implemented across all Imperial Trust sites and other areas within Virology. Lean delivered profound improvements in Virology.

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Calibration verification for Hitachi and Olympus automatic biochemistry analyzer with testing blood glucose

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Background: Since 1988, the College of American Pathologist (CAP) has been offering materials for calibration verification and has verified that the laboratories which have passed the calibration verification can perform very well in the proficiency testing. Clinical Laboratory Improvement Amendments of 1988 (CLIA'88) defined calibration verification is testing materials of known concentration in the same manner as patient specimens to assure the test system is accurately measuring samples throughout the reportable range. In this study, calibration verification for Hitachi and Olympus automatic biochemistry analyzer with testing blood glucose was perform in order to present a method of calibration verification and evaluate the results of calibration verification.

Methods: In accordance with the instruments used, the glucose data were divided into two groups: Hitachi (74 labs) and Olympus (73 labs) groups. The outliers in each group were removed using a robust statistical method which was to establish a TUKEY fence, i.e., Q_1-1.5*IQR to Q_3+1.5*IQR. One-sample Kolmogorov-Smirnov test was used to test the normality. The modified peer means were used as the assigned values. The performance of calibration verification was based on the approach of calibration verification currently used by the CAP, i.e., the slope and intercept of the calibration line were tested by one-sample t test and the percent differences were calculated, the percent differences were compared with allowable errors. If the calibration line has a slope and intercept which have no statistically significant difference from 1 and 0 respectively and the percent differences are within the limits of allowable errors, the evaluation is verified 1; if the calibration line has a slope and intercept which have no statistically significant difference from 1 and 0 respectively and at least one of the percent differences exceed the limits of allowable errors, the evaluation is different 1; if the calibration line has a slope or intercept which has statistically significant difference from 1 and 0 respectively and the percent differences are within the limits of allowable errors, the evaluation is verified 2; if the calibration line has a slope or intercept which has statistically significant difference from 1 and 0 respectively and at least one of the percent differences exceed the limits of allowable errors, the evaluation is different 2.

Results: In Hitachi group, verified 1, different 1, verified 2 and different 2 respectively were 5.4%, 0%, 68.9% and 25.7%. In Olympus group, verified 1, different 1, verified 2 and different 2 respectively were 16.4%, 0%, 58.9% and 24.7%. 3.0% and 7.6% outliers were removed in Hitachi and Olympus groups respectively.

Conclusion: The method of calibration verification used by the CAP is reasonable and feasible. The disadvantage of this method is how to delete outliers was not presented, although the CAP proposed that the outliers should be removed from the data set. In addition, Most of the participating laboratories (74.8%) can pass the evaluation of calibration verification.

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Should I repeat my 1:2s QC rejection?

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Background: Objective - Evaluate performance characteristics of QC strategies using a 1:2s rule that repeats if the initial evaluation fails. Relevance - Repeating a QC that is outside 2SD from the mean (1:2s rule) appears to be a common practice. Although this form of repeat sampling is frowned on by many, the comparative power of this approach has not been formally evaluated. We compare false rejection rates (P_{fr}), probability of error detection (P_{ed}), and expected QC consumption of a repeat sampling strategy to more commonly accepted QC rules.

Methods: Power functions are computed both mathematically and by simulation for a 1:2s rule, a 1:3s rule, a 1:3s/2:2s/R4s multirule, and two different 1:2s repeat sampling strategies. The first repeat sampling strategy uses 2 initial evaluations and repeats any initial evaluation outside 2SD. If any repeated evaluation is outside 2SD the rule rejects. The second repeat sampling strategy uses 2 initial evaluations and accepts if both are within 2SD and rejects if both are outside 2SD. Otherwise both evaluations are repeated and if any repeated evaluation is outside 2SD the rule rejects. Validation - The mathematically derived power functions were recomputed using simulation. The results matched the direct computations, validating the approach.

Results: See P_{ed} for QC Rules with 2 initial QC evaluations table.

Conclusion: The drawback of a 1:2s rule is its unacceptably high P_{fr}. The drawback of a 1:3s rule is its low power compared to the 1:2s rule. It is shown that a repeat sampling strategy provides a reasonable compromise with a P_{fr} similar to the1:3s rule, but with power to detect significant error conditions similar to the 1:2s rule. Additionally, the power of the repeat sampling strategy is greater than the 1:3s/2:2s/R4s multirule. These improvements come at a modest increase in the average number of controls tested - N_Q.

P_{ed} for QC Rules with 2 initial QC evaluations

SE	1:3s (N_Q = 2)	1:2s Repeat any (N_Q = 2.091)	1:2s Repeat all (N_Q = 2.174)	1:2s (N_Q = 2)	1:3s/2:2s/R4s (N_Q = 2)
0.0	0.0054 (P_{fr})	0.0041 (P_{fr})	0.0098 (P_{fr})	0.0889 (P_{fr})	0.0097 (P_{fr})
0.5	0.0128	0.0106	0.0244	0.1407	0.0190
1.0	0.0450	0.0505	0.1047	0.2944	0.0647
1.5	0.1292	0.1816	0.3182	0.5222	0.1865
2.0	0.2921	0.4375	0.6250	0.7500	0.4069
2.5	0.5219	0.7276	0.8642	0.9048	0.6685
3.0	0.7500	0.9147	0.9681	0.9748	0.8669
3.5	0.9048	0.9833	0.9950	0.9955	0.9632
4.0	0.9748	0.9980	0.9995	0.9995	0.9930

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Establishment and Confirmation of Reference Intervals for ten Assays on The Abbott ARCHITECT cSystems

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Background: Reference Intervals reported in Reagent Instructions for Use (IFUs) are relevant information available for correct interpretation of patient results. Additionally, the Laboratory must review reference interval data in order to account

for possible long-term shifts in the ethnicity and lifestyle habits of the patient population. Manufacturers are requested to support with reliable data for the periodic verification of Reference Intervals by the Laboratory in order to support a correct classification of the patient results.

Abbott Diagnostics and Sentinel CH. have revised and updated reference intervals for several analytes following the CLSI C28-A2 Guideline. The analytes included in this study were: UIBC, Pancreatic Amylase, Cholinesterase, Cystatin-C, Dibucaine Inhibited Cholinesterase, HBDH, Kappa and Lambda Light Chains, CK-MB Activity.

Methods: 260 serum samples (130 males and 130 females) were collected from individuals recruited as donors in a blood bank of a large Hospital in Northern Italy. Selection of suitable samples was made excluding all specimens containing substances known as interferents according to the IFUs enclosed in the Reagent Kits. Calculation of Reference Intervals was performed, the intervals defined as the 2.5th and 97.5th percentiles of each Sample Group with a 90% Confidence Level according to the non-parametric approach. As per the CLSI C28-A2 Guideline a minimum of 120 suitable results were obtained for each Reference Interval Calculation.

Assay		List Number	Unit	Male Individuals		Female Individuals	
				2.5th	97.5th	2.5th	97.5th
UIBC		4P79	µg/dL	69	240	70	310
Cholinesterase		6K24	U/L	4389	10928	2879	12669
Cystatin-C**	<=50y	1P93	mg/L	0.31	0.79	0.40	0.99
	>50y			0.41	0.99		
Dibucaine Inhibited Cholinesterase		6K92	U/L	800	2074	480	2479
HBDH		6K23	U/L	53	168	44	148
				All Subjects			
Dibucaine Number		6K92	D.N.	78.5 - 85.3			
CK-MB Activity		6K25	U/L	< 25			
Kappa Light Chains		6K96	mg/dL	122 - 437			
Lambda Light Chains		4P80	mg/dL	62 - 231			
Kappa/Lambda Ratio				1.30 - 2.61			
Pancreatic Amylase		6K22	U/L	8 - 51			

***New Standardisation vs ERM DA471/IFCC*

Conclusion: It is widely accepted that Reference Intervals reported in the Reagent IFU cannot be adopted without a previous verification study performed on the population afferent to the Clinical Laboratory. It's the responsibility of each laboratory to verify the appropriateness of reference intervals for its patient population, but manufacturers should assist by providing current interval estimates in Reagent IFUs.

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Effect of Clinical Laboratory Personnel Licensing on Wages

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Background: Professional licensing directly affects about 29% of U.S. workers compared to 15% for unionization (Kleiner & Krueger, 2008), and is considered a primary means to establish and maintain health care practitioner competence (Greiner & Knebel, 2003). Clinical laboratory personnel licensing and wages has been largely ignored with only two studies 30 years apart that provided conflicting conclusions. This was the first empirical study of clinical laboratory technologist licensing wage effects after controlling for human capital and individual characteristics wage determinants. Currently, 11 states and Puerto Rico require personnel licensing, with New York the most recent adopter (2006). Recently, several states including Massachusetts, Minnesota, Missouri, and Texas considered licensing bills (McCarty, 2009).

Methods: This nonexperimental correlational study used hierarchical wage regression. The DV (natural log Comparative Wage Index-adjusted wages/hr), IV (licensing), and Control variables (education, potential experience, gender, marital status, and children) data were obtained from Census 2000 5% Public Use Microdata Sample. Model 1 sample (*N*=4,061) included full-time clinical laboratory technologists, aged 25-65, BS or MS, annual salary $5K-$175K. Model 2 sample (*N*=1,665) included women aged 25-44.

Results: Clinical laboratory technologist wages were 5.8% higher ($p < .001$) on average in licensing states compared to nonlicensing states after controlling for these human capital and individual characteristics, R^2_{change} ($p < .001$). Women working in licensing states earned 5.0% higher wages ($p < .01$) compared to those in nonlicensing states, R^2_{change} ($p < .01$), see Table 1.

Table 1. Wage Regression Results for Effects of Licensing† on Clinical Laboratory Technologist Wages

	(1)[a]	(2)[b]
Licensing	.058*** (.011)	.050** (.018)
Gender	-.038*** (.010)	-------
Educational attainment	.034* (.014)	.017 (.025)
Potential experience (yrs)	.036*** (.002)	.043*** (.008)
Potential experience squared (yrs)	-.001*** (.000)	-.001** (.000)
Marital status	.016 (.010)	.025 (.015)
Number of own children < 18 yrs in the home	-.001 (.005)	-.019* (.008)
Observations (*N*)	4,051	1,665
Adjusted R^2	.160	.119
R^2 *change*	.006***	.004**

Note. Dependent variable: Natural log CWI adjusted wages/hr.
CWI = Comparative Wage Index (Taylor & Fowler, 2006). Standard errors in parenthesis. All data from 2000 U.S. Census 5% Public Use Microdata Sample.
[a]Model 1: Clinical lab technologists, aged 25-65
[b]Model 2: Female clinical lab technologists, aged 25-44
†Licensing states: CA, FL, HI, LA, MT, NV, ND, RI, TN, WV
*$p < .05$, ** $p < .01$, ***$p < .001$.

Conclusion: The findings suggested a statistically significant, but *practically* modest licensing premium for clinical laboratory technologists. This study has implications for state clinical laboratory personnel licensing policy by providing urgently needed empirical wage data for legislators, professionals, and other stakeholders to make informed decisions by weighting very modest wage costs against licensing benefits including higher average personnel quality and increased professional stature.

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Determining labor requirements for manual and automated sample processing for mass spectrometry testing through workflow analysis

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Background: Balancing process efficiency and labor requirements is important when optimizing methods for laboratory-developed tests. This is challenging for smaller laboratories that implement complex testing such as mass spectrometry (MS) with a limited number of full-time equivalents (FTE). Understanding processing workflow and labor resources required for MS sample preparation would assist these labs in determining the feasibility of implementing MS testing given limited labor resources.

Methods: A workflow model was built for a vitamin D assay in which samples are prepared for MS by a single technologist (FTE) through manual solid-phase extraction (SPE). We processed varying daily batch sizes (5 to 90 samples/batch) in a random manner over a 4 week period until 20 total batches were analyzed. Timing was recorded by observers to avoid bias. Technologist discomfort/fatigue was a subjective measure of work intensity. The manual sample preparation workflow was then compared to the published claims for workflow on the Tecan™ Freedom EVO 100® (EVO). Regression analysis was used to optimize batch sizes for manual processing and to determine volume thresholds for which front-end automated sample preparation should be considered.

Results: Batch preparation time, fraction of a FTE required to prepare a batch, and fatigue experienced by technologist for manual sample processing is shown in the Table. The fatigue experienced by the technologist was largely in the arm, neck, and shoulder areas. Mathematical regression analysis showed that efficiency and cost were balanced between manual and automated sample preparation when batch size reached 46 specimens. The labor "cost" at this batch size was 0.25 FTE/batch.

Conclusions: Increasing specimen batch sizes placed measurable increasing time demands on labor and raised ergonomic concerns when manually processing specimens for MS testing. For a single FTE, ideal batch sizes are approximately 50 specimens. Front-end automated sample preparation should be considered for larger batch sizes.

Workflow Analysis Result

Batch, *n*	5	15	30	60	90
Prep, mean ±CI (min)	57.7 ±1.2	75.3 ±1.5	90.7 ±3.5	145.3 ±4.0	184.0 ±18.2
Fatigue	None	None	Mild	Mod	Per
FTE Equiv	0.12	0.16	0.19	0.30	0.38
Regression	Batch	y=1.5048x+50.408; Fit (r)=0.9868			
	FTE	y=0.0031x+ 0.105; Fit (r)=0.9975			

CI= confidence interval; min = minutes; Mod = moderate ; Per = persistent; FTE = Full-time Equivalent

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Lean & Six Sigma implementation in a Central Laboratory to improve test turnaround time and reduce delays

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Background: Fleury Group is a large diagnostic company in Brazil. In 2010, 30 million tests were performed in its clinical laboratories, 32% of them in the Central Laboratory located in São Paulo city. In order to promote improvement of all processes to achieve production and administrative efficiency, we assemble a project team called Lean Team. This team examined the critical processes and promoted improvements through the use of Lean & Six Sigma.

Aim: Improvement of critical processes using Lean & Six Sigma tools to reduce turnaround time (TAT) and delays, increasing patient and physician satisfaction and market competitiveness.

Methods: All critical indicators are monitored monthly and are also present in the Balanced Scorecard (BSC) of the company. One of the indicators monitored, specifically chosen to begin this work because it has direct impact on patient care and satisfaction, is the indicator of results released on time. This indicator is calculated using all tests performed, comparing the promised TAT with the actual time the results are released. If any results are delayed, this will generate non-conformity for the section responsible for processing.

The Central Laboratory accounted for nearly 29,3% of all delays in 2009, and it was chosen to start the lean & Six Sigma work to reduce delays in test TAT. The 2009 indicator showed 0.60% of delayed results in relation to the total volume of tests done in this section during 2009.

Tools of Lean & Six Sigma were applied during 2010, allowing stratification of results, analysis and treatment of problems, standardization of processes and reducing points of failure. New processes were implemented, such as daily reports, Kanban, meetings with stakeholders, flows of activities and management board, which allowed the management analysis of the reasons that led to delays. They triggered immediate actions in different phases of the service delivery process, from requisition entering to blood drawing, from nurses to logistics, from information technology to result reporting.

Results: The first-year result of the implementation of this project was the reduction of the delays by 82%, from 0.60% in 2009 to 0.11% (4.57 Sigma) in 2010.

Several improvements were deployed to other laboratory sections, and also allowed improved TAT results for the clinical laboratory as a whole. In this process, all the requirements and standards of quality were met, ensuring the quality differentials established by Fleury Group.

Moreover, with more controlled, effective processes, it was also possible to reduce the TAT of 142 tests, resulting in increased patient and physician satisfaction and increased competitiveness.

Conclusion: Lean & Six Sigma tools are very important to the management of critical processes and their continuous improvement by encouraging the achievement of better TAT, patient and physician satisfaction, quality and others important goals present in the BSC of the company. Fast TAT in outpatient laboratory services is an important competitive indicator in Sao Paulo market, and another well-recognized competitive advantage of Fleury Group by different stakeholders, from patients and physicians to employers and health plans.

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Reducing Send out Reference Testing Cost: the power in negotiating the price of one test

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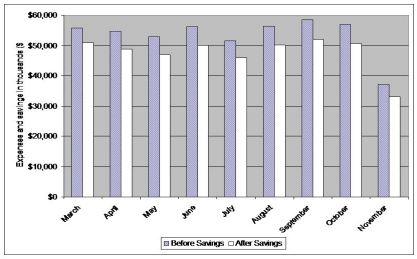
.**Background:** In 2010 it is estimated that the US health sector has spent $6.2 billions just for sendout reference testing. These are tests not offered in the main hospital-based core labs and need to be sent to commercial laboratories for esoteric testing. For this reason many hospital-based reference laboratories (RL) are faced with sky high expenses associated with RL testing. RL administrators and managers are striving to reduce and control sentout spending.Unfortunately, the amount of existing literature in the management and utilization of sentout tests is scarce with most publications are focused on describing the utilization management by ordering physicians, residents and other health care providers. They do not examine or provide ways to reduce test prices.

Objectives: In this report we attempt to give a successful simple strategy that we implemented in our RL to reduce the sentout testing cost.

Methods: A periodic review of utilization reports for 2008 showed that many tests were sentout in high volumes. The findings were based on the top 20 tests or the "hot list" we developed, with 25OH-VD resides at the top with a monthly send out of > 2000 tests per month. As a result, we negotiated a new price for 25OH-VD (11% discount) between March-November of 2010.

Results: During the 9-months period of 25OH-VD testing, currently performed in-house, we saved our institution a total of $52,400, an average of $5,816 per month, Figure.1.

Conclusions: Our data show that reducing RL sentout expenses is not a difficult task but requires periodic review of send out test volumes and dedicated efforts. Our simple could by adopted by any RL.



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Improving The Performance Of The Vitamin B12´Assay Using Methodology Dmaic- Six Sigma Metrics

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Background: The aim of this study was to evaluate the application of six sigma metric using DMAIC methodology (Define-Measure-Analyze-Improve-Control) to reduce the analytical variation of Vitamin B12 assay in a public tertiary hospital laboratory.

Methods: The serum Vitamin B12 assay was performed by eletrochemiluminescence method on COBAS e411 (Roche Diagnostics) . We fuse the DMAIC approach.

DEFINITION PHASE*:* we defined the responsibilities of the team work, we prepared the SIPOC diagram. The coefficient of variation of the PreciControl Anemia level I (CVA1) was considered as critical to quality (CTQ) and the upper limit of acceptability for the CV was 9%.

MEASURE PHASE: we measured the CTQ time series, with the descriptive statistic. Tests of repeatability and reproducibility (R&R) were performed. We gathered the preliminary data to evaluate current process performance and its capability (defects per million opportunities and sigma metric).

ANALYSIS PHASE*: We* used quality tools.

IMPROVEMENT PHASE*: T*o reduce the CVA1 we implementated of specific action plans .

CONTROL PHASE: during 5 months there were the consolidation this plans, we verifying the benefits, cost savings and the process capability .

The Minitab 15 statistical software was used .

Results: The improvement in the performance indicators were achieved. Others employees were trained. The process was revised and simplified. Many changes were done: the workflow, the handling of the samples and the reagents, critical analysis of internal controls. There were reduction of waste and economy to the laboratory.

Phase	Mean of CVA1 (%)	Process perfomance (Ppk) / SIGMA Capability	Evolution of DPMO
Measurement	10,90	-6,01 / 2,12	312500
Analyze	10,69	-2,15 / 2,19	281250
Improve	9,72	-0,29 / 2,56	156250
Control	6,28	1,48 / 5,50	31

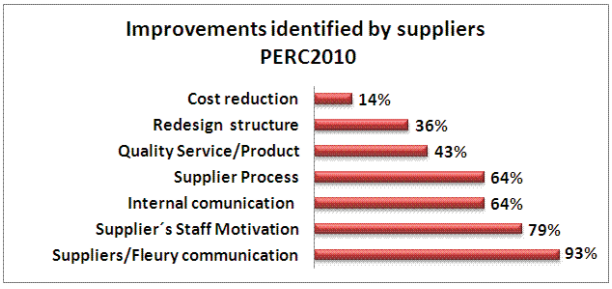
Conclusion: The factors that were interfering in the good performance of the Vitamin B12 assay were detected by the DMAIC methodology.The planning of actions at the beginning was the basis for the success of whole project.This method optimized the standardization, helped to solve the analytical problems, improved efficiency and effectiveness in the production.

A-146

Program for Excellence in Relationship with the Supply Chain - Implementation of a formal channel to give feedback to the supply chain

A. R. Haag, M. B. Rocha, P. Pedote. *Grupo Fleury S.A., Sao Paulo, Brazil,*

The Fleury Group, a 30-million test/year diagnostic company in Brazil, launched in 2010 a Supplier Relationship Management Program called PERC (Programa de Execelência no Relacionamento com a Cadeia de fornecedores), a pioneer project among Health Services providers. These are the main PERC **Objectives:** improve communication and integration between companies; engaged suppliers on Fleury´s medium and long-term goals; encourage supplier´s participation in continuous improvement and lean processes; Breakthrough innovation; ensure the quality and provide constant feedback to providers on their performance. In its first edition, 24 strategic suppliers (38% of total spent) were divided in two categories: Products and Services. After four quarterly reports the program recognized suppliers that had the best development and performance in five dimensions: 1-Quality, 2-OTIF (On-time in Full), 3-Terms and conditions, 4-Sustainability, 5-Creativity and innovation. Fleury's team conducted 24 visits to supplier's sites, totaling 72 meetings and more than 1,200 hours of work. It was possible to identify that the participants of PERC run their business in a sustainable way: 60% presented economic initiatives, 80% social initiatives and 70% environmental initiatives. In the dimension of Creativity and Innovation, suppliers presented 46 projects for process improvement, and 27 of them were fully implemented in 2010. The PERC also had a positive impact in participating suppliers, shown in the figure below:



In conclusion, a formal channel that provided frequent feedback to the Supply Chain enabled them to improve quality, processes, innovation and communication. There was also clear improvement in the governance of the supply chain (transparency, confidence, clear aims and goals, performance monitoring).The success achieved in the first year of the PERC confirms the maturity and alignment of Fleury Group and its Supply Chain. The second edition of PERC2011 was already launched increasing the number of participants to 40 (more than 50% of total spent).

A-147

Laboratory continuous quality improvement through Balanced Scorecard Strategies

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Background: Balanced Scorecard (BSC) four perspectives objectives and indicator results help to define laboratory strategies for continuous quality improvement.

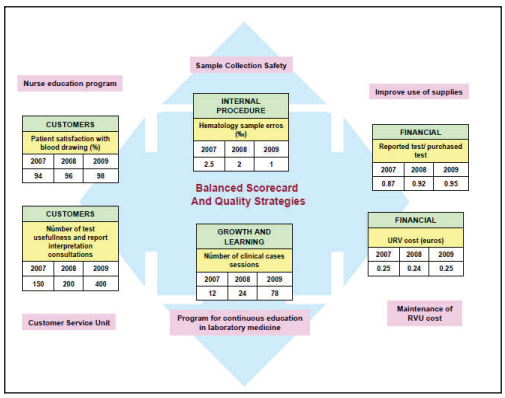
Methods: The laboratory serves a population of 234,403 inhabitants, attending Inpatients, Emergency Department and Primary Care patients. Three years strategies are showed related to BSC perspectives. Customer, and Growth and Learning are referred to Attention Unit that responded to consultations regarding the laboratory report interpretation, Clinical Cases Sessions that provide continuous education in laboratory medicine and Nurse Education Program (instructions for sample collection, and supervision by an experienced nurse), were developed. Customer and Growth and Learning indicators were assessed by means of internal surveys and manual registers.

The BSC Internal Procedure perspective is related to unacceptable haematology samples. Registers used to build indicator (the sum of coagulated, insufficient and unavailable samples, in errors occurred per 1000 samples) were collected and indicators calculated automatically from the LIS using a datawarehouse application.

Two strategies related to Financial perspective: The corrective measures established to improve the reported test/purchased test indicator consisted in changing the technology, switching from daily processing to processing one day a week in relation to the workload and clinical utility, outsourcing processing to external laboratories or adjustments in the number of quality controls processed. The indicator reported is the mean of every test indicator in the core biochemistry laboratory. An official Valencia Health Agency Catalogue application was used to obtain relative value unit (RVU) cost (serum glucose cost including supplies, human resources and general hospital and laboratory expenditures).

Results: The number of Clinical Case Sessions and report interpretation consultations increased, unacceptable haematology samples decreased and patient response to the question of whether they would be willing to have blood drawn again improved. Financial indicators also improved.

Conclusion: BSC strategic objectives and indicators were shown to be useful in evaluating laboratory continuous improvements.



A-148

In House Solutions to Routine Problems in Molecular Pathology Low Automation Level Process

G. S. Guimarães, F. H. Silva, M. A. Belmonte, M. S. Cunha. *Fleury Group, São Paulo, Brazil,*

Introduction: Fleury Group is a reference laboratory located at São Paulo, Brazil. With a rigorous quality control program, it is structured and prepared to perform high volume routines, as long as and low volume tests which have not been fully automated yet. Manual component of the operation requires a highly focused analyst, in order to avoid deviations of the process. At the Molecular Pathology Department, we perform fragmented processes, basically composed by sample sorting, nucleic acids extraction, reaction setup and thermal cycling, alternating manual and automated steps, all prone to errors and sample exchanges.

In order to work with processes that require tubes exchanges, sample position exchanges inside the plates, plate position exchanges inside the equipment and sample identification without workflow control, the creation of low cost mechanisms to reduce errors is necessary.

Methodology: The employment of *Poka-Yoke* concept (error proofing) through Lean & Six Sigma methodology in a 72,000 tests/year molecular pathology routine, associated to low cost resources and an innovative and enterprising spirit.

Results: Through bar codes employed in work batches, in samples and extraction plates, the process became connected in a way that it is possible to recover identifications and positions inside the robot plate and transfer them to the pipetage robot, through bar code scan and excel files. In manual process, it is possible to connect batches to samples, to intermediary and final tubes.

Customization of *Poka-Yoke* system in molecular pathology comprehends bar code labels, orientation signing in plate and rack positioning mediated by colorful signs, net resources to transfer independent lists generated by equipments, standardization of file nomenclature and employment of Excel software culminated in personalized conference checklists. This practice reduced sample exchanges to zero in molecular pathology process during the last six months of 2010. In the first semester of the same year, 12 sample exchanges were registered in two incidents.

The positive results are not only sensed by external client, but there is also massive improvement in organizational climate, once pressure levels are reduced.

Conclusion: Though we have not invested large money sums in error proof systems, the simple solutions presented above facilitate the operator's decision-making, because they require less focus in conferences and work station set up. The impression of more work required to complete all checkpoints changes rapidly once the analyst realizes that less time is wasted with doubts that lead to re-thinking of the process, or fixing unnoticed mistakes which had reached the final client.

Tuesday AM, July 26

Poster Session: 10:00 am - 12:30 pm
Pediatric/Fetal Clinical Chemistry

A-149

Harmonization of Measurements of Pregnancy Associated Plasma Protein-A (PAPP-A) and free beta-human chorionic gonadotrophin (FbhCG) on different automated analysers

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Pregnancy-associated plasma protein-A (PAPP-A) and free beta-human chorionic gonadotrophin (FbhCG) are the two established biochemical markers that are used, along with the ultrasound marker nuchal translucency, in the routine prenatal screening of chromosomal abnormalities in the first trimester of pregnancy.

The dominant method for the determination of the two biochemical markers is the time resolved amplified cryptate emission (TRACE) technology on Kryptor analyzer (Brahms Gmbh, Berlin, Germany).

Automated immunoassays for PAPP-A and FbhCG have also been released from Siemens (Siemens Healthcare Diagnostics Inc, Tarrytown, NY) and from Roche (Roche Diagnostics GmbH, Mannheim, Germany). The Siemens and Roche methods are enzyme-labeled chemiluminescent immunometric methods which run on the Immulite and Elecsys analyzers respectively. Recently, Siemens released a new recalibrated assay for PAPP-A in order to be aligned with the Brahms-Kryptor measurements.

The aim of our study was to evaluate the new Siemens Immulite 2000 PAPP-A and FbhCG assays as well as the old Siemens Immulite 2000 and Roche Elecsys 2010 assays in comparison to Brahms Kryptor method. For each one of the three comparisons we used different series of fresh serum samples from pregnant women in the first trimester of pregnancy.

We used linear regression analysis to calculate Pearson correlation coefficients (r), slopes and intercepts along with their 95% CI using Brahms Kryptor measurements as the independent variable. The results are shown in the following Table.

Compared method	N	Slope	Intercept	r
		FbhCG		
New Siemens Immulite	115	1.05 (1.01-1.08)	-0.53 (-2.8-1.7)	0.986
Old Siemens Immulite	141	1.03 (1.0 - 1.06)	-0.19 (-1.9 - 1.6)	0.988
Roche Elecsys	84	0.96 (0.88-1.05)	1.92 (-1.9 -5.8)	0.928
		PAPP-A		
New Siemens Immulite	115	0.99 (0.94-1.03)	-0.40 (-0.67- -0.12)	0.969
Old Siemens Immulite	140	0.60 (0.57-0.62)	0.05 (-0.04-0.14)	0.979
Roche Elecsys	86	0.97 (0.91-1.03)	0.01 (-0.3 -0.23)	0.961

The new recalibrated Siemens Immulite assay for PAPP-A seems now to be in agreement with the Brahms Kryptor assay (slope = 0.99) compared to the old Siemens Immulite assay which measured values almost 40% lower. The new and the old Siemens Immulite assays for FbhCG are both in agreement with the Brahms Kryptor assay. Roche Elecsys assays for FbhCG and PAPP-A are both in agreement with the corresponding Brahms Kryptor assays but with lower correlation.

A-150

Pediatric Reference Intervals for Alpha-Fetoprotein by 2 Immunoassay Analyzers

S. L. La'ulu[1], K. J. Rasmussen[1], W. L. Roberts[2]. *[1]ARUP Institute for Clinical and Experimental Pathology, Salt Lake City, UT, [2]Department of Pathology, University of Utah, Salt Lake City, UT,*

Increased concentrations of alpha-fetoprotein (AFP) are observed in a number of diseases. It is primarily used to aid diagnosis of hepatocellular carcinoma and as a marker of other tumors. A pediatric reference interval study was conducted for AFP using the Beckman Coulter Access 2 and Roche Diagnostics Modular Analytics E170 analyzers. Subjects undergoing elective surgical procedures at Primary Children's Medical Center were enrolled after obtaining parental permission. All subjects were excluded for known medical conditions, medication use, or if the parent did not provide consent. The study included 447 females and 466 males from 6 months through 6 years of age. Blood was collected into serum separator tubes, allowed to clot for 30 minutes at room temperature, and centrifuged. Aliquots were stored in liquid nitrogen prior to testing. An aliquot was thawed, mixed, and centrifuged prior to analysis by the Access and E170 analyzers on the same day. The results were analyzed and partitioned by age and gender. Due to insufficient sample size for some groups (n<120), transformed parametric reference intervals were established. When no statistically significant differences were observed, gender and/or age groups were combined. Analysis of group combinations were performed independently by analyzer and resulted in the same groups for both analyzers. The proposed reference intervals are summarized in the table. Significant differences were observed between males and females for 6 -11 months of age and 1 year of age. No significant difference was observed between males and females for 2 to 6 years, therefore genders were combined. However, there was a significant difference observed between the 2 year subject group from the 3 to 6 year subjects for both genders. AFP reference intervals decreased with increasing age and reached adult concentrations by age 3 years.

Gender(s)	Age Range	N	Upper Limit (ng/mL)	95% CI* (Upper Limit)	Proposed RI (ng/mL)
Beckman Coulter Access					
F	6-11 months	40	96.9	64.2 - 140.7	0 - 97
M	6-11 months	65	59.8	43.7 - 79.9	0 - 60
F	1 year	72	40.6	29.7 - 54.2	0 - 41
M	1 year	65	16.6	13.3 - 20.5	0 - 17
F&M	2 year	134	10.6	9.0 - 12.4	0 - 11
F&M	3-6 years	537	4.8	4.5 - 5.1	0 - 5
Roche E170					
F	6-11 months	39	118.3	71.6 - 195.4	0 - 118
M	6-11 months	63	69.1	48.2 - 99.0	0 - 69
F	1 year	67	38.1	27.6 - 52.7	0 - 38
M	1 year	64	21.0	15.8 - 28.1	0 - 21
F&M	2 year	131	10.7	9.0 - 12.8	0 - 11
F&M	3-6 years	522	5.2	4.8 - 5.6	0 - 5

*CI = Confidence interval

A-151

Alpha-1-antitrypsin Deficiency in Fraternal Twins Born with Familial Spontaneous Pneumothorax

D. N. Greene[1], M. Procter[2], P. Krautscheid[2], R. Mao[1], E. Lyon[1], D. G. Grenache[1]. *[1]University of Utah, Salt Lake City, UT, [2]ARUP Institute for Clinical and Experimental Pathology, Salt Lake City, UT,*

Background: Spontaneous pneumothorax is a rare condition that occurs when air accumulates in the pleural space without preceding trauma or obvious underlying lung disease. A positive family history is found in about 11.5% of cases and is referred to as familial spontaneous pneumothorax (FSP). In adults and adolescents, FSP has been mechanistically and genetically linked to Birt-Hogg-Dube syndrome, Marfan syndrome, alpha-1-antitrypsin (AAT) deficiency, homocystinuria, and Ehlers-Danlos syndrome. In neonates, the mechanism of FSP is poorly understood and is likely multifactoral. FSP is uncommon in neonates with four cases reported in the literature. There has not been an etiology established for neonatal FSP. We report a case of fraternal twin boys born with a spontaneous pneumothorax.

Case: The twins' family history is remarkable for reactive airway disease and a female sibling born with spontaneous pneumothorax. The family had no history of connective tissue disorders, renal cancer, or dermatologic diseases. AAT phenotype analysis revealed identical, uninterpretable patterns in both infants. Additional investigations were performed to definitively identify what appeared to be a novel AAT phenotype.

Methods: Total AAT was measured using an immunoturbidimetric assay. AAT phenotype was determined using isoelectricfocusing electrophoresis followed by AAT immunodetection. DNA was extracted from whole blood and all coding exons of the *SERPINA1* gene (which codes for the AAT protein) were bidirectionally sequenced.

Results: Total serum AAT concentrations were 117 and 132 mg/dL for twin A and B, respectively (reference interval 100-200mg/dL). Analysis of their AAT genotype revealed that they were both compound heterozygous for rare *SERPINA1* alleles. Additional serum and whole blood specimens were collected from the twins and their parents. Total AAT concentrations of the mother and father were 153 and 107 mg/dL, respectively. AAT phenotype analysis of the parents revealed that each expressed one normal and one abnormal AAT variant. Gene sequence analysis revealed that both parents carried one normal allele plus one rare *SERPINA1* allele and that each twin had inherited both of the rare parental alleles. Allele S330F was inherited from the mother and A60T;K129E from the father. The A60T ($M_{6passau}$) mutation has been

previously reported to be a benign mutation; A60T has never been reported in cis with K129E. S330F (S_{munich}) has also been reported as a benign allele. Homozygosity for the rare A60T and S330F has not been reported; thus, the pathogenicity of the variants cannot be confirmed. The total AAT concentration in the second sample collected from twins A and B were 72 and 92 mg/dL, respectively, values compatible with an AAT deficiency. Since AAT is an acute phase reactant, the decrease in AAT concentrations between the two samples (11 days apart) is not unexpected following a traumatic birth. It is also noteworthy that twin A, who consistently had the lower AAT concentration, had a more severe case of FSP, requiring chest tube insertion and needle thoracentesis; twin B's FSP resolved without additional intervention.

Conclusions: These findings suggest that the combination of A60T;K129E and S330F AAT alleles are likely deleterious and may play a role in neonatal FSP.

A-152

Validation of Lamellar Body Counts (LBC) on a Sysmex XE5000 Hematology Analyzer

D. A. Dalenberg, N. A. Baumann, D. R. Block, A. K. Saenger. *Mayo Clinic and Foundation, Rochester, MN,*

Background: Respiratory distress syndrome (RDS) in neonates is caused by insufficient lung surfactant production and can lead to morbity and mortality in newborns. Laboratory testing to assess fetal lung maturity enables physicians to assess the risk for RDS prior to delivery. Pulmonary surfactant is synthesized by type II pneumocytes and packaged into lamellar bodies that pass into the amniotic fluid. The concentration of lamellar bodies in amniotic fluid can be used to determine fetal lung maturity. Since lamellar bodies (1-5 μm) are of similar size as platelets (2-4μm), a standard hematologic counter can be used to quantify the concentration in amniotic fluid.

Objective: To validate lamellar body counts (LBC) on a Sysmex XE5000 hematology analyzer and compare results with those obtained using the Abbott TDx FLM II assay.

Methods: Concordance between the Abbott TDx FLM II assay (surfactant/albumin ratio) and LBC on a Sysmex XE5000 was assessed using residual amniotic fluid samples submitted for routine clinical testing (n = 76). Amniotic fluid samples that were centrifuged, frozen, or had visible meconium or blood contamination were excluded. A cutoff of ≥55 mg/g was used for the TDx FLM II assay and a cutoff of ≥50,000/uL was used for the Sysmex XE5000 LBC to predict maturity. The cut-offs suggesting fetal lung immaturity were <40 mg/g and 15,000/uL for the FLM II assay and LBC, respectively. Results between the immature and mature cut-offs are considered indeterminate

Results: Imprecision studies demonstrated intra-assay CV's of 4.6% and 2.8% at concentrations of 16,200/uL and 53,900/uL, respectively and inter-assay CV's of 6.7% and 4.0% at concentrations of 13,700/uL and 59,400/uL, respectively. The lower limit of quantitation (CV < 10%) was 6250/uL with a CV of 7.1%. The assay was linear over the range of 5000 - 105,000/uL (r^2 = 0.99, slope = 0.99). Overall concordance between the TDx FLM II method and the Sysmex XE5000 LBC results was 72.4%. Concordance between immature and mature results was 96.8% and 55.6%, respectively.

Conclusion: Lamellar body counts on the Sysmex XE5000 demonstrated excellent analytical performance. Overall concordance between the two methods was acceptable; however agreement between the methods for predicting maturity was not optimal. LBC is a rapid, widely available, and inexpensive method for assessing fetal lung maturity, however clinical cut-offs for LBC on the Sysmex XE5000 need to be verified with outcome studies due to poor concordance with the FLM II assay.

A-153

Pediatric Reference Intervals for Follicle Stimulating Hormone

S. L. La'ulu[1], K. J. Rasmussen[1], W. E. Owen[1], W. L. Roberts[2]. *[1]ARUP Institute for Clinical and Experimental Pathology, Salt Lake City, UT, [2]Department of Pathology, University of Utah Health Sciences, Salt Lake City, UT,*

In infants and children, follicle stimulating hormone (FSH) concentrations rise shortly after birth and then fall to very low concentrations by 6 months in boys and 1-2 years in girls. Later, concentrations begin to rise again before the beginning of puberty and the development of secondary sexual characteristics. FSH and luteinizing hormone are used to diagnose delayed or precocious puberty in children. Measurement of FSH is also used to evaluate pituitary function and aid in diagnosis of pituitary or hypothalamic disorders. A pediatric reference interval study was conducted for FSH using the Roche Diagnostics Modular Analytics E170 analyzer. Subjects undergoing elective surgical procedures at Primary Children's Medical Center were enrolled after obtaining parental permission. All subjects were excluded for known medical conditions, medication use, or if the parent did not provide consent. The study included 407 females and 440 males from 6 months up to 7 years of age. Blood was collected into serum separator tubes, allowed to clot for 30 minutes at room temperature, and centrifuged. Aliquots were stored in liquid nitrogen prior to testing. An aliquot was thawed, mixed, and centrifuged prior to analysis. The results were analyzed and partitioned by age and gender. When no statistically significant differences were observed, gender and/or age groups were combined. The proposed reference intervals are summarized in the table. Significant differences were observed between females and males. For females, there was a significant difference observed between the age groups 6 months to 2 years, 3 to 4 years and 5 to 6 years. No significant difference was observed between age groups for males, therefore all male subjects were combined to establish the reference interval. Reference intervals for FSH should be gender-specific throughout life. Establishment of pediatric reference intervals for FSH should be useful in clinical practice.

Gender(s)	Age Range	N	Lower Limit (IU/L)	90% CI* (Lower Limit)	Upper Limit (IU/L)	90% CI* (Upper Limit)
F	6 months-2 year	160	1.4	1.0 - 1.5	8.0	7.2 - 9.4
F	3-4 years	124	0.6	0.5 - 1.0	5.0	4.3 - 6.1
F	5-6 years	123	0.5	0.3 - 0.6	3.2	2.9 - 3.4
M	6 months-6 year	440	0.3	0.3 - 0.3	2.2	1.8 - 2.5

*CI=Confidence interval

A-154

Pilot assessment of use of color intensity distribution by image analysis to classify results of the phosphatidlyglycerol (PG) agglutination test for fetal lung maturity (Amniostat-FLM-PG)

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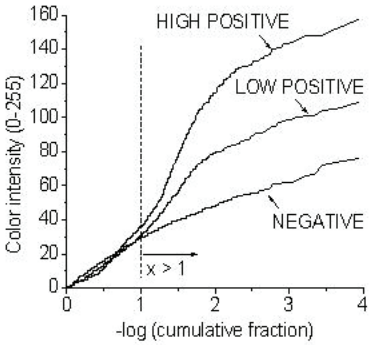
Background: The Amniostat-FLM-PG agglutination test (Irvine Scientific, Santa Anna, CA) is intended to indicate whether PG in an amniotic fluid sample is at least as great as that of a low-positive control (0.5 ug/mL). Test results are assessed visually based on particle size and color. Because qualitative visual assessment of weak agglutination relative to low-positive control is often difficult, we examined whether color intensity distribution of test results obtained by image analysis could provide an objective means of test interpretation.

Methods: Agglutination tests using kit-supplied negative, low-positive and high-positive control samples were conducted per kit instructions. After room-temperature drying (30 min), a digital image of each test was obtained using a scanner. Image analysis software was used to obtain the distribution of 8-bit (0-255) color intensities of pixels within the test area.

Results: An example of distributions of pixel color intensities are shown in the Figure (color intensity (y) vs. -log of cumulative fraction of pixels (x) above y). Areas of above-negative color intensity for positive controls involved only approximately 10% of total pixels (x>1). The color intensity distributions and areas-under curve (AUCs) for x>1 consistently distinguished negative, low-positive and high-positive controls in correct order. However, the ratio (R) of AUCs for low-positive controls relative to negative controls had high intra-assay variability. For example, R calculated for 9 possible combinations of 3 low-positive controls vs. 3 negative controls within a single run of CAP survey samples showed intra-assay CV of 14% (R=1.55+/-0.21, n=9).

Conclusions: Color intensity distributions can readily distinguish negative, low-positive and high-positive controls in the PG agglutination test. However, there was high intra-assay variation of AUCs for low-positive controls relative to negative controls. For this reason, AUC comparison to low-positive control as an absolute cutoff for designating weakly agglutinating samples as positive or negative in a singleton assay is not recommended.



A-155

First-trimester reference intervals for thyroid function tests for the Beckman Coulter UniCel® DxI 800 and the Roche Modular Analytics E170 analyzers

S. P. Wyness[1], S. L. La'ulu[1], W. L. Roberts[2]. *[1]ARUP Institute for Clinical and Experimental Pathology, Salt Lake City, UT, [2]Department of Pathology, University of Utah, Salt Lake City, UT,*

Thyroid function in pregnant woman is important to monitor, because abnormalities of the thyroid can lead to complications during pregnancy and lead to neonatal pathology. The objectives of our study are to establish method specific first-trimester reference intervals (RIs) for thyroid stimulating hormone (TSH), free thyroxine (FT4), free thyroxine index (FTI), thyroxine (T4) and thyroid hormone binding ratio (THBR). In our study we analyzed 2,090 surplus serum samples which had been previously submitted for first-trimester maternal screening (median age 31, weeks gestation 10-13). RIs for 134 healthy non-pregnant subjects were determined (91 females, 43 males, 18 to 64 years of age, median age of 29). All samples were previously tested for thyroglobulin autoantibodies (TgAb) and thyroid peroxidase autoantibodies (TPOAb) using an Abbott ARCHITECT $i2000_{SR}$ and only negative samples were included. Samples were analyzed by both Beckman Coulter UniCel® DxI 800 and Roche MODULAR Analytics E170 analyzers for TSH, T4, FT4 and T-Uptake. T-Uptake was used to calculate THBR and FTI for both methods as described in their respective package inserts. Non-parametric, central 95% RIs for TSH were calculated for all subjects. RIs for FT4, FTI, T4 and THBR were determined by analyzing only those samples which fell within the TSH RI for each method (table). Significant differences were observed between methods for both lower and upper RIs for FT4 and FTI and the upper RIs for TSH and T4. Lower and upper non-pregnant reference limits for TSH, FT4 and THBR were higher than those for pregnant subjects for both methods. Non-pregnant reference limits for FTI were higher for the DxI 800 and lower for the E170 compared to those for pregnant subjects. Method specific RIs are necessary for thyroid function tests for the first trimester of pregnancy.

Summary of Reference Intervals

Analyte/Subjects	DxI 800			E170		
	N	2.5th percentile (90% CI)	97.5th percentile (90% CI)	N	2.5th percentile (90% CI)	97.5th percentile (90% CI)
TSH (uIU/mL)						
Pregnant	2,090	0.04 (0.03 - 0.06)	2.98 (2.79 - 3.11)	2,090	0.03 (0.02 - 0.05)	3.40 (3.24 - 3.69)
Non-pregnant	134	0.32 (0.01 - 0.59)	4.21 (3.13 - 5.16)	134	0.36 (0.01 - 0.72)	4.77 (4.14 - 5.30)
Manufacturer	217	0.34	5.60	516	0.27	4.20
FT4 (ng/dL)						
Pregnant	1,987	0.57 (0.56 - 0.59)	1.04 (1.02 - 1.05)	1,987	0.86 (0.85 - 0.87)	1.42 (1.41 - 1.44)
Non-pregnant	128	0.61 (0.56 - 0.65)	1.10 (1.06 - 1.19)	128	0.89(0.80 - 0.93)	1.58 (1.50 - 1.65)
Manufacturer	316	0.61	1.12	801	0.93	1.7
1st trimester manufacturer	131	0.52 (0.47 - 0.57)	1.08 (1.08 - 1.27)	418	0.94	1.52
FTI						
Pregnant	1,987	4.93 (4.83 - 5.06)	10.00 (9.83 - 10.26)	1,987	5.71 (5.60 - 5.82)	11.78 (11.63 - 12.12)
Non-pregnant	128	5.11 (4.41 - 5.50)	11.43 (9.37 - 15.68)	128	4.77 (4.50 - 5.02)	10.77 (9.90 - 11.97)
T4 (ug/dL)						
Pregnant	1,987	6.67 (6.50 - 6.85)	13.00 (12.71 - 13.27)	1,987	6.70 (6.51 - 6.92)	14.59 (14.36 - 14.78)
Non-pregnant	128	6.08 (5.49 - 6.44)	13.71 (12.00 - 14.80)	128	5.10 (4.63 - 5.32)	12.46 (11.78 - 14.00)
Manufacturer	533	6.09	12.23	90	4.5	11.7
THBR						
Pregnant	1,987	0.61 (0.60 - 0.61)	0.92 (0.92 - 0.93)	1,987	0.72 (0.72 - 0.73)	0.95 (0.94 - 0.96)
Non-pregnant	128	0.68 (0.60 - 0.73)	1.03 (0.97 - 1.12)	128	0.79 (0.75 - 0.81)	1.16 (1.10 - 1.32)

A-156

Pediatric reference values for commonly used biochemical tests in central Ghana

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Background: Our aim was to establish age-specific pediatric reference values for commonly used biochemical tests that can be used for routine patient care and the screening and monitoring of children participating in clinical trials in central Ghana.

Methods: Serum samples from apparently healthy children <1 year (n=271), 1-4 years (n=422) and 5-12 years (n=485) were analysed for alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin, urea and creatinine on the Vitalab Selectra E Clinical Chemistry analyzer (Dieren, The Netherlands). Median (50% percentile) and reference values (2.5th and 97.5th percentiles) were determined nonparametrically for each analyte and age group using Stata 11 statistical software (Stata Corp, TX, U.S.A) according to Clinical Laboratory and Standards Institute / International Federation Clinical Chemistry C28-A3 guidelines. Outliers were removed using the Dixon test.

Results: The median and reference values for each analyte and age group are presented in Table 1 below:

Table 1: Median (and reference values) of biochemical and haematological tests for children in the middle belt of Ghana

Median (reference values)			
Age group	< 1 year	1 - 4 years	5 - 12 years
Biochemistry			
ALT (U/L)	17 (7-40)	21 (7-51)	20 (5-53)
AST (U/L)	41 (23-67)	37 (21-71)	31 (19-57)
Total Bilirubin (µmol/L)	6.3 (1.8-23.2)	5.9 (1.6-19.5)	6.7 (1.7-18.9)
Urea (mmol/L)	1.6 (0.6-3.6)	1.7 (0.5-4.2)	1.9 (0.6-4.5)
Creatinine (µmol/L)	31 (16-46)	34 (18-54)	50 (33-74)

Conclusion: We have established age-specific pediatric biochemical reference values that would be used to improve healthcare and the enrolment and management of children during clinical trials in central Ghana.

A-157

Serum Calcium and Phosphate levels in Ghanaian Children with Sickle Cell Disease

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Background: Biochemical abnormalities have been associated with sickle cell disease. Studies on serum phosphate and calcium levels in sickle cell disease have been conflicting and the paucity of information on the role of these ions in the pathogenesis and management of sickle cell disease. This study was aimed to determine the serum calcium and phosphate levels in Ghanaian Children with sickle cell disease.

Methods: Ninety six children (48 Hb SS and 48 Hb SC) aged 1 year to 12 years attending the sickle cell clinic of the Child Health Department of Korle Bu Teaching Hospital and 96 aged matched controls (Hb AA) were recruited for the study after an informed consent . Venous blood samples was collected from each subject and the serum was used for calcium, phosphate and albumin analysis using Jas reagents (Jas Diagnostics Inc, USA) on Atac 8000 random chemistry auto analyzer (Elan Diagnostics, USA). Statistical analysis was done by simple parametric methods.

Results: The mean serum calcium level in sickle cell disease patients was 2.08 mmol/L (+/-0.30) while that of the control group was 2.28 mmol/L (+/- 0.15). The mean serum phosphate level in sickle cell disease patients was 2.18mmol/L (+/- 0.70), while that of the control group was 1.48mmol/L (+/-0.60). The mean serum albumin level in sickle cell patients was 3.88 +/-0.78 g/dL, and that of the control group was 3.82 +/- 0.52 g/dL.

Conclusion: There was significantly lower calcium level in sickle cell disease patients compared with the control s at p<0.01. There was a significantly higher phosphate

level in sickle cell patients compared with the controls at p<0.001. There was no statistical difference in the serum albumin levels in patients versus the controls, thus eliminating any difference in the albumin binding to calcium. Hyperphosphatemia and hypocalcaemia was found in the sickle cell disease patients. Lowering the serum phosphate levels may enhance the prognosis of sickle cell disease patients.

A-159

Multicenter Performance Evaluation of the RAPIDPoint® 405 Neonatal Bilirubin Method

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Objective: To evaluate the accuracy and imprecision of the RAPIDPoint 405 neonatal bilirubin* (nBili) whole blood method when used by point-of-care (POC) operators.

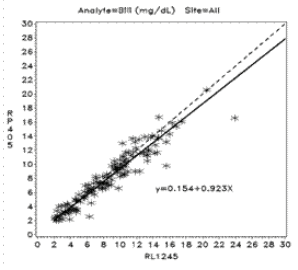
Relevance: Bilirubin is a bile pigment derived from the decomposition of hemoglobin. Measurement of nBili aids in the assessment of jaundice and the risk of kernicterus.

Methodology:The RAPIDPoint 405 (RP405) and RAPIDLab 1245 (RL1245) blood gas analyzers (Siemens, Tarrytown, NY, US) both feature a CO-oximetry module for reporting CO-ox fractions and total hemoglobin. The systems employ optical absorbency on nonhemolyzed whole blood specimens. At each of three clinical testing sites, four POC operators tested the RP405 and RL1245 analyzers with the following minimum design requirements: 5 days of reproducibility using three QC levels in quadruplicate, 15 days of method correlation using 40 whole blood neonatal bilirubin specimens in singleton, and continuous automated QC. Recommendations outlined in the CLSI EP05-A2 and EP09-A2-IR documents were used to assess imprecision and method correlation, respectively.

Results: RP405 nBili total (and within-run) %CV estimates were 2.6%-9.6% (2.3%-3.1%) for manual QC and 1.9%-3.1% (1.7%-2.6%) for automated QC. For the method correlation (all-sties combined); the RL1245 nBILI results (range = 2.1 - 23.9 mg/dL) resulted in the following Deming regression equation: RP405 = 0.92 (RL1245) + 0.15, R = 0.95 (Figure 1).

Conclusion: The RP405 nBili method demonstrated clinically acceptable imprecision and correlation with the RL1245. The RP405 offers a viable alternative for measurement of neonatal bilirubin suitable for use at the point of care.

* Not available in the US. Pending 510K clearance. For investigational use only. The performance characteristics of this product have not been established.



A-160

Prevalence of Impaired Kidney Function in Hospitalized Pediatric Patients

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Background: The severity of chronic kidney disease (CKD) is classified into 5 stages based on the calculation of an estimated glomerular filtration rate (eGFR). Recently, the Schwartz formula, used to estimate GFR in children, was found to routinely overestimate the eGFR due to a change in the methodology of serum creatinine determination. A new estimating equation has been generated by the Chronic Kidney Disease in Children (CKiD) study, which more accurately estimates GFR based on an IDMS standardized enzymatic creatinine measurement. The new equation makes it possible to estimate the GFR of children undergoing an assessment of their serum creatinine, blood urea nitrogen (BUN) and height. The goal of this study was to calculate the eGFR of the inpatient population over 6 months and estimate the prevalence of impaired renal function in a hospital setting Our program automatically calculated the eGFR from height, urea and IDMS-calibrated serum creatinine values from patients admitted to our institution..

Methods: Collection of eGFR and demographic data from hospitalized children between 1-22 years over 6 months. When multiple eGFR values were available on a single patient, the highest value was included in the analysis.

Results: During the 6 months, 1512 eGFR values were recorded. Mean age: 8.5 years, mean serum creatinine: 0.51 mg/dL, and mean eGFR: 101.82 mL/min/1.73m^2. Prevalence of low eGFR by CKD stage was: stage 4: 0.7%, stage 3: 3.1%, and stage 2: 27.4%. Data on the patients in each group are presented in Table 1.

Conclusion: Based on this initial study, more than one-quarter of hospitalized patients have impaired renal function, as determined by an automated estimation of GFR. The majority of these patients had an eGFR between 60-90 mL/min/1.73m^2. Further study of these patients will determine the ability of this screening process to detect CKD early in its course.

Table 1.

eGFR group (mL/min/1.73m^2)	Number of Patients	Age, Year Mean (SD)	Height, cm Mean (SD)	Serum Creatinine, mg/dL Mean (SD)	eGFR, mL/min/1.73m^2 Mean (SD)
>90	1043	8.2 (5.5)	125.5 (32.7)	0.4 (1.3)	113.9 (51.2)
60-90	410	9.4 (6.7)	128.1 (38.1)	0.6 (0.2)	79.8 (7.1)
30-59	47	10.4 (6.9)	113.6 (53.7)	0.9 (0.5)	46.1 (8.6)
15-29	10	11.0 (8.9)	129.7 (51.4)	3.4 (1.8)	22.1 (4.8)
<15	2	13.5 (0.7)	154.5 (0.7)	8.7 (4.8)	10.1 (6.8)

A-161

Cystatin C: election marker in chronic renal failure in pediatric patients

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Background: Glomerular filtration rate (GFR) is considered the best index for assessing renal function in both healthy subjects and in patients.

In clinical practice, creatinine is the most commonly endogenous marker used FG despite being subjected to different sources of biological variability and not sensitive enough to identify early stages of renal damage (serum concentration does not rise until the FG is not below 50%).

Therefore, the need for a simple marker of GFR, accurate and minimally invasive, remains a limiting factor in clinical practice to evaluate renal function.

Cystatin C is a non-glycosylated protein of low molecular weight, synthesized in all nucleated cells of the body, which has a wide tissue distribution.

Thanks to their physiological properties and that its plasma concentrations are not affected by muscle mass, diet and body surface area, Cystatin C has been proposed as a marker of glomerular filtration rate since 1985.

Methods: 52 children followed in pediatric nephrology diagnosed with chronic kidney disease (Stage I, II, III, predialysis), in which is determined by comparing creatinine and cystatin C for assessment of GFR. Added to the assessment values of weight, height, BMI, complete renal assay, microalbuminuria, and TA.

Cystatin C is determined in serum by intensifying particle immunonephelometry, using the BN. Siemens

Results: We confirm that there is no relationship between Cystatin C, weight, height and sex.

Of the 52 children studied by assessing the glomerular filtration rate by creatinine found the following **Results:** 50% in IRC stage I, stage II 36.5% and 13.4% stage III. Rating filtering by Cystatin C, 73.1% would be in stage I, stage II 17.3% and 9.6% stage III.

Conclusion: The glomerular filtration rate with Cystatin C in pediatric patients better discriminate the early stages of CKD, leading to changes in monitoring, treatment and prognosis

A-162

Comparison of Total and Direct Bilirubin Measurements Among Four Different Analyzers

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Background: Pathologic hyperbilirubinemia in neonates can result in acute bilirubin encephalopathy and permanent brain damage. Accurate and prompt measurement of neonatal bilirubin values is therefore essential to guide clinical treatment and prevent brain damage and neonatal death. Neonatal bilirubin values measured on one analyzer may not correlate with bilirubin values obtained using a different analyzer. This could negatively impact management and treatment of patients transferred between different care sites.

Objective: We aimed to evaluate the comparability of total and direct bilirubin results obtained on four different analyzers used at three sites within the Dartmouth-Hitchcock healthcare system, and two local hospitals outside the system. This difference between bilirubin values based on analytical test systems/methodologies is of particular importance as patients are often referred to our facility and follow-up testing may be performed in their home communities.

Methods: Total and direct bilirubin measurements were obtained on each of the four analyzers for fifty patient samples. All the Roche analyzers were in the Dartmouth-Hitchcock sites and the Dimension Vista analyzers were in the local hospitals. Results from each analyzer were compared with the other three analyzers. Comparability was assessed using linear regression and Bland-Altman plots.

Results: Linear regression results and mean percent biases for the comparisons are listed in the table below.

	Total Bilirubin	Direct Bilirubin
Roche Modular vs Roche Cobas 6000	y = 0.9673x + 0.1091 r^2 = 0.9985 Average % bias = 3.1%	y = 1.0265x - 0.0271 r^2 = 0.9976 Average % bias = -0.4%
Roche Modular vs Roche cobas c111	y = 0.942x + 0.1137 r^2 = 0.9992 Average % bias = 1.8%	y = 0.9983x - 0.0508 r^2 = 0.9770 Average % bias = -10.3%
Roche Cobas 6000 vs Roche cobas c111	y = 0.9729x + 0.0108 r^2 = 0.9988 Average % bias = -1.3%	y = 0.972x - 0.0235 r^2 = 0.9783 Average % bias = -9.5%
Roche Modular vs Dimension Vista	y = 0.8977x + 0.2724 r^2 = 0.9948 Average % bias = 4.7%	y = 1.0172x + 0.1129 r^2 = 0.9894 Average % bias = 11.3%
Roche Cobas 6000 vs Dimension Vista	y = 0.9276x + 0.1727 r^2 = 0.9954 Average % bias = 1.7%	y = 0.9897x + 0.1421 r^2 = 0.9893 Average % bias = 12.4%
Roche cobas c111 vs Dimension Vista	y = 0.9526x + 0.1653 r^2 = 0.9949 Average % bias = 3.1%	y = 1.0019x + 0.1964 r^2 = 0.9793 Average % bias = 31.4%

Conclusions: There was good correlation (r^2 >0.99) and limited bias (<5%) for total bilirubin measurements among all analyzers. For direct bilirubin measurements, there was good correlation (r^2 >0.97) but variable bias (0.4 - 31.4%) among most analyzers. The comparison data can serve as a useful tool for clinicians when bilirubin specimens are measured in different sites.

A-163

Performance Evaluation of the Minolta/Drager JM-103 Jaundice Meter

M. G. Rush, C. L. Wiley. *Providence Sacred Heart Medical Center and PAML, Spokane, WA,*

Background: In 2004 the American Academy of Pediatrics released a revision of their Clinical Practice Guideline, Management of Hyperbilirubinemia in the Newborn Infant 35 or More Weeks of Gestation, with its focus on reducing the incidence of severe hyperbilirubinemia and bilirubin encephalopathy. Bilirubin must be measured to assess the neonate's status. A transcutaneous bilirubin (TcB) measurement is a non-invasive method of obtaining that bilirubin measurement.

Objective: To assess the agreement between the crib-side Minolta/Drager JM-103 Jaundice Meter (TcB meter) and the core lab's Abbott Architect ci8200 Neonatal Bilirubin assay, which utilizes a spectrophotometric differential wavelength method.

Methods: Daily reports of neonatal bilirubin results from the Architect were printed. Candidates (newborns) for transcutaneous bilirubin measurement were taken from the list and tested within 2 hours over a five week period. Meter measurements were taken on the newborns' foreheads and the instrument gave an average of 3 readings. Newborns with a gestational age <30 weeks, readmits and those in phototherapy were excluded from the study.

Results: From the 110 measurements made with the TcB meter, 10 were excluded by the criteria noted above. The results of 100 measurements were analyzed using EP Evaluator. Architect results ranged from 0.9 to 13.2 mg/dL while the JM-103 range was 0 to 11.2 mg/dL. The regression statistics were: Slope = 1.21, Intercept = -2.4, Correlation Coefficient = 0.945. The Abbott ci8200 N. Bilirubin mean = 6.0 ± 2.8 (1 SD) and the JM-103 mean = 4.9 ± 3.3 (1 SD). Additionally, we evaluated accuracy at different levels and found that, overall, 81.0% of results were within ± 2.0 mg/dL of the lab value. Above 7.0 mg/dL, 91.4% were within ± 2.0 mg/dL and above 8.0 mg/dL, 92.3% of the meter values were within ± 2.0 mg/dL of the lab results.

Conclusions: The manufacturer reports that 66% of results fall within ± 1.5 mg/dL. In this study, 61% of results fall within that range. The JM-103's performance is acceptably close to the manufacturer's claims. Due to the positive slope and negative intercept, at lower values, the JM-103 shows a greater divergence from the lab Bilirubin results.

A-164

Metabolomics And Urine Ngal For The Early Prediction Of Kidney Injury In Healthy Adults Born Early Low Birth Weight (ELBW)

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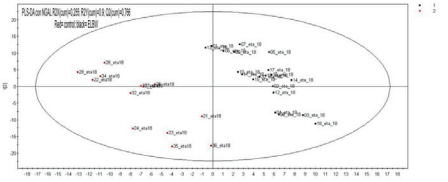
Background: metabolomic is a recent "omic" technology which is defined as "the quantitative measurements of the dynamic multyparametric response of living systems to the pathophysiological stimuli or genetic modification". In other words, a urine sample is considered as a person's metabolic fingerprint status at certain point of time. We have previously shown the metabolic differences between a group of 24 years old who were born with extremely low birth weight (ELBW) and a group of appropriate for gestational age (AGA) as control. The impairments found using ^{1}H-NMR were related to many pathways involving kidney injury. Recently, a new biomarker has been associated to kidney injury: NGAL. Therefore, we hypothesized measuring urine NGAL, (uNGAL) as kidney damage biomarker, in both groups (ELBW, AGA) with the aim to test and confirm our previous results.

Methods: the study was performed on two groups of people from the Pediatrics Division, Cagliari, and San Martino hospital, Genoa. The first group constituted of 18 ELBW (black spots, mean: 24years), while the second of 13 AGA (red spots, mean 25 years). A urine sample was collected from each patient, analyzed using 500 MHz spectrometer and spectra information was then subjected to multivariate analysis using SIMCA-P+ software. uNGAL was measured by chemiluminescent microparticle method, optimized on a fully-automated analytical platform (ARCHITECT, Abbott Diagnostics, Rome, IT); the cutoff was >130 µg/g urine creatinine. Groups are statistically different in uNGAL median value (p-value= 0,004)

Results: using a PLS-DA (Partial least squares discriminant analysis) we could correlate ELBW metabolic profiles with uNGAL concentration (median 577,34, range: 31,58-10223,88 µg/g creatinine), conversely uNGAL could not be correlated to AGA (median 10,6, range: 2,20-53,40 µg/g creatinine).

Conclusion: This study demonstrates the relevance of uNGAL as a biomarker which may predict a subclinical pathological process in the kidney.



Tuesday PM, July 26

Poster Session: 2:00 pm - 4:30 pm
TDM/Toxicology/DAU

B-01

Development of a Free Phenytoin assay by modification of the Siemens ADVIA 1800 Total Phenytoin Method

H. T. Richard, L. M. Bachmann, G. Gin. *Virginia Commonwealth University, Richmond, VA,*

Background: The commonly prescribed anti-epileptic drug, phenytoin, has a narrow therapeutic range and small changes in concentration may lead to toxicity. Phenytoin is approximately 90% bound to serum albumin and only the unbound, or "free" phenytoin is pharmacologically active. Estimation of free phenytoin from total phenytoin measurements using binding constants is inaccurate in patients with various disease states. Consequently, direct measurement of free phenytoin is clinically important. We developed a novel free phenytoin assay for use on the Siemens ADVIA 1800 system, for which total phenytoin (but not free phenytoin) is available.

Methods: To separate free from bound phenytoin, 750 µL of a serum specimen was added to a Millipore Centrifree YM 30 Spin Filter and centrifuged at 711 x *g* for 10 minutes at ambient temperature. The filtrate was used for further testing. Because the unmodified ADVIA Total Phenytoin method lacked adequate sensitivity for measurement of free phenytoin, the reaction mixture was modified by increasing the sample volume to 16 µL with a total reagent volume of 120 µL to produce an approximate 2-fold increase in calibrator absorbance response. Working calibrator solutions were prepared by dissolving purified phenytoin (≥ 99%, Sigma) into 50% MeOH/50% dH_20 for final concentrations of 0, 0.5, 1.0, 2.0, 3.0, and 4.0 µg/mL. Precision and accuracy were evaluated according to CLSI EP15-A2. Linearity was evaluated according to CLSI EP6-A. For interference testing, serum pools were prepared that contained total phenytoin concentrations of 10, 20 and 30 µg/mL. The pools were then spiked with exogenous hemolysate, ditaurobilirubin or intralipid. Interference testing was performed according to CLSI EP7-A2.

Results: The CV at the LoQ (0.5 µg/mL) was 10%. CVs for QC materials with concentrations of 1.5, 2.5, and 3.5 µg/mL were 4.8%, 4.4%, and 3.9%, respectively. Comparison of results for 33 patient specimens (range 0.5 - 3.0 µg/mL) to the Abbott Diagnostics TDx free phenytoin gave Deming regression equation: Advia (modified) = 1.0919 [TDx] - 0.0621 µg/mL, Sy.x = 0.21 µg/mL. The 95% CIs for the slope and intercept were 0.95 - 1.23 and -0.22 to 0.09 µg/mL, respectively. The assay had a linear response from 0.5 - 4.0 µg/mL with an r^2 of 0.9991. The control pools (equivalent volume of dH_20) for interference evaluation had free phenytoin concentrations of 0.9, 1.9 and 2.8 µg/mL. Addition of 1000 mg/dL hemolysate-equivalent hemoglobin resulted in <3% bias. However, 30 mg/dL of conjugated bilirubin resulted in an average bias of +38% for all three free phenytoin concentrations. 1000 mg/dL of intralipid resulted in an average bias of +18% for all three concentrations.

Conclusion: The Siemens Total Phenytoin procedure modified to measure free phenytoin with the ADVIA 1800 system met requirements for clinical use. However, interferences from icteric and lipemic samples were observed.

B-03

Potential Pitfalls in Free Phenytoin Measurements include Centrifugation Temperature

S. M. Truscott, K. Rappe, C. Daniel, S. A. Jortani. *University of Louisville, Louisville, KY,*

Background. Phenytoin is an anticonvulsant that is 90% protein-bound in serum. Only the free, non-protein bound fraction is pharmacologically active, so measurement of free phenytoin levels is critical when a patient's protein concentration is abnormal, or when co-medications might affect binding of phenytoin to serum proteins. *Ex vivo* conditions could also affect this equilibrium. Separation of free and bound forms of phenytoin is accomplished by centrifugal ultrafiltration. Our laboratory noted unexpected fluctuation of free phenytoin results and considered centrifugation temperature as a possible source of error. *The objective of this study was to determine the effect of centrifugation temperature on free phenytoin measurements in patient samples, quality control material, and in proficiency testing samples.*

Methods. De-identified remnant serum samples, commercial quality control materials, and proficiency testing samples were tested for free phenytoin concentrations after 15-minute centrifugation at either 25°C or 10°C. For some samples, we simulated technical difficulties with temperature regulation on the centrifuge. This was done by starting with the centrifuge at 10°C, adjusting the temperature setting to 25°C without allowing for rotor and centrifuge equilibration to 25°C, then immediately loading the samples and centrifuging for 15 minutes. Ultrafiltrates were loaded onto the Roche COBAS INTEGRA 800 automated analyzer with Roche Free Phenytoin in vitro diagnostic reagent pack, which quantifies phenytoin by fluorescence polarization immunoassay.

Results. Less free phenytoin was recovered from all sample types centrifuged at the lower temperature. Free phenytoin recovery in patient samples had a median decrease of 22% (range 11-47%, n=12). In quality control samples the median decrease was 27% (range 19-34%; n=4). Proficiency testing samples had the greatest decrease with a median of 31% (range 29-37%; (n=3). The decreases observed in remnant serum samples were statistically significant by Wilcoxon matched-pairs test ($p<0.003$, n=12). Low centrifuge temperature produced error which exceeded 3 SD based on our quality control data and on proficiency testing comparative statistics. When the centrifuge was not allowed time to equilibrate to 25°C before running the samples, free phenytoin measurements were still low and not significantly improved from the previous set of experiments in which the centrifuge was held at 10°C.

Conclusions. Decreased centrifugation temperature during ultrafiltration led to lower free phenytoin recovery. Decreases in recovery tended to be more pronounced in quality control materials and proficiency testing samples than in patient samples, presumably due to differences in matrix components. The Roche method is calibrated to quantify free phenytoin after centrifugal ultrafiltration at 25±3°C. Therefore, centrifuge temperature must be carefully monitored. Potential pitfalls include (1) failure to bring stored samples to room temperature before centrifugation, (2) failure to allow sufficient time for temperature equilibration of centrifuge and rotor, (3) failure of temperature sensors or temperature control in the centrifuge. Centrifuge temperature monitoring or verification is not specifically addressed in laboratory accreditation checklists produced by the College of American Pathologists. Thus it is advisable for laboratories to verify centrifuge temperature of their own accord.

B-04

Ultra-fast, Simultaneous Analysis of a Panel of Benzodiazepines in Human Urine Using an SPE-TOF System

M. V. Romm, N. Parikh, V. P. Miller, W. A. LaMarr, C. C. Ozbal. *BIOCIUS Life Sciences, Wakefield, MA,*

Background: Benzodiazepines are widely prescribed drugs for the treatment of anxiety, sleep disorders and other illnesses. Because addiction and abuse can occur with these drugs, efficient screening methods are critical to clinical, forensic and toxicology laboratories. We evaluated the ability of an ultra-fast SPE-MS system (RapidFire) to analyze a panel of benzodiazepines in human urine with much faster sample cycle times and similar analytical results compared to traditional LC-MS systems.

Methods: Calibration standards were prepared by spiking blank urine with a panel of 9 benzodiazepines to final concentrations ranging from 10 ng/ml to 5,000 ng/ml. Calibration standards and urine samples were spiked with internal standards (deuterated and ^{13}C labeled benzodiazepines) and processed using a liquid-liquid extraction (LLE) procedure. Sample analysis was performed at a rate of 9.5 seconds per sample using a RapidFire ultra-fast autosampler/in-line SPE system coupled to an Agilent 6530 Q-TOF. The SPE method consisted of a C4 column and elution with 100% acetonitrile. Data analysis was performed using RF*Integrator* software. This methodology is capable of throughputs >370 samples per hour.

Results: Feasibility was assessed using a panel of commercially available benzodiazepines spiked into blank human urine. A single generic SPE-MS condition was developed for the analysis of all compounds. Quality control standards were run in triplicate over a series of days to establish both intra- and inter-day precision and accuracy. Temazepam, for example, had a linear range of 10ng/ml to 5,000 ng/ml with an R^2 of 0.9998. The limit of quantitation for temazepam was approximately 3 ng/ml with a precision (%CV) of less than 10% and accuracies within 15% . Similar results were seen for all of the compounds investigated. Due to the high dynamic range and resolution of the Q-TOF, simultaneous analysis of the entire panel of benzodiazepines was investigated. Multiplexing the panel together into a single run (with a sample cycle time of 9.5 seconds) produced similar results to those obtained using singleton sample runs. These analytical results are comparable to those using LC-MS/MS, however the analysis time for SPE-MS/MS was approximately 20 times faster. Blinded samples will be evaluated to further validate this method.

Conclusion: A single 9.5s SPE-TOF method was developed that provided analysis results (precision and accuracy) for a panel of 9 benzodiazepines which were similar to LC-MS/MS methods. The simultaneous analysis of all 9 benzodiazepines in human

urine could also be achieved while retaining accuracy, precision and speed using the same methodology. SPE-MS/MS may be useful for the fast and efficient analysis of similar clinical research assays.

B-05

Metabolic ratios of excreted pain medications obtained by LC-MS/MS analysis can be used to determine aberrant drug metabolism

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Background: Patients on chronic opioid therapy are monitored for adherence using either qualitative or quantitative urine drug testing. LC-MS/MS analyses for an individual patient can also measure one or more metabolites along with the parent drug in the urine, such as morphine/hydromorphone, hydrocodone/hydromorphone, and oxycodone/oxymorphone. If a patient database of the metabolic ratio (metabolite divided by parent drug molar concentrations) of these pairs is established in a treated population, the usual inter and intrapatient expected range values can be defined and used to monitor variances in an individual's metabolism of a specific drug over time.

Object of the study: To establish a database of metabolic ratios for the parent drugs morphine, hydrocodone, oxycodone, methadone, and carisoprodol.

Methods: Urine from 250,000 patient samples sent to monitor patient adherence were analyzed by LC-MS/MS as previously published. (Ther Drug Monit 2009;31:746-748, Pain Physician. 2010;13;273-281, Pain Physician 2010; 13:71-78).

Results: Inter and intrasubject metabolic ratios were established for the following: hydromorphone/morphine, hydromorphone/hydrocodone, oxymorphone/oxycodone, EDDP/methadone, and meprobamate/carisoprodol as shown in the table.

Table of intersubject and intrasubject metabolic ratios

Drug pair	Intersubject geomean* metabolic ratio ×÷ GSD§	Intersubject 95% lower and upper limit	Intrasubject average geomean metabolic ratio ×÷ average GSD	Intrasubject average 95% lower and upper limit
hydromorphone/ morphine	0.007×÷1.9	0.002, 0.05	0.007×÷1.5	0.003, 0.02
hydromorphone/ hydrocodone	0.6 ×÷3.3	0.01, 1.7	0.2×÷2.4	0.03, 0.8
oxymorphone/ oxycodone	0.5×÷4.2	0.04, 4.4	0.4×÷1.6	0.04, 2.5
EDDP/ methadone	1.7×÷2.1	0.4, 13.8	1.7×÷1.7	0.6, 6.7
meprobamate/ carisoprodol	70.8×÷3.6	8.5, 504.0	63.0×÷3.4	9.9, 311.0

*geometric mean § geometric standard deviation

Discussion: We propose that the values in the table be used in the following manner: After the administration of any of the drugs in the table, the results of a patient's metabolic ratio should be expected to fall within the 95% confidence limits of the intersubject population. After this initial determination the variance of a patient on the second and subsequent visits should be within the 95% confidence limits. If this is not true, the treating physician should look for a cause. If this is not true, the treating provider should take a more detailed history and assessment of the patient to look for a possible cause keeping in mind that drug-drug interactions might be the most plausible.

B-06

Everolimus Measurement: Evaluating the Waters MassTrak Immunosuppressant XE Kit* for use with LC-MS/MS

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Background: The mTOR inhibitor, everolimus, is a candidate for therapeutic drug monitoring (TDM). Reflecting the trend observed in many clinical areas, there is an increasing use of liquid chromatography with tandem mass-spectrometric detection (LC-MS/MS) for TDM. Lack of standardisation of reference material and preparation of in-house calibrators have been highlighted as two main reasons for within- and between-centre variability. The objective of this study was to evaluate the performance of the Waters MassTrak Immunosuppressant XE Kit* (Kit) for use with LC-MS/MS to quantify everolimus in blood samples from renal and cardiac transplant recipients.

Methods: Precision (5-Day), linearity, recovery, dilution accuracy and potential interferences were investigated. Testing was performed on the Waters ACQUITY TQD LC-MS/MS system and methodology carried out as specified in the Kit's Directions for Use. Protein precipitation (200µL 0.1M zinc sulphate, 500µL acetonitrile containing 2ng/mL $^{13}C_2D_4$-everolimus internal standard) was used to extract everolimus from whole blood calibrators/quality controls/patient samples (50µL). Chromatography was on a MassTrak TDM C_{18} column (2.1x10mm) at 55ºC with a binary gradient (0.4mL/min) comprising de-ionised water and methanol, both supplemented with 2mM ammonium acetate and 0.1% formic acid. Positive electrospray ionisation was used to monitor everolimus (975.6/908.3,926.4) and $^{13}C_2D_4$-everolimus (981.6/914.3). Total run time was 1.5 mins. Calibration curves were constructed using six non-zero calibrators (0.8-32.5ng/mL) with 1/x linear regression. A method comparison study was performed for samples from renal (n=50) and cardiac (n=50) transplant recipients using the Kit and a fully validated in-house LC-MS/MS method (Comparator method), used routinely at St George's - University of London.

Results: The linearity of the Kit was confirmed using patient pools over the range 2.7-24.3ng/mL. Within-run and total imprecision (5-Day) was <6.9% and <7.5%, respectively. Recovery of everolimus from spiked drug-free whole blood over the analytical range 0.5-30ng/mL varied between 100 to 120% and from 103.7% to 106.0% for supplemented patient samples. Dilution accuracy ranged between -8.3 to 8.8% and -9.2 to 8.8% when the Kit's Calibrator 0 and drug-free whole blood were used as diluents, respectively. No significant interference on the performance of the Kit was observed related to either the anticoagulant or haematocrit. Deming regression was performed on the method comparison data obtained for the renal and cardiac samples following analysis by both the Kit and Comparator methods in order to predict the bias at the two medical decision points for everolimus. The predicted bias for both graft types, renal (3ng/mL: -13.3%, 8ng/mL: -3.8%) and cardiac (3ng/mL: -6.7%, 8ng/mL: -2.5%) data, met the acceptance criterion of being within +/-15%.

Conclusions: This evaluation indicates that the Kit is suitable for TDM of everolimus in renal and cardiac transplant recipients and that the use of this device could be a positive step in achieving harmonization of calibration for clinical laboratories using LC-MS/MS.

*NOTE: The MassTrak Immunosuppressant XE Kit is not available for commercial distribution in any market.

B-07

Evaluation of Thermo Scientific ClinSpec™ Immunosuppressants Test Kit

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Objective: We evaluated ClinSpec Immunosuppressants Test Kit manufactured by Thermo Fisher Scientific (Freemont, CA). This is a research use only (RUO) kit for simultaneous determination of tacrolimus (Tac), sirolimus (Sir), everolimus and cyclosporine A (CsA) levels in whole blood. The kit contains all the necessary reagents, including calibrator and control materials. The chromatography column as well as the recommended liquid chromatography and mass spectrometry (LC-MS/MS) parameters are also included. To assess the accuracy of the methodology, we compared patient sample results for Tac, Sir and CsA obtained using this kit to our in-house developed LC-MS/MS method. We did not evaluate everolimus at this time due to the lack of patient samples.

Methods: The sample preparation and chromatographic separations were carried out as per manufacturer's instructions. Briefly, 50 µL of sample is mixed with 150 µL of protein precipitation solution and analyzed after homogenization and centrifugation. The chromatographic separations were performed using the provided Thermo Hypersil Gold (5 mm, 3x10 mm) column. The MS/MS parameters were modified slightly to better suit our specific instrument, Thermo TSQ Vantage triple-quadrupole mass spectrometer attached to the APCI source.

Results: Inter-assay CVs were : Tac, 13.0% (3.3 ng/mL), 10.0% (11.9 ng/mL) 10.0% (28.5 ng/mL); Sir, 15.0% (3.0 ng/mL), 16.0%(11.7 ng/mL), 15.0%(28.9 ng/mL); CsA, 8.0-18.0% (31.3-1333 ng/mL). Functional sensitivities (20% CV) were 2.5 ng/mL for Tac and Sir and 20 ng/mL for CsA. No carryover was observed. All three drugs demonstrated good correlation with our current clinical assay ($r^2 > 0.90$). The following correlations were observed: y = 0.86x + 0.50 (Tac), y = 0.92x - 0.46 (Sir) and y = 0.78x + 4.71 (CsA) where y is the method we evaluated and x is our current LC-MS/MS clinical method.

Conclusion: We evaluated a commercially available kit for simultaneous measurement of common immunosuppressive drugs. This kit is suitable for any clinical laboratory, regardless of previous mass spectrometry experience, since it provides all the necessary components and starting conditions. We found it very easy and fairly straightforward to use. The biases observed between the ClinSpec method and our current clinical method are most likely result of calibration differences. The precision was acceptable, albeit not optimal. We feel that, if necessary, improvements in precision could be achieved by further optimizing chromatographic conditions.

B-08

Performance characteristics of a simple LC-MS/MS method for measuring fractionated urine metanephrines

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Background Fractionated urinary metanephrines measurement is one of the primary tests in pheochromocytoma diagnosis or exclusion. Liquid chromatography with electrochemical detection (LC-ECD) is commonly used to measure urinary metanephrines. Recently, however, LC-tandem mass spectrometry (LC-MS/MS) has been demonstrated as the most specific and accurate technology for this assay. Our objective was to develop a simple LC-MS/MS method for measuring fractionated metanephrines in urine with simpler sample preparation, and better specificity and accuracy as compared to a commercial LC-ECD method.

Methods Boronate complexes with the metanephrines were formed by treating urine samples with diphenylboronic acid. Then, each sample was extracted using a Varian Bond-Elute Plexa SPE cartridge. The final eluent was concentrated and injected on a short Atlantis T3 column (100x2.1mm, 3µ) where elution of metanephrine and normetanephrine was achieved using a gradient method with 10mM ammonium formate 1% formic acid, and 100% methanol for mobile phases A and B, respectively. Metanephrines and their deuterated internal standards were monitored in positive electrospray ionization mode by multiple reaction monitoring.

Results Due to potential isobaric interference between epinephrine and normetanephrine, we achieved baseline chromatographic separation of the two analytes within 8.5 minutes. Absolute ion suppression was observed, however, was compensated for by the internal standards. Using spiked patient urine samples, the analytical measurement range was determined by serial dilution and found to be 0.2-27.4 µmol/L and 0.3-14.6 µmol/L for metanephrine and normetanephrine, respectively. Precision was assessed based on CLSI EP10-A3 protocol. The intra-assay and total coefficients of variation throughout the linear ranges were 2.03-2.11% and 2.20-3.80% for metanephrine, and 4.50-8.09% and 9.00-10.00% for normetanephrine, respectively. A comparison of the current LC-MS/MS method with a commercial LC-ECD method was performed by analyzing duplicate patient samples (n=65). Passing-Bablok regression gave a slope of 1.000 and 1.014, y-intercept of -0.080 and -0.067, a correlation coefficient of 0.8830 and 0.9022, and a mean difference of 14.0% and -0.43% for metanephrine and normetanephrine, respectively.

Conclusion This LC-MS/MS method for measuring fractionated urine metanephrines requires simple sample preparation and is suitable for clinical use.

B-09

Comparison of Six Extraction Methods for Emergency Toxicology Screening

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Background A goal of emergency toxicology testing is to incorporate comprehensive test results into patient diagnosis and treatment. Therefore, rapid and comprehensive sample preparation techniques should be employed whenever possible. In this work, six methods of extraction are compared for emergency toxicology analysis via gas chromatography/mass spectrometry (GC/MS).

Objectives The objective of this study was to determine the best method of sample extraction for emergency screening of urine by GC/MS. The laboratory's current method of extraction (method A) was compared to other methods in order to incorporate a more comprehensive and timely protocol for emergency testing. Each method was evaluated for the number of drugs detected, timeliness, and chromatographic cleanliness.

Methods Fifty clinical and forensic urine specimens were de-identified and stored at -80°C until analysis as approved by the Institutional Review Board at the University of Mississippi Medical Center, Jackson, MS. Samples were neither hydrolyzed nor derivatized on the premise that drugs of abuse immunoassay screens would be performed prior to GC/MS analysis. Each specimen was extracted using four solid phase extraction (SPE) and two liquid-liquid techniques as follows: A) Bond Elute Certify (Agilent Technologies Inc.), B) Strata X Drug B (Phenomenex Inc.), C) Bond Elute PCX (Agilent Technologies Inc.), D) Clean Screen Excel I (United Chemical Technologies Inc.), E) Toxi-Tube A (Agilent Technologies Inc.), F) Generic Liquid-Liquid Extraction (1-chlorobutane). Each extraction technique was used as per manufacturer's recommendations or published methods for the extraction of four or five milliliters of urine. Each sample extract was evaporated to dryness using compressed nitrogen prior to reconstitution with 50 µL ethyl acetate and addition of the internal standard (2-amino 5-chlorobenzophenone) in dichloromethane. All samples were injected onto a single ion trap GC/MS (Thermo ITQ, Thermo Fisher Scientific Inc.). Spectral analysis was performed by a medical technologist with >5 years GC/MS experience. Spectral matches were determined using the Pfleger/Maurer/Weber library with positive cutoff criteria set to the following measures of spectral fit and purity: >500/1000 SI, and >750/1000 RSI. The time required for extraction was determined as the mean (n=5) +/- Standard Deviation. Caffeine was excluded from data analysis.

Results More drugs were detected using methods B and C than the other methods as follows: A=119, B=140, C=141, D=132, E=117, F=81 drugs. Methods B and C were relatively equivalent in the number of drugs detected, but a greater variety of drugs was detected using method C. The time (in minutes) required for each method was: A=47.3 (3.7), B=41.2 (4.2), C=32.7 (3.6), D=27.9 (2.9), E=19.3 (2.1), and F=29.4 (2.8). Method E exhibited the cleanest chromatograms.

Conclusion Methods B and C were the most comprehensive techniques evaluated in this study. Of these, a greater variety of drugs was detected using method C, which also required 8.5 minutes less time than method B.

B-10

Prospective Validation of Plasma S-Warfarin Concentration-Time Profiles Adjusted for CYP2C9 Genotype

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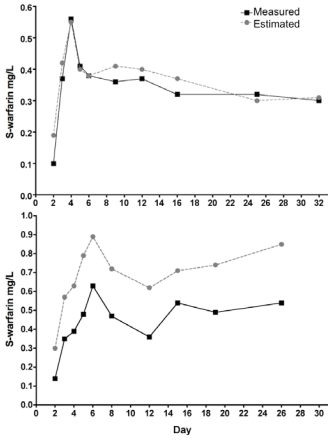
Background: We have previously validated PerMIT:Warfarin (Linder et al., 2009), a pharmacokinetic modeling tool which adjusts for changes in *CYP2C9* clearance resulting from genetic polymorphism. The purpose of this study was to measure plasma *S*-warfarin concentrations in patients to prospectively validate the consistency between actual and estimated concentrations.

Methods: Approximately ten trough plasma samples were taken during the first month of therapy for each patient. A robust chiral HPLC assay was developed and validated for measuring *S*-warfarin with inter-assay CV less than 5 percent for warfarin controls at 0.25 and 0.5 mg/L. The absolute agreement between measured and estimated concentrations was evaluated in addition to analysis of concentration-time profile trends.

Results: Overall, 29 patients (*CYP2C9**1/*1 = 16; *1/*2 = 8; *1/*3 = 5) were evaluated resulting in a total of 281 samples. *CYP2C9**1, *2 and *3 allele frequencies were 0.78, 0.14, and 0.09, respectively, consistent with expected frequencies in a Caucasian population. Based on previous retrospective studies, a goal of at least 55% of the estimates within 0.15 mg/L of the measured value was set. Overall, 63.4% of estimated values were within 0.15 mg/L. By genotype, *CYP2C9**1/*1 had 66.5% (n=158), *CYP2C9**1/*2 had 50.7% (n=75) and *CYP2C9**1/*3 had 72.9% (n=48) within the limit (top panel case example). In 8 cases, it was evident that the model was consistently under estimating the clearance (longer half-life than actual) of *S*-warfarin resulting in inflated estimates (bottom panel case example). However, the concentration-time profile trends remained in agreement such that overall 77.8% measured and estimated values followed the same trend and accurately predicted steady-state status.

Conclusion: Concentration modeling performance of PerMIT:Warfarin met or exceeded analytical goals, prospectively validating pharmacokinetic adjustments for *CYP2C9* genotypes. This data supports the use of this model in prospective trials of pharmacogenetics-guided warfarin therapy. (Supported in part by ARUP Laboratories and NIH 2R44 HL090055)



B-11

A novel liquid chromatography-tandem mass spectrometry method for quantification of nineteen drugs and metabolites important for pain management

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Background: Monitoring pain management and illicit drugs/metabolites (Table 1) in urine is an important tool to pain management physicians in order to ensure compliance and detect drug abuse. Mass spectrometry-based methods have the advantage of high specificity.

Methods: 200µL urine was vortex mixed with 100µL internal standard solution and 100µL limpet glucuronidase in 1M sodium acetate solution. Samples were incubated at 60ºC for 16-24 hours after which 50% methanol (500µL) was added. The samples were then centrifuged at 15,500 g for 10 minutes. 100µL of supernatant was loaded onto a Cyclone Max TurboFlow column and a Cyclone-P TurboFlow column (both 0.5 x 50mm) followed by a Hypersil Gold PFP analytical column (150 x 4mm, 5u) monitored by a Quantum Ultra mass spectrometer in selective reaction monitoring mode. Identification was achieved by calculating the ratio of another transition to the base peak and quantification was based on peak area ratios of analytes to internal standards (Table 1).

Results: This method had a wide linear range for each analyte (Table 1) with an analytical recovery of 83.6-119.8%. Precision was based on EP10-A2 protocol. For spiked urine samples the within-run coefficients of variation (CV) were ≤9.8% and the total CV were ≤12.5% for all analytes at 3 levels. No significant carryover was observed. Commercial controls containing >100 therapeutic drugs and common endogenous substances were tested and showed no interference with this method. Comparison using 152 de-identified patient samples and spiked urine samples whose values spanned over linear ranges were concordant with commercially mass spectrometry-based methods.

Conclusion: This liquid chromatography-tandem mass spectrometry method was validated for measuring nineteen drugs and metabolites important in pain management with high sensitivity and specificity, and a wide analytical measurable range for each analyte.

Analyte	MRM Transitions	Internal Standard	Linearity (ng/mL)	Analyte	MRM Transitions	Internal Standard	Linearity (ng/mL)
Amphetamine	136.12→91.12, 136.12→119.13	d5-amphetamine 141.14→93.2	5-5365	Morphine	286.1→173.1, 286.1→201.05	d3-morphine 289.17→201.1	5-5365
Benzoylecgonine	290.13→105.04, 290.13→168.07	d3-benzoylecgonine 293.14→171.1	24-5410	Norfentanyl	233.17→84.1, 233.17→150.1	d5-norfentanyl 238.17→84.2	6-5514
Codeine	300.1→165.1, 300.1→215.06	d3-codeine 303.16→215.1	11-5484	Norpropoxyphene	308.22→100.07, 308.22→128.04	d5-norpropoxyphene 313.22→147.1	24-5734
Dihydrocodeine	302.101→199.03, 302.101→201.2	d6-dihydrocodeine 308.16→202.1	5-5184	O-desmethyl-Tramadol	250.2→58.2, 250.2→250.1	d3-tramadol 268.24→58.2	20-2066
2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP)	278.15→234.08, 278.15→249.09	d3-EDDP 281.2→234.2	6-4178	Oxycodone	316.1→241.05, 316.1→256.07	d3-oxycodone 319.18→259.2	5-4719
Fentanyl	337.2→105.06, 337.2→188.08	d5-fentanyl 342.27→188.2	6-5971	Oxymorphone	302.1→198.19, 302.1→227.1	d3-oxymorphone 305.17→230.2	5-4581
Hydrocodone	300.102→171.06, 300.102→199.1	d3-hydrocodone 303.19→199.1	5-5270	Propoxyphene	340.2→58.24, 340.2→266.3	d5-propoxyphene 345.26→58.2	11-2826
Hydromorphone	286.11→157.1, 286.11→185.1	d3-hydromorphone 289.17→185.1	5-4980	Tramadol	264.21→58.2, 264.21→264.2	d3-tramado 268.24→58.2	25-5208
Methadone	310.5→105.04, 310.5→265.1	d3-methadone 313.25→268.2	6-4897	11-nor-9-carboxy-delta9THC	343.2→245.2, 343.2→299.2	d3-11-nor-9-carboxy-delta9THC 236.2→302.3	21-5008
Methamphetamine	150.14→91.11, 150.14→119.1	d5-methamphetamine 155.14→92.2	5-5339				

B-12

New ARK IMMUNOASSAY FOR Methotrexate

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Background: Methotrexate (MTX), a classical antifolate, can be safely administered over a wide dose range as maintenance chemotherapy for acute lymphoblastic leukemia and treatment of nononcologic diseases including rheumatoid arthritis or psoriasis. When combined with leucovorin (LV) rescue, high-dose MTX (HDMTX; doses of 1,000-33,000 mg/m^2) is usually administered as a prolonged i.v. infusion for a variety of cancers, including acute lymphoblastic leukemia, lymphoma, osteosarcoma, breast cancer, and head and neck cancer. HDMTX can be safely administered to patients with normal renal function by vigorously hydrating and alkalinizing the patient to enhance the solubility of MTX in urine. Serum levels may reach 1000 µmol/L or more. Pharmacokinetically guided LV rescue by monitoring MTX serum levels is required to prevent potentially lethal MTX toxicity. Ability to measure MTX accurately at 0.05 µmol/L enables clinical determination of non-toxic status.

Objective: Evaluate the analytical performance of a new ARK Methotrexate Assay.

Methods: The ARK™ Methotrexate Assay is a homogenous enzyme immunoassay for quantifying MTX in human serum or plasma. The assay was evaluated on the Roche/Hitachi 917 system. Increasing reaction rate correlates to increasing MTX concentration for a six point calibration curve (0 to 1.20 µmol/L). Six-level (0.07, 0.40, 0.08, 5.0, 50.0, and 500.0 µmol/L) quality controls were run. Performance of the assay was determined by assessing precision, limit of quantitation, linearity, endogenous interference, specificity, proficiency samples from the Heath Control scheme and method comparison to Abbott TDx® MTX II Assay.

Results: Total Precision (%CV) for controls was 7.2% (0.07 µmol/L), 3.7% (0.40 µmol/L), 5.6% (0.80 µmol/L), 4.9% (1:10 of 5.0 µmol/L), 5.2% (1:100 of 50.0 µmol/L), and 6.4% (1:1000 of 500.0 µmol/L) respectively. Limit of Detection and Quantitation were comparable to that of the TDx **Methods:** LOD ≤ 0.02 µmol/L and LOQ was 0.04 µmol/L (12%CV, 98.8% analytical recovery). Analytical recovery was within 10% for nominal values 0.15 to 1.00 µmol/L. The ARK assay was linear from 0.035 to 1.26 µmol/L. Endogenous substances did not interfere with measurement of MTX at the levels tested. Crossreactivity to the major metabolite, 7-hydroxy MTX, was equivalent to that of TDx; 20 µmol/L of 7-hydroxy MTX in the presence of its parent molecule MTX (0.2 µmol/L in serum) resulted as 0.12% crossreactivity in both assays. Recoveries of MTX in proficiency samples from the Heath Control scheme were within 10% of spiked and consensus values. For method comparison (35 samples): ARK = 0.99 TDx - 0.00 (r^2 = 0.99) using Passing Bablok regression analysis.

Conclusion: The ARK Methotrexate Assay provided quantitative measurement MTX in serum and plasma on the Roche/Hitachi 917 and correlated with TDx Methotrexate II Assay. Its homogeneous enzyme immunoassay technology is well-suited for routine TDM of MTX on automated clinical laboratory systems.

B-13

Analytical Performance Assessment of a Thermo QMS Immunoassay for Everolimus on the Roche/Hitachi MODULAR Analytics P800 and cobas c501Systems

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Background: Everolimus is kinase inhibitor used primarily in preventing organ rejection in kidney transplant and also in the treatment of advanced renal cell carcinoma. It is a rapamycin derivative that inhibits mammalian target of rapamycin (mTOR) which reduces cell proliferation and is primarily protein bound *in vivo*.

Objective: This study examined the analytical performance of a new Quantitative Microsphere System (QMS) immunoassay developed by Thermo Scientific for everolimus on the Roche/Hitachi MODULAR Analytics P800 clinical chemistry module and Cobas c501 analyzer.

Methods: The Thermo QMS Everolimus immunoassay is based upon competition between drug in the sample and drug coated onto a microparticle for antibody binding sites in the reagent. In the absence of everolimus in the sample, the everolimus-coated microparticle is agglutinated in the presence of the anti-everolimus antibody in the reagent. The rate of absorbance change is measured photometrically, and is directly proportional to the rate of agglutination of the particles. If the sample contains everolimus, the agglutination reaction is partially inhibited, slowing down the rate of absorbance change. A concentration-dependent agglutination inhibition curve can be obtained, where the rate of agglutination is inversely proportional to the concentration of everolimus in the sample.

Results: Within-run precision for the Thermo method using three levels of Thermo quality control material (n = 21, mean = 4.0, 7.8 and 15.5 ng/mL) ranged from 4.1 to 5.8% CV for P800 and 6.7 to 10.0% CV for c501. Between-day precision was analyzed in replicates of six, two times per day over a 5 day period for three levels of quality control material (n = 60, mean = 4.0, 7.8 and 15.5 ng/mL) and provided results that ranged from 4.6 to 7.9% CV for P800 and 11.0 to 13.7% CV for c501. The limit of detection was evaluated using materials devoid of everolimus and determined to be 0.43 ng/mL for P800 and 1.68 ng/mL for c501. Reportable range was evaluated using materials of known everolimus concentration analyzed in replicates of four and demonstrated analytical recoveries ranging from 72 to 121% for P800 (0.75 to 20 ng/mL) and 65 to 109% for c501 (1.5-20 ng/mL). Comparison of results from patient specimens using the Thermo method on both the P800 and c501with results from an LC-MS/MS method (n=105, range 1.8-19.8 ng/mL) demonstrated a Passing-Bablok regression equation of y=0.88x -0.08 (Pearson's derived r^2=0.89) for P800 and y=0.85x -0.62 (Pearson's derived r^2=0.84) for c501. Comparison between the P800 and c501 methods demonstrated a Passing-Bablok regression equation of y=1.01x -0.64 (Pearson's derived r^2=0.90). Bland-Altman bias analysis demonstrates a significant bias of -17.4% on P800 and -34.0% on c501 when compared to LC-MS/MS.

Conclusions: Overall, we find the Thermo everolimus QMS assay demonstrates acceptable analytical performance characteristics on both the P800 and c501 analyzers. However, the P800 appears to outperform c501 in analytical precision and sensitivity; both analyzers demonstrating significant bias when compared to an LC-MS/MS method.

B-14

Development of an immunoassay for the detection of meprobamate and the parent compound carisoprodol in biological samples

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Introduction. Meprobamate is the major active metabolite of carisoprodol and is used as a sedative, anxiolytic agent and muscle relaxant. It has a longer half life (6-17 hours) than the parent compound (1-3 hours). In the United States, meprobamate was listed as a schedule IV drug by the Controlled Substances Act. However, carisoprodol is not subject to federal control. Both meprobamate and carisoprodol are frequently encountered drugs in impaired driving casework.

Relevance. The availability of immunoassays enabling the detection of both compounds in biological samples is relevant for monitoring purposes. We report the development of a new immunoassay for the detection of meprobamate and carisoprodol, which is of value in test settings.

Methodology. An immunogen was developed for meprobamate and was administered to adult sheep on a monthly basis to provide target-specific polyclonal antisera. IgG was extracted from the antisera and evaluated via competitive immunoassay. The purified antibodies were immobilised on a biochip platform (9mm x 9mm), which is also the vessel for the immunoreactions. This chemiluminescent immunoassay is based on competition for binding sites of a polyclonal antibody between free meprobamate present in samples and horseradish peroxidase labelled conjugate. The semi-automated analyser Evidence Investigator was used. Analytical parameters were evaluated and agreement with GC/MS was assessed.

Results. The specificity of the assay, expressed as % cross-reactivity, was 100% for meprobamate and 57% for carisoprodol. The sensitivity value, expressed as IC50, was 8.2 ng/ml for meprobamate. The intra-assay precision (n=6), expressed as %CV was <10% for different concentration levels. Assessment of 58 whole blood samples and 72 spiked whole blood/plasma samples showed 100% and 96% agreement with GC/MS respectively.

Conclusion. The data indicate that this immunoassay is applicable to the detection of meprobamate and its parent compound carisoprodol in whole blood samples. This is of value for monitoring purposes.

B-15

The clinical utility of LC-TOF/MS drug screening in emergency intoxication cases referred to the San Francisco Poison Control Center

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Background: Stat serum and urine drug testing for emergency intoxication cases currently employ immunoassays and LC-MS/MS assays. Immunoassays, however, suffer from a limited scope of compounds they can screen and lack of sensitivity and specificity. LC-MS/MS provide high sensitivity and specificity but is limited to targeted screening. This has limited utility in frequently referred cases to Poison Control Centers where enough historical information is lacking to narrow down analytical testing to a target group of analytes. Current advances in the mass accuracy, resolution, sensitivity, and dynamic range of liquid chromatography- time-of-flight mass spectroscopy (LC-TOF/MS) have allowed its suitability for both targeted and non-targeted drug screening. This feature makes it an ideal platform for emergency toxicological screening.

Methods: We have set-up a rapid, generic, non-targeted urine and serum drug screening method using "dilute-and-shoot" or "crash-and-shoot" protein precipitation followed by chromatography on a C18 column with gradient elution (8 min run time) in Agilent LC 1200 and detection on Agilent TOF/MS 6230 with electrospray ionization in positive and negative polarity. The data were analyzed using Agilent MassHunter software and Forensic database by imposing a compound- mass tolerance ≤ 10 ppm, area count ≥ 5000, and a target score ≥ 70 (numerical comparison of theoretical and measured isotopic patterns) for compound hits. The combination of molecular formula matches to drug and/or drug metabolite obtained from the urine, serum, and when available, drug taken by the patient are used to report presumptive intoxicant data in real time.

Using the same generic method for non-targeted drug screening, we have also set-up semi-quantitative, targeted serum drug screenings for 44 seizure-inducing drugs (Seizure panel) and 205 drugs-of-abuse (DOA panel) to facilitate the resolution of cases involving unexplained seizures and patients with mentally altered status, the two most common referrals to the PCC . For these targeted screenings, a retention time window of ± 0.15 min was added as search criteria for compound hits.

Results: The over-all turnaround time (tat) for the targeted screening methods is 1-2h. The methods developed are analytically sound. Analytes in the seizure panel, for example, have LOD of 0.5-20ng/mL, linear concentration range of 200-500, %CV of 3-12% and 8-20% for within run and between run precision, respectively, and % recoveries of 70-105%. Similar analytical characteristics were obtained for the DOA panel. For more complicated cases requiring non-targeted screening, the over-all tat is 2-6h to obtain preliminary qualitative data. Using both approaches we were able to solve >70% of the 55 cases referred to the San Francisco PCC last year. These cases include 9 unexplained seizures, 10 therapeutic drug overdoses of which 5 are suicide attempts, 15 illicit drug overdoses, 3 new designer drug intoxications, 4 intoxications involving fraudulent drug sellers and 2 adverse drug reactions.

Conclusion: Except for a narrower dynamic range, analytically sound semi-quantitative, targeted drug screening methods with rapid turnaround time can be established using LC-TOF/MS. Combined with its ability to facilitate non-targeted screening, LC-TOF/MS makes an ideal platform for toxicology screening of emergency intoxications referred to the PCC.

B-16

Detecting Promethazine Use in the Methadone Maintenance Population by Liquid Chromatography Tandem Mass Spectrometry

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Background: Numerous drugs are known to augment the effects of methadone, providing the user with a euphoric effect. Promethazine, an antihistamine typically used to treat nausea and migraines, is one of the more commonly known "potentiators." One health concern associated with combining methadone with promethazine is the risk of QT prolongation, as both medication are known to block hERG K+ channels. In fact there has been a significant rise in the number of cases of Torsades de Pointe associated with methadone use in the past decade. No studies to date, however, have looked into the incidence of promethazine use in the methadone maintenance population.

Methods: Urine was collected from patients at the San Francisco General Hospital Opiate Treatment Outpatient Program over a one-month period. A 500uL aliquot

of each urine sample was alkalinized with NaOH, spiked with chlorpromazine-d3, an internal standard, and then extracted with ethyl acetate. The organic layer was evaporated to dryness and reconstituted in 100uL of 0.05% formic acid to achieve a 5-fold concentration of sample. Chromatographic separation was performed on a Phenomenex C18 2.6-um column (50x2.1mm) using a gradient of 0.05% formic acid and 1:1 acetonitrile/methanol with a run time of 6 minutes. Mass spectral analysis was performed on a 3200 QTrap LC-MS/MS from ABSciex. Each of the three compounds were monitored in MRM using the following transitions: 285.1-->86.0 (promethazine), 301.2-->198.0 (promethazine sulfoxide) and 322.1-->253.0 (chlorpromazine).

Results: Recovery for the liquid-liquid extraction was >90% for both analytes. For the LC-MS/MS method, four calibrators were used between 0 and 20 ng/ml. The assay was linear over the range of 0.625 - 25 ng/ml (R2=0.998) for promethazine and 0.16 - 20 ng/ml (R2=0.999) for the sulfoxide. The limit of detection for promethazine was 1.25 ng/ml, and 0.16 ng/ml for the sulfoxide. Intraday precision for both analytes fell between 5-10% and interday precisions between 12-14% (two sets of controls were used: 3 and 9 ng/ml for promethazine, 1 and 15 ng/ml for the sulfoxide). Carryover for the sulfoxide was observed at as low as 400 ng/ml, but none was detected for promethazine at levels up to 2000 ng/ml. Approximately 400 urine samples have been collected from the methadone clinic, and 100 have been assayed to date. So far, promethazine or its sulfoxide metabolite has been detected in 22 (22%) samples. After analysis of the urine is completed, positive results will be compared with corresponding demographic data - including age, sex, ethnicity, and clinic enrollment history - to identify any correlative patterns. Urine samples will then be subjected to general screening on the LC-MS/TOF to determine whether other drugs are also being used with any prevalence in this population.

Conclusion: We have developed a robust LC-MS/MS assay for detecting promethazine in urine. Premilinary results at The San Francisco General Hospital suggest that there is a substantial incidence of promethazine abuse in the methadone maintence population. Given the potential health risks associated with coingestion of methadone and promethazine, this assay plays a clinically useful role, particularly with regards to drug screening in the methadone clinic.

B-17

High-Throughput Analysis of Tacrolimus in Whole Blood Using Ultra-fast SPE-MS/MS

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Introduction: Fast, sensitive and accurate therapeutic drug monitoring of immunosuppressants is critical for transplant patient care. In many clinical laboratories, LC-MS/MS methods of analysis have replaced traditional immunoassays for monitoring immunosuppressant drugs because of their increased sensitivity and selectivity. However, LC-MS assays have slower turnaround times compared to immunoassays. We evaluated the ability of an ultra-fast SPE-MS/MS system to analyze tacrolimus in whole blood with much faster sample cycle times and similar analytical results compared to LC-MS/MS assays.

Methods: MS methods for tacrolimus and its internal standard ascomycin were optimized for analysis by QqQ MS. Calibration standards for tacrolimus (1-50 ng/ml) were prepared in bovine whole blood. The whole blood samples (500 µl) were mixed with water and precipitated using a (0.9 mM) zinc sulfate and methanol solution containing the internal standard. Precipitated samples were gently mixed and then centrifuged. Following centrifugation, supernatants were transferred to a 96-well plate for analysis. Samples were analyzed at a rate of 9.5 seconds per sample using a RapidFire (RF300) system coupled to a QQQ mass spectrometer. The SPE method consisted of a Phenyl column and elution with 100% acetonitrile. Data analysis was performed using RF*Integrator* software. This methodology is capable of throughputs >370 samples per hour.

Results: Prepared calibration standards and commercially available quality controls were run in triplicate over a series of days to establish both intra- and inter-day precision and accuracy. Tacrolimus had both intra- and inter-day accuracies within 15% and coefficient of variation values less than 10% for all concentrations within the linear range. This method had excellent linearity within the measured range of 1-50 ng/ml with an R^2 value greater than 0.999. Blank whole blood was treated and analyzed in the absence of internal standard in the same manner as the other samples to establish signal windows which were found to be greater than 60 to 1. These analytical results are comparable to those using LC-MS/MS, however the analysis time for SPE-MS/MS was approximately 20 times faster. Blinded samples will be evaluated to further validate this method.

Conclusions: Based on these results, Tacrolimus can be accurately and precisely measured in whole blood using ultra-fast SPE-MS/MS at rates of 9.5 seconds per sample. While the analytical results were comparable to LC-MS/MS, the analysis time was approximately 20 times faster. SPE-MS/MS may be useful for the fast and efficient analysis of similar clinical research assays.

B-18

Evaluation of Syva EMIT® and Microgenics DRI® Acetaminophen Assays on cobas c501 analyzer, in comparison to Roche Acetaminophen assay on COBAS INTEGRA® 800 System

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Background: Acetaminophen has a wide therapeutic range between 10 and 20ug/dL. However, liver damage does occur with chronic acetaminophen therapy and overdose. Accurate measurement of serum acetaminophen levels plays a vital role in the management of acetaminophen overdoses guiding treatment with antidotes to avoid hepatic necrosis. This study evaluated the Syva EMIT® and Microgenics DRI® acetaminophen assays on a Roche cobas c501 analyzer in comparison to the Roche acetaminophen assay on a COBAS INTEGRA® 800 system.

Methods: Both EMIT® and DRI® assays are homogenous enzyme immunoassay techniques based on glucose-6-phosphate dehydrogenase activity measured spectrophotometrically at 340nm. Within-day and between-day imprecisions were evaluated by measuring three levels of controls ten times a day or once a day for 25 days. Accuracy was determined by comparing acetaminophen measurements from EMIT® and DRI® assays from 30 serum samples to the results obtained from Roche method. Linearity was calculated based on results from five serum samples at acetaminophen 0, 50, 100, 150, and 200ug/dL. Limit of detection (LOD) was defined as the mean plus three times standard deviation of the blank serum measured five times and limit of quantification (LOQ) was defined as the acetaminophen concentration which gave 10% coefficient of variation (CV) calculated from the regression derived from CVs of the samples containing acetaminophen at 1, 2, and 2.5ug/dL. Hemolytic (H), icteric (I) and lipemia (L) interference effects were determined in sera containing acetaminophen 5, 10, and 30ug/dL and hemolyzed serum, bilirubin and intralipid to achieve different levels of H/I/L indices up to 1000, 60, and 1000, respectively.

Results: The within-day precision for EMIT® and DRI® at low, medium, and high controls were 5.3%, 2.5%, 4.5%, and 7.3%, 4.9%,5.4% CV, respectively. The between-day precision for both assays were 3.3%, 4.2%, 3.9%, and 7.5%, 5.9%, 6.8% CV, respectively. Correlation between the COBAS INTEGRA® assay and the Syva and DRI assays were both 0.9. LODs for EMIT® and DRI® were 0.4 and 3.0ug/dL, while LOQs were 1.1 and 3.1ug/dL. The Syva assay was linear to 240ug/dL with a slope of 0.99 and intercept of 2.71 (R2= 0.9993). The Microgenics assay was linear to 200ug/dL with slope of 1.05 and intercept of 1.15 at (R2=0.9998). No hemolytic or icteric interference was observed for the Syva or Microgenics assays at any concentration of acetaminophen. Roche assay was affected significantly by hemolysis. The Roche assay was affected by icterus when the icteric index was greater than 20, 30, and 50 at acetaminophen concentrations of 5, 10 and 30ug/dL, respectively. High turbidity levels showed slight interference with Roche acetaminophen assay. The Syva and Microgenic's assays were affected by turbidity (L index) in a negative fashion particularly at higher acetaminophen levels.

Conclusion: Both Syva EMIT® and Microgenics DRI® assays demonstrated excellent precision, accuracy, linearity, and interference performance on our cobas c501 analyzer. Samples with high L index, however, were recommended to be tested after ultracentrifugation. Cautions should be taken for samples with hemolysis and icterus when analyzed on COBAS INTEGRA® 800 system, although it seemed to be less susceptible to lipemia interference.

B-19

Simultaneous Extraction of Five Nucleoside Reverse-transcriptase Inhibitors

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Therapeutic drug monitoring is currently offered for numerous pharmaceuticals but the market lacks an efficient way to monitor nucleoside reverse-transcriptase inhibitors (NRTI). This need has been addressed with the following efficient, quantitative, simultaneous extraction of five nucleoside reverse-transcriptase inhibitors with subsequent HPLC UV-vis detection. Stavudine, Emtricitabine, Zalcitabine, Lamivudine, and Abacavir are extracted from plasma in less than ten minutes. The extraction involves the addition of Prothionamide (PTA) as an internal standard, the acid precipitation followed by centrifugation, the neutralization of the sample, and the

final centrifugation. Samples are then injected on a Phenomenex Luna 250 x 4.60 mm 5 micron column with an HPLC buffer comprised of sodium phosphate and 1-hexane sulfonic acid with a pH of 3.0. Baseline resolution of all compounds is achieved in 20 minutes at a pressure of 2500 psi. Table 1 outlines pertinent statistical factors from the assay validation. With linearity maintained over the range of 10.0 µg/mL to 0.05 µg/mL, one can quantitatively measure the concentration of these five drugs over their respective therapeutic range. The HPLC method was tested for interferences against 58 commonly used drugs and compounds. Although baseline resolution was not achieved for all interfering compounds, accurate integration of the desired peak was maintained. This new assay provides a simple, time-efficient method for the simultaneous extraction of five nucleoside reverse-transcriptase inhibitors.

Table 1.

Compound	Linearity (R^2)	Slope	Intercept	Range (µg/mL)	Within-Sample Precision (%CV)
Stavudine	0.9996	0.1962	0.0182	10.0-0.05	1.60
Emtricitabine	0.9997	0.1675	0.0083	10.0-0.05	1.43
Zalcitabine	0.9997	0.1342	0.0030	10.0-0.05	0.82
Lamivudine	0.9997	0.1656	0.0068	10.0-0.05	0.88
Abacavir	0.9998	0.2132	0.0140	10.0-0.05	1.01

B-20

Drug monitoring and toxicology: a procedure for the monitoring of levetiracetam by HPLC-UV

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Background: Levetiracetam is an anticonvulsant drug used to treat epilepsy. It is the S-enantiomer of etiracetam, structurally similar to the prototypical drug piracetam. Levetiracetam has been used as a monotherapy treatment for epilepsy in the case of partial seizures, or as an adjunctive therapy for partial, myoclonic and tonic-clonic seizures. The exact mechanism by which levetiracetam acts to treat epilepsy is unknown.

Levetiracetam has potential benefits for other psychiatric and neurologic conditions such as Tourette syndrome, autism, bipolar disorder and anxiety disorder, but its most serious adverse effects are behavioral and its benefit-risk ratio in these conditions is not well understood. It is also sometimes used to treat neuropathic pain.

Therapeutic drug monitoring of levetiracetam concentration is helpful to physicians in evaluating patient compliance with treatment, in providing guidance to achieve well-tolerated and effective dosing, and in identifying drug-drug interactions when drugs are given as polytherapy. Serum levetiracetam is generally measured by high-performance liquid chromatographic (HPLC) method. In some HPLC methods, multi-step extraction techniques and extensive sample pretreatment are used. Previously, measurement of serum levetiracetam was performed at the reference laboratory. The results turnaround time were not always satisfied and service of therapeutic drug monitoring was, therefore, lagging. The need for a quick measurement of levetiracetam in serum and the need for a cost-effective procedure prompted the development of a rapid HPLC method. Here, a reliable HPLC method is described for determination of levetiracetam in serum.

Methods: Serum (100µL) was vortex-mixed with methanol and the internal standard phenylethylmalonamide (300µL) for 1 minute and centrifuged at 10,350 g for 10 minutes at room temperature. The supernatant (ca. 300µL) was transferred to an autosampler vial, A small portion of supernatant (10µL) was injected directly onto the HPLC system. Separations of levetiracetam and phenylethylmalonamide were achieved by using a 5-µm Microsorb-MV reversed-phase C18 column (250 x 4.6 mm) and a mobile phase consisting of phosphate buffer (pH = 6.9, 0.05 M) and acetonitrile. The flow rate of HPLC run was at 1.1 mL/min and column temperature at 50°C. Peaks of levetiracetam and phenylethylmalonamide were monitored at 200 nm.

Results: The method achieved a linear concentration range of 1-100 mg/L, which covered the proposed therapeutic range of 12-46 mg/L for seizure control. The limit of detection was 0.2 mg/L. Both within-run and between-run precision for three fortified controls (20, 40, and 80 mg/L) in serum were lower than 7%. Analytical recoveries were greater than 95%. No interference was observed from the most commonly administered antiepileptic drugs (such as primidone, carbamazepine, ethosuximide, felbamate, lamotrigine, oxcarbazepine, phenobarbital, phenytoin, rufinamide, topiramate, valproic acid, and zonisamide). The method was compared to a reference laboratory HPLC assay using 30 samples ranging from 5 to 90 mg/L. The correlation showed a slope of 1.04, an intercept of 0.25 mg/L and an *r* of 0.94.

Conclusion: This method provides excellent reproducibility, requires no solid-phase extraction and one step deproteinization prior to chromatography. No interferences with other common antiepileptic drugs were observed. It is suitable for routine analysis of levetiracetam in serum.

B-21

CEDIA Tacrolimus Applications for the Ortho Clinical Diagnostics VITROS Systems

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Background. Tacrolimus (FK506) is an antibiotic with potent immunosuppressive function as prescribed for patients with kidney and liver transplantation. A therapeutic range of 5 to 20 ng/mL in whole blood is generally recommended for patients at standard risk of rejection. Monitoring of tacrolimus is thus important for effective use of the drug in the prevention of renal or liver allograph rejection. The Ortho Clinical Diagnostics VITROS™ 5600 Integrated System and VITROS™ 5,1 FS Chemistry System are new applications for the CEDIA Tacrolimus Assay. The CEDIA Tacrolimus assay uses the bacterial enzyme -Galactosidase that has been genetically engineered into two fragments. The assay is based on the competition of tacrolimus in the sample with tacrolimus conjugated to the Enzyme Donor (ED) fragment of -Galactosidase for antibody binding sites. In the presence of tacrolimus, the drug binds to the antibody, leaving the ED fragment free to form active enzyme with the Enzyme Acceptor (EA) fragment. In the absence of tacrolimus, the antibody binds to tacrolimus conjugated on the ED fragment, inhibiting the re-association of EA and ED. The amount of active enzyme formed and resultant absorbance change are directly proportional to the amount of drug present in the sample.

Methods. The performance of the CEDIA Tacrolimus application on the VITROS 5600 and 5,1 FS Systems was determined for precision ,linearity and accuracy against the Hitachi 917 System.

Results. Tests for within-run and total precision (N=80 per level) were run over 20 days. With-in run CVs were 14.1, 6.5 and 5.3% and total CVs were 19.4, 11.0 and 9.8% at 6.65, 13.9 and 18.9 ng/mL on the VITROS 5600 Integrated System. Within run CVs were 11.5, 6.5 and 5.9% and total CVs were 21.4, 9.2 and 9.8% at 6.74, 14.0 and 19.1 ng/mL on the VITROS 5,1 FS Chemistry System. Linearity was good across the reportable range of 2.0 ng/mL to 30 ng/mL. Agreement with the predicate Hitachi 917 also running the CEDIA Tacrolimus Assay was good using patient samples spanning the reportable range:

VITROS 5600 = 1.02 (Hitachi 917) + 1.0 with a correlation coefficient of 0.984

VITROS 5,1 FS = 1.00 (Hitachi 917) + 1.1 with a correlation coefficient of 0.975

Conclusions We conclude that the performance of the CEDIA Tacrolimus Assay on the VITROS 5600 Integrated System and the VITROS 5,1 FS Chemistry System warrants their introduction in clinical practice.

B-22

Development and Validation of a Mass Spectrometry Method for the Quantitation of Docetaxel Directly from Serum

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Background: Docetaxel (MW = 816.9 Da) is a semi-synthetic agent commonly used in cancer therapeutics. It is commonly implemented as a treatment option for breast and lung carcinomas. Docetaxel is largely plasma protein-bound (>98%), interacting with a high affinity to alpha$_1$-acid glycoprotein, lipoproteins and albumin. When administered intravenously at high doses, docetaxel causes significant adverse side effects, most notably severe neutropenia. In recent years, however, a more frequent, lower dose regimen of docetaxel has been implemented to combat the adverse side effects previously observed. This shift in drug administration has led to further investigation of the pharmacokinetic and pharmacodynamic profiles of docetaxel under these conditions. Thus, we describe the development and validation of a method for quantitation of serum docetaxel concentrations using turbulent-flow liquid chromatrography-tandem mass spectrometry and direct serum injection.

Methods: 25 µl docetaxel-spiked (Toronto Research Chemicals, Inc.) human sera and 10 µl deuterium-labeled internal standard (10 µg/ml) were directly injected onto the Aria TLX2 turbulent flow liquid chromatrography system (Thermo Fisher Scientific), which consists of a Cyclone-P column for on-line solid phase extraction and a Hypersil Gold C18 column for analytical separation. Samples were eluted with methanol (MeOH) containing 10 mM ammonium formate and 0.1% formic acid. Docetaxel was detected over a 7 minute run time using a TSQ Quantum Access tandem

mass spectrometer (Thermo Fisher Scientific) with a heated electrospray-ionization (HESI) source in positive ionization mode with selected reaction monitoring (SRM). Validation of this method was determined through the characterization of precision (five replicates twice a day over five days), linearity and recovery, and limit of quantitation. Additionally, carryover studies were conducted using high (2000 ng/ml) and low (20 ng/ml) calibrators in randomly alternating runs. All statistical parameters were evaluated with EP Evaluator 8.

Results: Precision was defined as running three levels of docetaxel (35 ng/ml, 350 ng/ml and 1500 ng/ml). The within run precision was evaluated giving a % CV of 16.7%, 13.7% and 13.2% for the aforementioned levels, respectively. Between run and between day precision were resulted with a % CV of 7.3%, 8.4% and 13.4% (between run) and 20.7%, 7.6% and 10.2% (between day) for 35 ng/ml, 350 ng/ml and 1500 ng/ml docetaxel, respectively. The described method was linear from 31.3 ng/ml to 2000 ng/ml (slope of 1.1) and mean recovery ranged from 101.1-108.1% over the linear range. The limit of quantitation (the lowest concentration with a CV <20%) was determined to be 31.3 ng/ml for this method. Carryover studies using high and low docetaxel calibrators resulted in a minimal carryover of 4.2 ng/ml, which is less than three times the SD of the low calibrator (1 SD = 8.8).

Conclusions: This developed and validated LC-MS/MS method allows for the quantitation of docetaxel from direct serum injections. Although this method uses the direct injection of a small quantity of specimen (25 µl) for drug analysis, more studies are being conducted to increase the sensitivity of the assay.

B-23

Development of a Rapid Quantitative/Semi-quantitative LC-MS/MS Method to Monitor Opioids and Glucuronide Metabolites

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Background: Managing chronic pain patients with opioid therapy is a difficult and controversial issue. Random urine drug screens are recommended by the APS and AAPM to help physicians detect aberrant behavior in patients, such as divergence (not taking prescribed drug, and selling or exchanging it for another drug). Several opioids are metabolized primarily to glucuronide metabolites; detecting only the parent compound can lead to false negative results. Regular screening is used to confirm patient compliance, and patients reported as negative are at risk for losing their therapy. Immunoassays are commonly used to screen, but they suffer from a lack of specificity. We have previously developed a quantitative LC-MS/MS method for 14 opioids that offers superior specificity and sensitivity compared with immunoassays. In this work, we aimed to develop a novel LC-MS/MS method to quantify the same 14 opioids and 6 new glucuronide metabolites with minimal sample preparation. While all of the opioids could still be quantified by isotope dilution, internal standards are not universally available for all of the glucuronide metabolites. In addition, several of the glucuronide metabolites are not stable in calibration mixtures. Moreover, it is more important to specifically detect an opioid or its metabolite than it is to accurately quantify the analyte. As a result, we aimed to develop a new semi-quantitative assay by LC-MS/MS using a 50ng/mL cut-off.

Methods: Urine specimens, calibrators, or controls were centrifuged and added in equal volume to 100 µL internal standard solution containing 14 dueterated opioids and 3 dueterated glucuronide metabolites in water. Ten µL were injected for analysis by LC/MS/MS. Separation was performed by UPLC on an Acquity HSS T3 column (2.1 x 50 mm, 3 µm). Mobile phase A contained 2 mM ammonium acetate in water, 0.1% formic acid and mobile phase B contained 0.1% formic acid in acetonitrile. The separation of all compounds was complete in 9 minutes.

Results: The method was evaluated for linearity, precision, and analytical recovery. The assay was linear between 10 and 1000ng/mL. Intra-assay imprecision (150ng/mL) ranged from 1.0 to 8.4% CV. Inter-assay precision ranged from 1.0 to 16%. Recovery was determined by spiking five patient specimens with opioid and glucuronide standards at100ng/mL. The patient specimens contained varying degrees of protein, bilirubin, and pH ranges. Recoveries ranged from 82 to 107% (median 98.9%). The method correlated well with our current quantitative LC-MS/MS assay for opioids. It is very important to point out that during our correlation study we found several patient samples that tested positive for glucuronides that would have tested negative when measuring the parent compound alone.

Conclusion: We have developed a quantitative/semi-quantitative method to simultaneously monitor 14 opioids and 6 of their glucuronide metabolites with minimal sample preparation. The assay can be used to identify non-compliance and diversion with high specificity in the chronic pain population.

B-24

A Highly Specific 6-Acetylmorphine Immunoassay for Detecting Heroin Use

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Background: Immunoassays directed against the unique metabolite of heroin, 6-acetylmorphine (6-AM), are increasingly used to screen for heroin use. These assays are more specific for assessing heroin use than opiate immunoassays which target morphine. However, in our population of patients treated for chronic pain, we observed a high false positive rate with the CEDIA 6-AM immunoassay, which was suspected to be due to interference from other structurally-related opioids.

Objective: To evaluate whether a recently approved automated urine 6-AM immunoassay is more specific than our current CEDIA 6-AM assay for detection of 6-AM.

Methods: A total of 214 urine samples sent to our laboratory from patients treated for chronic pain (192 consecutive, 22 previously screened positive for 6-AM by CEDIA) were analyzed by the Microgenics CEDIA Heroin Metabolite (6-AM) assay (Thermo Fisher Scientific, Inc.) and the 6-AM enzyme immunoassay (EIA) (Lin-Zhi International, Sunnyvale, CA) on the Olympus AU480 analyzer (Beckman Coulter, Inc.) using the manufacturers' recommended 10 ng/mL cutoffs. All positives by CEDIA and/or EIA were tested for 6-AM by GC/MS (5 ng/mL reporting limit). Samples were also tested for the presence of morphine (free and conjugated forms) and other opioids by LC-MS/MS (100 ng/mL reporting limit). Water spiked with free morphine concentrations up to 50,000 ng/mL was tested to determine the morphine cross-reactivity of both assays.

Results: CEDIA and EIA demonstrated equivalent sensitivity for 6-AM detection, both correctly identifying the eight 6-AM positive specimens. However, the EIA 6-AM assay provided improved specificity for 6-AM (100%) compared to CEDIA (91%). The CEDIA false positive rate was 69%. Upon investigation all but one of the eighteen CEDIA false positive samples were found to contain high levels (>30,000 ng/mL) of morphine. Subsequent spiking experiments revealed that spiked morphine concentrations as low as 12,500 ng/mL generated positive CEDIA results, whereas EIA showed no morphine cross-reactivity up to 50,000 ng/mL.

Conclusion: The EIA assay offers sensitive and specific 6-AM detection without the significant morphine cross-reactivity of the CEDIA assay. Implementation of the EIA 6-AM screening assay should facilitate accurate detection of recent heroin use in clinical laboratories that do not perform confirmatory testing.

B-25

Performance Charectersitcs Of Ark Diagnostics Quantitative Immunoassay For Levetiracetam On The Beckman Random Access Unicel® Dxc System & Comparsion To Lc-Ms-Ms Batch Analysis

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Background: Levetiracetam [(-)-(S)-α-ethyl-2-oxo-1-pyrrolidine acetamide] is a second generation anticonvulsant medication indicated as adjunctive therapy in the treatment of certain types of seizures in people with epilepsy. It is marketed under the trade name Keppra®. Levetiracetam is a single enantiomer and the precise mechanism(s) by which levetiracetam exerts its antiepileptic effect is unknown. However, high doses of levetiracetam can induce adverse effects, including dizziness, somnolence, asthenia, headache, behavioral problems, depression, and psychosis (Kanner et al., 2004). The therapeutic drug monitoring of levetiracetam concentrations plays an important role as an aid in management of patients treated with levetiracetam for toxicity issues.

Objective: To evaluate the performance characteristics of the new ARK Diagnostics Levetiracetam Assay on the Beckman Random Access UniCel DxC system for routine clinical laboratory use.

Methods: The ARK Levetiracetam Assay is a homogeneous immunoassay used in the quantitative determination of levetiracetam in human serum or plasma. When sample and reagents are mixed, drug in the sample competes with drug labeled by the enzyme glucose-6-phosphate dehydrogenase (G6PDH) for antibody binding sites. Enzyme activity decreases upon binding to the antibody so that the drug concentration in the sample can be measured in terms of enzyme activity. Active enzyme converts nicotinamide adenine dinucleotide (NAD) to NADH, resulting in an absorbance change that is measured spectrophotometrically. The NADH absorbance is directly proportional to drug concentration in the sample. Endogenous serum G6PDH does

not interfere because the coenzyme functions only with the bacterial enzyme (from Leuconostoc mesenteroides) used in the assay. The ARK Levetiracetam Assay was evaluated using the Random Access UniCel DxC 600 analyzer. The assay was calibrated using a six point calibration curve (0 to 100 µg/mL). Performance of the assay was determined by assessing precision, limit of quantitation, linearity, endogenous interferences, Heath Controls (NEQAS, UK) proficiency samples performance and correlation studies using LC/MS/MS batch analysis.

Results: Precision on tri-level controls was 7.3% CV (7.9 µg/mL), 6.5% CV (29.5 µg/mL) and 7.8% CV (79.1 µg/mL). Limit of Quantitation (LOQ) was 2.0 µg/mL. Linearity was demonstrated from 2.0 to 100.0 µg/mL. No common endogenous substances (Hb, bilurubin, gamma globulin, uric acid, albumin, cholesterol and triglyceride) interference was observed with the measurement of levetiracetam at the levels tested. Recovery experiment using spiked samples at 1.0, 2.0, and 3.0 µg/mL showed acceptable recovery. Correlation studies were done using 50 patient samples with level ranging from 2.0 to 61.0 µg/mL were analyzed using reference the LC/MS/MS method showing acceptable statistical results (Passing Bablok regression analysis: y (Beckman DxC) = 1.01 (LC/MS/MS) - 0.03, r2 = 0.95).

Conclusions: The ARK Levetiracetam Assay is suitable for the quantitative measurement of Levetiracetam in serum and plasma on the UniCel Random access DxC 600 System. This assay correlated with LC/MS/MS and is well-suited for routine TDM use on the UniCel DxC 600 Random Access Clinical System. Compared to LC/MS/MS method, which is time consuming and expensive and requires highly skilled technical staff, the ARK Levetiracetam Assay can be used in routine clinical chemistry laboratories and can generate results within 30 minutes.

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Global Tacrolimus Assay Proficiency Study

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Background: Current LC/MSMS and immunoassay test methods used to monitor tacrolimus concentrations in whole blood of allograft recipients are not standardized due to the lack of an internationally recognized tacrolimus reference material and reference method. The aim of this study was to assess the need for tacrolimus assay standardization.

Methods: A 40 member whole blood tacrolimus proficiency panel (2-30 ng/mL) was sent blinded to 23 clinical laboratories in 14 countries to be tested by the following assays: LC/MSMS (n=9), Abbott ARCHITECT (n=17), Siemens/Dade Dimension (n=5) and Microgenics (n=1). Select LC/MSMS laboratories (n=4) also received a calibrator panel (MassTrak kit, Waters Corp.). Test results from each laboratory were compared to the values of the blinded panel members obtained by a validated LC/MSMS method, which was designated as the provisional reference method.

Results: The range of CVs observed with the tacrolimus proficiency panel was as follows: LC/MSMS 11.4-18.7%; ARCHITECT 3.9-9.5%; Siemens/Dade 5.0-48.1%. The range of historical within-site QC CVs using controls was as follows: LC/MSMS low=3.8-8.9%, medium=2.0-6.0%, high=2.3-6.3%; ARCHITECT low=2.5-9.5%, medium=2.5-8.6%, high=2.9-18.6%; Siemens/Dade low=8.7-23.0%, medium=7.6-13.2%, high=4.4-10.4%. Assay bias observed between 4 LC/MSMS sites was not ameliorated by implementation of a common calibrator set.

Conclusion: The ARCHITECT assay gave better precision than either the LC/MSMS or Siemens/ Dade Dimension assays for the tacrolimus proficiency panel. Use of a common calibrator did not improve agreement between LC/MSMS methods. Tacrolimus assay standardization is required in order to provide optimized drug dosing and consistent care across transplant centers globally.

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High Throughput Analysis Using TFC-LC-MS/MS: Breaking the 2000 samples/system/day barrier in Quantitative Clinical Toxicology

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Background: Over the last 4 years, our laboratory has consolidated a number of previously utilized technologies (LC-UV, GC-MS) to a single analytical workflow, incorporating dilute and shoot/direct injection of analytically cassetted multi-analyte clinical toxicology panels. We have employed a generic 4-channel turbulent flow chromatography-LC separation modality (TFC-LC) with tandem mass spectrometric detection (AB Sciex API 5000) for real-time and batch oriented processing of targeted clinical toxicology confirmatory/quantitative analysis.

Methods: Generic sample preparation involves automated (Tecan Evo) 10-fold sample dilution of patient samples with appropriate isotopically labeled internal standards in 0.1/1% formic acid solution followed by mixing, centrifugation and injection. Each channel of the 4-channel Aria Transcend system (Thermo) is formatted identically, with a 0.5x5mm, 60micron particle size Cyclone P TFC column for analyte enrichment, and sample loading under turbulent flow conditions with 0.1% formic acid solution at 2mL/min for 15 seconds. Enriched analyte transfer to the chromatographic separation occurs via chromato-focussing mode in 20 seconds, with class specific loop composition elution conditions tuned to resolve co-extracted phospholipids, salts and high molecular weight proteins. Following refocusing, analytical separation is performed using an XDB C18, 50 x 2.1mm, 5micron LC column (Agilent) at 1-1.5mL.min, under class specific gradient conditions using 0.1% formic acid (elute pump A) and 90:10 methanol: water (0.1% formic acid) elute pump B. Increased analytical throughput is generated using method-folding principles to realize inject-to-inject cycles per channel of 2.5 minutes, together with acquisition windows per channel of 25-40 seconds in a class specific manner.

Results: The multi-factorial net results of these throughput enhancements are: 80% reduction in FTE labor, real-time and batch mode operation, open-access utility through common analytical setup (for each of the 4-channels) and 2400 samples per day capacity in operational utility.

Analytical and clinical validation parameters of accuracy, precision, linearity, carry-over, specificity and inter-assay correlation will be shown for each class (determined for each analyte independently within each class), conforming to CLIA, NCCLS and FDA method validation guidance. As an example, the tricylcic antidepressants panel assay (Amitryptiline, Nortryptiline, Desipramine, Imipramine, Doxepin, Desmethyldoxepin, Clomipramine and Desmethylclomipramine) exhibited selectivity (measured concentration <20% LLOQ) against >250 exogenous/endogenous analytes at supra-physiological levels. Blank defibrinated plasma (6 independent lots) was free of contribution to analyte/internal standards. Assay matrix effects were <10%, as determined by sample mixing, standard addition and post-column infusion. Assay carry-over was < 0.1% for all analytes and internal standards. Intra and inter-assay imprecision and bias was <11.4% for all analytes (n=20 replicates/batches) from 20 - 1000ng/mL; recoveries were between 94.99 and 114.83% for all analytes. Assay linearity (calibrator verification over 5 batches) and serial dilution indicated bias < 10% for each analyte. Inter-assay correlation statistics using deming methods (n>20 samples/analyte) indicated mean bias < 15%, correlation coefficient > 0.99 and deming slopes between 0.9 and 1.1.

Examples of clinically validated and operationally utilized multi-cassette assays for benzodiazepines, tricyclic antidepressants, fast-acting opiates, analgesics and other commonly measured clinical toxicology analytes will be shown.

Conclusion: Currently, the analytical system performs screening/confirmation for >50 analytes per patient sample in < 5 minutes.

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Simultaneous screening of tricyclic antidepressants, buprenorphine, MDMA and other drugs of abuse in urine and blood with matrix dedicated Evidence biochip array kits

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Background. Simultaneous screening of tricyclic antidepressants (TCAs), buprenorphine, MDMA and other drugs of abuse is relevant for applications in therapeutic drug monitoring, toxicology, forensic settings. The use of different sample types is now commonplace. There are limitations with generic methodologies for the analysis of different matrix types-typically using calibrator and control materials, which require large dilutions to different cut-offs. This may result in sub-optimal analytical reagent mixture for a particular matrix type. Evidence biochip array technology provides a platform for the simultaneous determination of TCAs, buprenorphine, MDMA and other drugs of abuse from a single sample using matrix dedicated kits. The miniaturizacion of the immunoassays, using this multi-analytical approach, reduces the volume of the sample and reagent per test and increases the results output.

Relevance. We report the applicability of this technology to the simultaneous determination of these compounds in urine and blood with matrix dedicated biochip array kits. This represents a valuable analytical tool for the rapid screening of batches of samples in test settings.

Methods. Competitive chemiluminescent simultaneous immunoassays are applied for the determination. The core of this technology is the biochip (9mm x 9mm), which represents the chemically activated solid phase where the ligands are immobilised and

stabilised defining microarrays of test sites and also the vessel where the reactions take place. The assays were applied to the fully automated Evidence analyser. The system incorporates the software to process, report and archive the data generated.

Results. Application to urine: The TCA assay detects approximately 12 compounds in this class including amitryptiline, desipramine, nortryptiline and trimipramine (% cross-reactivity 72%, 131%, 100%, 375% respectively). The sensitivity of the TCAs assay was 3.9 ng/ml. The buprenorphine assay exhibited a sensitivity value of 0.04 ng/ml and the MDMA assay 7.06 ng/ml. For other drugs of abuse the sensitivity values ranged from 0.04 ng/ml (opiates) to 50.9 ng/ml (methamphetamine). The intra-assay precision and inter-assay precision, expressed as %CV, were <13% and <19% respectively for all the assays.

Application to blood: The TCA assay detects approximately 14 compounds in this class including desipramine, nortryptiline and trimipramine (%cross-reactivity: 206%, 100% and 238% respectively). The sensitivity value of the TCAs assay was 2.04 ng/ml, the buprenorphine assay 0.03 ng/ml and the MDMA assay 1.31 ng/ml. The sensitivity values for the drug of abuse assays ranged from 0.07 ng/ml (oxazepam) to 13.19 ng/ml (methamphetamine). The intra-assay and total precision, expressed as %CV were <17.7% and <20% respectively for all the assays.

Conclusion. Data show applicability of biochip array technology to the simultaneous determination of TCAs, buprenorphine, MDMA and other drugs of abuse in urine and blood with matrix dedicated kits. This is of value for applications in therapeutic drug monitoring, toxicology and forensic settings.

B-29

EDDP Screening is Superior to Methadone Screening for Compliance Monitoring in Patients Treated for Chronic Pain

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Background: Methadone is a potent analgesic that is increasingly prescribed for the treatment of chronic pain. Urine methadone screening is frequently performed to monitor compliance and/or detect undisclosed use. A separate immunoassay measuring methadone's primary metabolite, 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP), has also been suggested to detect compliance in fast metabolizers and identify patients who spike their urine to simulate compliance.

Objective: To determine if EDDP screening can replace methadone screening as a more sensitive and reliable method for monitoring methadone use in patients treated for chronic pain.

Methods: A total of 1247 consecutive urine specimens from patients treated for chronic pain were screened by Microgenics Methadone DRI and EDDP CEDIA assays (Thermo Fisher Scientific, Inc.) on the Olympus AU480 analyzer (Beckman Coulter, Inc.) using 300 and 100 ng/mL cutoffs, respectively. All discrepant specimens were tested for methadone by GC-MS with a reporting limit of 100 ng/mL; select specimens were also tested for EDDP by LC-MS/MS with a reporting limit of 10 ng/mL.

Results: Of the 1247 specimens screened for methadone and EDDP, 200 were positive by both assays, 10 were positive by the EDDP screen only, and 5 were positive by the methadone screen only. The ten additional samples screening positive for EDDP only contained relatively low levels of methadone and/or EDDP (Table). These samples were considered to be true positives. The five samples screening positive for methadone only were unusual because they contained high concentrations of methadone (> 3000 ng/mL) but undetectable or very low concentrations of EDDP (<13 ng/mL). These five samples likely represent adulterated specimens from patients simulating compliance.

Conclusions: Overall, EDDP screening identified 5% more patients taking methadone than the methadone assay. The EDDP assay is a highly specific and more sensitive assay than methadone for monitoring methadone compliance in patients treated for chronic pain.

Table. Discrepant urine samples.

Group	Methadone (DRI)	EDDP (CEDIA)	Methadone (GC-MS) (ng/mL)	EDDP (LC-MS/MS) (ng/mL)
I (n=5)	POS	NEG	3406 - 28475	<10 - 13
IIa (n=7)	NEG	POS	116 - 2615	Not Performed
IIb (n=3)	NEG	POS	<100	160 - 276

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Analytical Performance Characteristics of the Abbott Architect i2000 Sirolimus assay: Comparisons with Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS) and Abbott IMx methods

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Background: Sirolimus, also known as rapamycin and Rapamune, is a macrocyclic antibiotic with immunosuppressive activity that is used extensively in solid organ transplantation, along with other immunosuppressive drugs. Its pharmacokinetics vary significantly among individuals, and response is correlated with blood concentration, thus making it a prime candidate for therapeutic monitoring, to avoid dose-related graft rejection or serious toxicity.

Objective and **Methods:** To support therapeutic monitoring, we have evaluated performance characteristics of the Abbott Architect i2000 assay (CMIA, chemiluminescent magnetic particle immunoassay) and compared its accuracy with LC-MS/MS (reference method) and another immunoassay, the Abbott IMx (MEIA, microparticle enzyme immunoassay).

Results: The limit of detection of CMIA is 0.25 ng/mL, and the analytical measurement range of the assay is 0.5-30 ng/mL. Total imprecision (%CV) was 7.6, 6.0 and 6.1 at concentrations of 4.81, 10.47 and 20.7 ng/mL respectively. Sirolimus concentration in specimens collected from various organ transplant patients was compared between CMIA, LC-MS/MS and MEIA methods. A comparison was also made based on storage of specimens at 2-8 ºC and -20 ºC. The Table below summarizes the the method comparison data.

Conclusions: We conclude that the CMIA method is a sensitive and precise method. Studies comparing the two different temperatures indicated that although the drug is stable at both temperatures, precision is improved with storage at -20 ºC than at 2-8 ºC. In addition, agreement of results is closest between the CMIA and LC-MS/MS.

Statistical Analysis of Patient Specimen Results:

Deming Regression Analysis Method Pair (y vs x)	n	r	Slope (CI) Intercept (CI)	Mean Bias* y-x (ng/mL)
Stored at 2 - 8° C				
CMIA vs. MEIA	66	0.808	1.48 (1.26 to 1.71) 1.73 (-0.33 to 3.78)	5.76
CMIA vs. LC-MS/MS	47	0.936	0.83 (0.74 to 0.92) 2.14 (0.75 to 3.54)	-0.37
MEIA vs. LC-MS/MS	47	0.788	0.58 (0.46 to 0.71) 0.29 (-1.62 to 2.21)	-5.72
Stored at - 20° C				
CMIA vs. MEIA	70	0.993	1.24 (1.20 to 1.28) -0.33 (-0.73 to 0.06)	1.94
CMIA vs. LC-MS/MS	20	0.895	1.11 (0.86 to 1.36) 1.23 (-1.60 to 4.05)	2.29
MEIA vs. LC-MS/MS	23	0.918	0.43 (0.35 to 0.52) 2.69 (1.28 to 4.11)	-4.91

*Bland Altman Analysis, n= Number of Specimens, r= Spearman Correlation Coefficient, CI= 95% Confidence Interval

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New Emit® II Plus 6-Acetylmorphine Assay+ on the Roche HITACHI 747 and 917 Chemistry Analyzers

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Background: 6-Acetylmorphine (6-AM) is a heroin metabolite and its presence in urine specifically confirms the illicit use of heroin. Applications for a new Emit® II Plus 6-Acetylmorphine Assay (6-AM) for human urine screening are currently being developed on the Roche HITACHI 747 and 917 Analyzers. The 6-AM assay has a cutoff of 10 ng/mL. It will meet the new Substance Abuse and Mental Health Services Administration (SAMHSA) Mandatory Guidelines for Federal Workplace Drug Testing Programs. The assay consists of two reagents that will provide qualitative and semi-quantitative results. The data presented in this study was generated on the Roche HITACHI 747 and 917 Analyzers.

Methods: Precision was evaluated using the cutoff and ± 25% controls according to CLSI EP5-A2. Recovery was studied by spiking 6-AM into human urine at levels that span the calibration range (0-20 ng/mL). Calibration stability and on-instrument stability were assessed by testing the cutoff and ± 25% controls over a 31-day period. Urine specimens were analyzed and the results compared to those from the GC/MS. Cross-reactivity with

structurally related drugs was assessed at different cross-reactant concentrations. The effect of common interferences was assessed by spiking the interferents into human urine in the presence of 6-AM at levels of +/- 25% of the cutoff.

Results: The qualitative repeatability precision CV's (rate) for the +/- 25% controls and cutoff ranged from 0.47 - 0.61%. The Within-Lab precision CV's ranged from 0.99 - 1.62%. The semi-quantitative repeatability precision (ng/mL) CV's ranged from 2.1-3.5% and the Within-Lab precision CV's ranged from 3.1 - 11.1%. The analytical sensitivity of the assay was found to be ≤ 2.2 ng/mL. The overlap rate between the +/- 25% 6-AM controls and the 10 ng/mL cutoff was less than 5%. Semi-quantitatively, the assay quantified 6-AM spiked samples between 2.5 - 20 ng/mL within +/- 20% of nominal values. At the 10 ng/mL cutoff, the percent agreement of 109 specimens between the new assay and GC/MS was> 99%. The assay reagents had minimal cross-reactivity (≤0.03%) with the structurally related drugs, morphine, morphine-3-glucuronide, morphine-6-glucuronide, and codeine. Potential interfering substances resulted in no false responses for the spiked ± 25% controls relative to the cutoff. A minimum of 7 (HITACHI 747) and 14 (HITACHI 917) days calibration stability was demonstrated. The reagents are stable on-board the analyzers for at least 30 days.

Conclusion: The new Emit® II Plus 6-AM Assay applications for the Roche HITACHI 747 and 917 Analyzers will provide suitable screening methods for urine specimens in both the qualitative and semi-quantitative analyses of 6-AM.

+ Product under development - Not available for sale

B-32

Validation of a Syva 6-Acetylmorphine Method on the AU5400 Clinical Chemistry Analyzer

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Introduction and Objective: 6-Acetylmorphine (6-AM) is a unique metabolite of heroin and its presence in urine is an indication of heroin use. Starting in October, 2010 the revised Mandatory Guidelines for Federal Workplace Drug Testing Programs require testing of all samples for 6-AM in order for labs to be SAMHSA certified. We describe the results of testing performed to assess analytical performance of a new Emit assay for 6-AM with a cutoff of 10 ng/mL. The assay performance was validated at LabCorp and PAML. This study validates the use of Emit® II 6-Acetylmorphine assay as an application on the AU5400 series of instruments.

Methods: The Emit 6-AM assay can be run in either qualitative or semi-quantitative modes. Studies performed for the Emit 6-AM assay included repeatability and within-laboratory precision over 20 days (CLSI EP5-A2) with + 25%, -25% controls and cutoff calibrator, concordance to GC/MS, proficiency sample and QC testing, calibration stability, and daily QC.

Results: Repeatability and within-laboratory precision CVs for the 6-AM assay were all less than 7.2 % at 6-AM concentrations ranging from 7.75 to 13.28 ng/mL (semi-quantitative). Both qualitative and semi-quantitative modes demonstrated 100% concordance to GC/MS on 84 negative urine samples (<5 ng/mL), 11 samples below the cut-off (5-10 ng/mL), 13 samples near the cut-off (10-15 ng/mL), and 87 positive urine samples (> 15 ng/mL). During daily quality control testing (23 days at site 1 and 21 days at site 2), all QC samples recovered as expected (Pos/Neg). Calibration stability (<15% drift) of at least 12 days was demonstrated. Testing of CAP proficiency samples and BDI[3] ±25% QC samples at both sites demonstrated complete agreement with expected results. At PAML, 24 additional frozen proficiency samples were tested by the Emit assay. Two samples were discordant based on initially high GC/MS values; one of these discordant samples demonstrated concordance with the Emit 6-AM result upon repeat by GC/MS. Degradation of 6-AM in the frozen proficiency samples is the likely reason for the initial discordance.

Conclusions: Precision for the Emit 6-AM assay is well within the clinical requirements for this assay. Concordance between the Emit 6-AM assay and GC/MS is excellent. Proficiency sample testing and Daily QC demonstrated acceptable performance. In conclusion, the Emit® 6-AM assay is suitable for use in screening urine samples for 6-Acetylmorhpine in accordance with recently enacted Federal requirements.

[1]Product under development, not available for sale.

[2]AU5400 series of clinical chemistry analyzers is a registered trademark of Beckman Coulter, Inc.

[3]Biochemical Diagnostics, Inc. DETECTABUSE® Liquid control urine, Confirm 2 (new SAMHSA guidelines) Edgewood, NY 11717.

B-33

Ordering practices for vancomycin measurement at a university hospital: comparison to practice recommendations

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Background: Numerous studies have documented overutilization of vancomycin with respect to prescription practice guidelines, but few studies have addressed appropriateness of utilization of vancomycin measurement. Vancomycin therapeutic guidelines (Clin Infect Dis 2009;49:325-7) present only general recommendations for monitoring, most specifically that "frequent monitoring (>1 measurement of trough concentration before the fourth dose) for short-course therapy (<5 days)...is not recommended." Use of random (non-trough) vancomycin measurements may be recommended by pharmacy, but only in circumstances of significantly impaired kidney function. We analyzed vancomycin test orders (random vs. trough), test intervals, and creatinine-based estimated glomerular filtration rates (eGFR) over a one-year period at our institution to determine whether laboratory data might indicate departures from recommendations on frequency of orders and use of non-trough measurements.

Methods: Lists of vancomycin results (with designations as trough, random or peak) and eGFRs were obtained for all patients over a 1-year interval (Dec 2009-Nov 2010). Data matching, categorizations and counting analyses using the two lists were performed using Visual Basic and Excel.

Results: There were a total of 10438 vancomycin measurements from among 3048 patients (average results per patient = 3.4). Trough (T), peak (P) and random (R) designations were T=63.7%, P=0.2% and R=36.1%. Median results for T and R were 14 ng/mL and 18 ng/mL, respectively. Only 2551 vancomycin patients (83.7% of total) were identifiable in the eGFR database. Among these patients, utilization of R measurements was dependent on eGFR: among patients with eGFR<30 mL/min (24.8% of patients, 37.8% of all vancomycin measurements), R comprised 67.9% of their vancomycin measurements (70.0% of all R measurements), whereas among patients with eGFR>60 mL/min (33.4% of patients, 26.4% of all vancomycin measurements), R comprised 11.2% of their vancomycin measurements (8.2% of all R measurements). Defining an encounter as any series of single patient measurements in which no measurement interval between successive samples was greater than 5 days, vancomycin measurements comprised 4224 encounters (1.4 encounters/patient), of 0 to 42 days duration. 1864 encounters (44.1%) were of single measurements only. 1530 encounters (36.2%) were of 1-5 days duration, for which the rate of vancomycin measurements was 1.22±0.46 per day. This ordering rate for 1-5 day encounters was uniform across eGFRs: for eGFR<30 mL/min (443 encounters, 10.5% of total), patients had 1.28±0.49 measurements per day; for eGFR>60 mL/min, (502 encounters, 11.9% of total), patients had 1.19±0.43 measurements per day.

Conclusions: The majority (70%) of R measurements were associated with severely impaired GFR; however, the remainder (30% of R, 10.8% of total) were outside of recommended practice. Even considering a maximum vancomycin dosing frequency of q8h (which would apply to only a minority of patients), the high frequency of vancomycin measurements (>1/day) for 5-day encounters across all patients suggests that monitoring frequency is considerably greater than that recommended by current guidelines.

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CEDIA Mycophenolic Acid Applications for the Ortho Clinical Diagnostics VITROS Systems

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Background. Mycophenolic Acid (MPA), metabolized from pro-drug mycophenolate mofetil or mycophenolate sodium, is widely used for the prevention of rejection in patients receiving renal, heart or liver transplants. Monitoring of MPA may be important for the effective use of the drug and for minimizing adverse side effects in patients. The Ortho Clinical Diagnostics VITROS™ 5600 Integrated System and VITROS™ 5,1 FS Chemistry System are new applications for the CEDIA Mycophenolic Acid Assay. The CEDIA Mycophenolic Acid assay uses the bacterial enzyme -Galactosidase that has been genetically engineered into two fragments. The assay is based on the competition of MPA in the sample with MPA conjugated to the Enzyme Donor (ED) fragment of -Galactosidase for antibody binding sites. In the presence of MPA, the drug binds to the antibody, leaving the ED fragment free to form active enzyme with the Enzyme Acceptor (EA) fragment. In the absence of MPA, the antibody binds to MPA conjugated on the ED fragment, inhibiting the re-association of EA and ED. The amount of active enzyme formed and resultant absorbance change

are directly proportional to the amount of drug present in the sample.

Methods. The performance of the CEDIA Mycophenolic Acid application on the VITROS 5600 and 5,1 FS Systems was determined for precision, linearity and accuracy against the Hitachi 917 System.

Results. Tests for within-run and total precision (N=80 per level) were run over 20 days. With-in run CVs were 2.0, 1.0 and 0.9% and total CVs were 5.9, 2.8 and 2.3% at 1.1, 3.4 and 7.0 µg/mL on the VITROS 5600 Integrated System. Within run CVs were 2.0, 1.2 and 0.7% and total CVs were 12.0, 6.0, 4.4% at 1.1, 3.3 and 6.9 µg/mL on the VITROS 5,1 FS Chemistry System. Linearity was good across the reportable range of 0.3 µg/mL to 10 µg/mL. Agreement with the predicate Hitachi 917, also running the CEDIA Mycophenolic Acid Assay, was good using patient samples spanning the reportable range:

VITROS 5600 = 1.04 (Hitachi 917) + 0.2 with a correlation coefficient of 0.998

VITROS 5,1 FS = 1.03 (Hitachi 917) + 0.3 with a correlation coefficient of 0.998

Conclusions We conclude that the performance of the CEDIA Mycophenolic Acid Assay on the VITROS 5600 Integrated System and the VITROS 5,1 FS Chemistry System warrants their introduction in clinical practice.

B-35

Biochip array-based immunoassays for the determination of zaleplon, zolpidem, zopiclone in biological samples

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Background. The Z drugs -zaleplon, zolpidem, zopiclone- are members of a nonbenzodiazepine class of drugs with similar effects to the benzodiazepines. They are used for the treatment of insomnia and when consumed for a prolonged period, induce tolerance and dependence.

The pyrazolopyrimidine zaleplon is rapidly adsorbed following oral administration, the blood concentration peaking after approximately one hour. Because of its rapid action and short half-life, zaleplon is increasingly being used in drug facilitated crimes and recreational abuse.

The imidazopyridine zolpidem, is metabolised to 4-[3-(2-N, N-dimethylamino-2-oxoethyl)-6-methylimidazo[1,2-a]pyridin-2-yl]benzoic acid (80%) and to a lesser extent to 3-(2-N,N-dimethylamino-2-oxoethyl)-2-(4-methylphenyl)imidazo[1,2-a] pyridin-6-yl carboxylic acid.

The zopiclone from the pyrazolopyridine class is extensively metabolised in the liver via decarboxylation, demethylation, and side chain oxidation. The major metabolites are zopiclone N-oxide and N-desmethyl zopiclone. Approximately 50% of the dose is converted to other inactive metabolites via decarboxylation.

Relevance. For therapeutic monitoring and also due to the increasing number of reports dealing with “drug-facilitated crimes” (robbery, mugging, sexual assault) related to benzodiazepines and benzodiazepines-like hypnotics, methods are required for the determination of these drugs. We report the development of three biochip array-based immunoassays for the detection of low levels of Z drugs and metabolites in biological samples.

Methods. Three polyclonal antisera were generated from adult sheep after separate administration of the following immunogens: zaleplon hapten conjugated to bovine thyroglobulin (BTG), zolpidem-hapten conjugated to bovine thyroglobulin (BTG) and zopiclone-hapten conjugated to bovine thyroglobulin (BTG) as carrier. The resulting antisera were used in the development of biochip immunoassays for the detection and quantification of the corresponding Z drugs. The competitive chemiluminescent immunoassays were applied to the semi-automated analyser Evidence Investigator.

Results. The specificity of the zaleplon biochip assay, expressed as % cross-reactivity, was 100% for zaleplon and the sensitivity value, expressed as IC50, was 0.27ng/ml. For the zolpidem biochipo assay the specificity was 100% for zolpidem and 71% for the metabolite with sensitivity values of 0.75ng/ml for zolpidem and 1.056ng/ml for the metabolite. The zopiclone biochip assay showed specificity of 100% for zopiclone, 112% for N-desmethyl zopiclone and 55% for zopiclone N-oxide with a sensitivity value of 0.72 ng/ml for zopiclone.

Conclusion. Results indicate that these immunoassays determine low levels of Z drugs and metabolites and are applicable to monitor the use or misuse of these compounds.

B-36

Immunoassays for the determination of drugs of abuse in whole blood on a biochip platform: comparison of analytical parameters with other immunoassay technique

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Background. Evidence biochip array technology provides a platform for the simultaneous screening of drugs of abuse from a single whole blood sample. The core of the technology is the biochip (9mm x 9mm) and represents not only the platform in which the capture ligands are immobilized and stabilised defining arrays of discrete test sites, but is also the vessel where the immunoassays are performed. Miniaturised simultaneous chemiluminescent immunoassays are employed with this technology. This leads to an increase of results output and a reduction of sample/reagent consumption.

Relevance: The availability of reliable matrix-dedicated immunoassays for the determination of drugs of abuse is relevant for clinical, regulatory, toxicological and forensic applications. We report the comparison of analytical parameters of five biochip array-based immunoassays for the determination of amphetamine, methadone, opiates, phencyclidine and tetrahydrocannabinol (THC) in whole blood with commercially available ELISAs.

Methodology. The chemiluminescent biochip immunoassays were applied to the Evidence Investigator analyser. The analytical parameters considered for comparison were limit of detection (LOD) and intra-assay precision. Both methodologies -biochip array technology and ELISA were carried out according to the manufacturers’ instructions.

Results. For the amphetamine, methadone, opiates, phencyclidine and THC biochip array-based immunoassays, the LOD values in whole blood were 5.68 ng/ml, 1.41 ng/ml, 1.64 ng/ml, 2.52 ng/ml, 7.34 ng/ml respectively. The intra-assay precision values for biochip array technology, expressed as %CV, were <12% (amphetamine biochip assay), <13% (methadone biochip assay), <20% (opiates biochip assay), <16% (phencyclidine biochip assay), <11% (THC biochip assay). Amphetamine, methadone, opiates, phencyclidine and THC analysis with the ELISAs showed LOD values of 3.84 ng/ml, 2.06 ng/ml, 1.01 ng/ml, 1.47 ng/ml and 9.11 ng/ml respectively. The intra assay precision for the ELISAs were >20% (amphetamine ELISA), >16% (methadone ELISA), >27% (opiate ELISA), >19% (phencyclidine ELISA), >17% (THC ELISA).

Conclusion: The results indicate superior reproducibility of the five Evidence biochip immunoassays for drugs of abuse and lower LOD values in whole blood of the methadone and THC biochip immunoassays when compared with the respective ELISAs.

B-38

Performance Evaluation of DAT Oral Fluid Barbiturates Assay on Roche Hitachi

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Objective: The study goal was to evaluate analytical performance of the DAT Oral Fluid Barbiturates Assay for determination of amobarbital, butabarbital, phenobarbital, secobarbital, and pentobarbital, in oral fluid under routine laboratory conditions. Imprecision and agreement with routine immunoassay and reference method was evaluated according to a standardized protocol.

Methods: Roche oral fluid turbidimetric immunoassay is based on kinetic interaction of microparticles in solution (KIMS). In conjunction with the Intercept® Oral Specimen Collection Device from OraSure Technologies, Inc. (OTI), the assay utilizes a single cutoff concentration of 20ng/mL. The assay has semi-quantitative and qualitative applications; only semi-quantitative was used in this trial. MODULAR *ANALYTICS* <P> module results are compared, with those of OTI Intercept® Microplate EIA. A combination of routine drug-of-abuse oral fluid samples and spiked samples were used for method comparison. Discrepant samples were analyzed by LC-MS/MS.

Results: Intra-assay imprecision (21 replicates/run; 3 runs):

Assay	Cutoff (ng/mL)	Sample Concentration (ng/mL)	Performance (SD)	(%CV)
Barbiturates	20	10	0.48-0.85	4.5-7.7
		20	0.60-0.90	2.9-4.5
		30	0.58-1.00	2.0-3.5

Method Comparison: 303 specimens were analyzed. All positives, all discordant specimens, and 10% of all negative specimens were confirmed. Overall agreement between Roche and OTI screening method prior to confirmation was 97.4%. Agreement between Roche method and LC-MS/MS was 100%.

Conclusion: Roche DAT Oral Fluid Barbiturate assay yielded a high level of agreement with OTI Intercept® Micro-plate EIA (>97%) and with LC-MS/MS (100%) in this study.

For Investigational Use Only. The performance characteristics of this product have not been established. MODULAR is a trademark of Roche. All other product names and trademarks are the property of their respective owners.

B-39

An Improved, No-Manual-Extraction Immunoassay for Tacrolimus on the Siemens Dimension® Clinical Chemistry System

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Background: Therapies combining lower dosages of tacrolimus with other immunosuppressant drugs to reduce nephrotoxicity in transplant patients require more sensitive methods for therapeutic monitoring of tacrolimus. We describe an improved, fully automated immunoassay for the measurement of tacrolimus* in whole blood on the Dimension system (Siemens Healthcare Diagnostics, Deerfield, IL, US).

Methodology: An EDTA whole blood sample (15 µL) is automatically lysed onboard, then incubated first with antibody-β-galactosidase conjugate and later with chromium dioxide particles coated with a tacrolimus analog. Tacrolimus molecules in the sample form immunocomplexes with the antibody conjugate, and the excess molecules of antibody conjugate are bound by the chrome particles. The chrome-conjugate complexes formed in the incubation are magnetically separated from the supernatant, which contains the tacrolimus-antibody conjugate complexes. The supernatant is then transferred to a photometric cuvette where the enzyme tag is detected using a sensitive chromogenic substrate.

Results: This new assay showed improved precision at lower tacrolimus concentrations over the original tacrolimus method on the Dimension system. Analytical and functional sensitivity were less than 0.2 and 1.2 ng/mL, respectively. The method was linear to 30 ng/mL, and calibration was stable for 30 days. Repeatability and within-lab reproducibility (%CV) on whole blood patient pools were measured to be 3.7% and 4.2% at 20.6 ng/mL, 3.0% and 3.7% at 10.7 ng/mL, 3.7% and 4.0% at 5.2 ng/mL, 3.7% and 7.1% at 2.4 ng/mL, and 6.0% and 12.0% at 1.2 ng/mL tacrolimus, respectively, per the CLSI EP5-A2 protocol over a 20-day testing interval. A correlation study comparing values for the revised Dimension method (TAC) and LC/MS/MS (LCMS) on split samples (n = 104, range = 1.2 to 25.4 ng/mL) yielded the following linear regression statistics: TAC = 1.05 × (LCMS) - 0.28; r = 0.98. No significant cross-reactivity was detected in samples spiked with 5000 ng/mL sirolimus, 5000 ng/mL everolimus, 1000 ng/mL CSA, and 200 µg/mL MPA. No significant interference (<10%) was found for 60 mg/dL conjugated or unconjugated bilirubin, 1000 mg/dL triglyceride, and 400 mg/dL cholesterol.

Conclusion: The new tacrolimus method showed improved accuracy and precision, especially at low drug concentrations. It provides fast measurements of tacrolimus on the Dimension system.

* Product under development. Not available for sale.

B-40

LC-MS/MS Determination of Cocaine Metabolites and the Adulterant Levamisole in Urine

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Background: Levamisole, an anti-helminthic agent, is increasingly being discovered as an adulterant of street cocaine. The US Drug Enforcement Agency reported that, in July 2009, levamisole was found in as much as 69% of seized cocaine. The previous human uses of levamisole were as an immunomodulator in treatment of rheumatoid arthritis and as an adjuvant treatment with fluoracil for colorectal cancer. Recent reports have linked levamisole found in street cocaine to agranulocytosis and retiform purpura in cocaine users.

Objective: Develop a LC-MS/MS method for the determination of the major urinary cocaine metabolites: benzoylecgonine, cocaethylene, and ecgonine methyl ester; and the adulterant levamisole and its urinary metabolite 4(OH) levamisole. Use this method to investigate the incidence of levamisole contamination of samples positive for cocaine, and the incidence of agranulocytosis in those patients.

Methods: We performed these assays on a Waters ACQUITY UPLC with an ACQUITY TQ Detector equipped with a Waters ACQUITY UPLC BEH C18 column using an ammonium acetate/formic acid mobile phase. Urine was extracted using methanol after the addition of Benzoylecgonine-D3, Ecgonine methyl ester-D3, Cocaethylene-D3, and aminorex ((*RS*)-5-phenyl-4,5-dihydro-1,3-oxazol-2-amine) as internal standards. MS/MS operates with positive Electrospray Ionization (ESI). Imprecision was tested using drug-free urine spiked with known low, medium, and high concentrations of the analytes. Acceptable allowable error was viewed as being <20%.

Results: Both within-run (4.7%-17.1%) and between-run (3.7%-7.3%) imprecision were acceptable. The limit of detection ranged from <1ng/mL to 6.3ng/mL. We tested 69 urine samples sent for toxicology screening at Grady Memorial Hospital. 51 samples tested positive for cocaine and 18 tested negative, by both immunoassay and LC-MS/MS. 25 patients tested positive for opiates as well. Levamisole and 4(OH) levamisole were detected in 70.6% of the cocaine positive urine samples, while the metabolite cocaethylene (indicator of concomitant alcohol consumption) was detected in 31.4% of cocaine positive urine samples. Levamisole was not detected in opiate positive urines in the absence of cocaine.

Conclusions: This method was successfully used to detect cocaine metabolites and its adulterant levamisole in urine, and determine the incidence of levamisole contamination of cocaine in the Atlanta region. The method will also allow the investigation of the incidence of agranulocytosis in the setting of levamisole-tainted cocaine.

B-42

Improved Sensitivity for Methotrexate Analysis Using Enzyme Multiplied Immunoassay Technique on the Siemens Viva-E Instrument

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Background: The available assay kit for methotrexate (MTX) using Syva enzyme multiplied immunoassay technique (EMIT) reagent allows for detection of MTX in serum or plasma to concentrations as low as 0.3 umol/L. Current clinical decision points for MTX therapeutic drug monitoring and subsequent leucorvorin rescue exist at concentrations below that limit. The goal of the current study was to lower the limit of MTX quantitation with acceptable precision to 0.05 umol/L using EMIT assay technology on the Siemens Viva-E Drug Testing instrument.

Methods: EMIT MTX assay parameters were modified on the Viva-E instrument to increase the sample volume to 7.0 uL from 3 uL. EMIT methotrexate kit calibrators were used at concentrations of 0, 0.2, 0.5 and 1.0 umol/L. Additionally, calibrators of 0.02 and 0.05 umol/L were made by dilution. Calibration of the instrument was achieved using modified cubic spline regression. Precision of this modified assay was assessed using a UTAK Laboratories custom MTX control with a target of 0.05 umol/L as well as Biorad Lyphochek TDM controls with targets of 0.3, 1.34 and 8.49 umol/L. Concentrations of samples greater than 1.0 umol/L were diluted using kit-supplied buffer reagent. All other assay procedures were followed according to manufacturer instructions.

Results: Intra-assay precision was assessed using the four control materials and concentrations listed above (n=10). Mean values (umol/L MTX) and percent coefficient of variation (CV) for the respective controls were 0.05, 9.4%; 0.38, 1.8%; 1.48, 2.0%; and 8.98, 0.9%. Inter-assay precision (n=25) was assessed using the same controls with mean values and percent CV as the following 0.04, 15.7%; 0.37, 3.9%; 1.47, 2.5%; and 8.54, 2.2%. Measured values of the 0.05 umol/L control ranged from 0.03-0.06. Small fluctuations in values at this low concentration resulted in a standard deviation of 0.006 and thus a larger CV at low concentration. The CV at the low end was comparable to reported values for the Abbott TDx MTX II assay with a 14% CV at 0.07 umol/L. This modified EMIT MTX assay as well as the unmodified approved assay version were compared using patient specimens with the current in-house Abbott TDx MTX assay. Linear regression of correlation data revealed that both EMIT assays produced positive bias compared with the reference method. However, the modified EMIT assay had the best correlation in the low range (0.03-2 umol/L) with an equation of Modified EMIT = 1.12 TDx + 0.03, r^2=0.987. Furthermore, over a larger range (0-20 umol/L) the correlation remained consistent with slope = 1.06 and r^2= 0.997. We further demonstrated that carryover has no effect in the assay.

Conclusion: The modified Syva EMIT assay for MTX was successful at measuring concentrations down to 0.05 umol/L with acceptable precision and can be used in clinical practice for monitoring methotrexate therapy. This assay can provide clinically-required sensitivity on a bench top immunoassay instrument attractive to many clinical laboratories.

B-43

Falsely Elevated Immunosuppressant Concentrations: Reversible Adsorption to a Variety of Central Venous Catheters

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Background: Reversible adsorption to intravenous catheter systems has been reported to cause erratic high concentrations of immunosuppressants (1). We systematically examined some of the most widely used central venous catheter systems (CVC) in Europe and the USA.

Methods: We tested 4 types of catheters, 2 made from polyurethane: Arrow-Howes TM "Quad-Lumen Central Venous Catheterization Set" (n=4) and Intra "Trilucath" (n=3). One type of catheter was coated with a silver ion-based antimicrobial agent: Vygon "Multicath Expert 4 lumen" (n=3). The last type was made from silicone: Vygon "Lifecath Apharesis Plus" (n=3). Either 2mg of Tacrolimus (TAC, n=7) in 50 mL (40µg/ml) over 22h or 250mg of Cyclosporin A (CsA; n=6) in 100mL NaCl (2,5mg/ml) over 6h were infused into one of the lumina. After rinsing the lumina with increasing volumes of NaCl (0.9%), we repeatedly mimicked sampling blood with Fresh Frozen Plasma (FFP) discarding the first 6mL as performed in clinical practice.

Results: For all materials significant evidence of adsorption was shown for TAC (n=7, p=0.016) and CsA (n=6, p=0.031, Wilcoxon signed rank test). Immediately after infusing the drugs and discarding 6mL of FFP, median concentrations of 152 ng/mL (range:25-447 ng/ml) of TAC and 6290 ng/mL (range:2360-10400 ng/ml) of CsA respectively were measured via LCMS (usual reference ranges: 4-11 ng/ml for TAC and 75-325 ng/ml for CsA). After rinsing with 10mL of NaCl and discarding another 6mL of FFP, the median concentrations were lowered to 44 ng/mL (range: 11-200 ng/ml) of TAC and 108 ng/mL (range: 102-620 ng/mL) of CsA. Further rinsing with NaCl led to a further reduction of concentration. However, even extensive rinsing with volumes as much as 24.01L still produced concentrations of up to 5.9 ng/mL of TAC. When cross contamination between the catheter outlets was strictly avoided, adsorption of the drugs was only observed for the lumen utilized for immunosuppressant infusion. Higher drug levels for TAC and CsA were found for silicone compared to polyurethane and silver throughout the entire experiment (TAC: t-test: p-mean=0.064, p-range 0.000-0.426, CsA: t-test: p-mean=0.017, p-range 0.000-0.079). At 37°C, higher drug concentrations were measured as compared to room temperature.

Conclusion: TAC and CsA reversibly adsorb to all CVCs tested and lead to falsely elevated drug concentrations. Raised levels can be demonstrated even after extensive rinsing. This indicates that catheter lumina used for immunosuppressant infusion should be permanently avoided for blood sampling. The clinical practice of discarding a limited volume of blood prior to blood sampling from a CVC line is insufficient. In clinical practice slightly elevated concentrations (e.g. after rinsing) may result in dose reduction and insufficient immunosuppression. This may be more dangerous for patients than erratic toxic concentrations which usually result in further investigation. We recommend venipuncture in order to obtain correct blood samples. If other lumina of the CVC are to be used for blood sampling, avoidance of lines previously used for infusion has to be ensured long term. Schneider H, Menzel H, Steimer W. Falsely elevated levels of tacrolimus or intoxication or both?, Clinical Chemistry, 53(6): A101-A101 B-154 Suppl. S JUN 2007

B-44

Measuring Ethyl Glucuronide in Human Urine in Order to Determine the Effects of the UGT1A1*28 and UGT2B7*2 Polymorphisms

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Background: Ethyl glucuronide is a minor metabolite of ethanol which has been utilized as a marker to monitor ethanol abuse. It is superior to measuring ethanol itself since ethyl glucuronide can be detected in urine for up to 5 days, while ethanol can only be detected for approximately 12-18 hours. However, there is much debate over the use of this test because of recent studies demonstrating that incidental exposure to products containing small amounts of ethanol can led to false positives depending on the cutoff utilized. In addition, the principal enzymes that catalyze the formation of ethyl glucuronide from ethanol, UGT1A1 and UGT2B7, are highly polymorphic and mutations in these genes such as the UGT1A1*28 and UGT2B7*2 have been demonstrated to affect enzyme activity. These polymorphisms are common in the human genome but it is unclear whether their presence will significantly alter ethanol metabolism in humans. If the *28 and *2 alleles do significantly affect the formation of ethyl glucuronide, then individuals that harbor these mutations could be predisposed to false positives or false negatives when measuring ethyl glucuronide.

Objective: Develop an LC-MS/MS assay to detect ethyl glucuronide in human urine. 2) Develop a PCR based method to genotype UGT1A1 (*1,*28) and UGT2B7 (*1,*2). 3) Conduct an IRB-approved controlled ethanol consumption study and measure ethyl glucuronide formation in fifty genotyped individuals.

Methods: An assay for measuring ethyl glucuronide was developed utilizing an AB Sciex 3200 LC-MS/MS system. Urine was diluted 1:10 in H20 with internal standard (D5-ethyl glucuronide). Liquid chromatography was performed on an Agilent 1200 using a Waters Xterra C18 column. An isocratic elution was used (10% methanol, 90% H2O). MS/MS analysis was performed using an MRM/IDA/EPI method in negative mode. Genotyping was performed on a BD Max thermocycler for UGT1A1 and an Abbott M2000rt thermocycler for UGT2B7. Ethanol consumption studies were performed at the General Clinical Research Center at San Francisco General Hospital. Urine collections were taken at predetermined time points for 36 hours. Total area-under-the-curve was used to calculate ethyl glucuronide formation.

Results: We developed an LC-MS/MS assay to measure ethyl glucuronide in human urine. The assay was linear from 0.1 ug/mL to 100 ug/mL with a LLOQ (S/N of 20:1) of 0.25 ug/mL and a LLOD (S/N 5:1) of .05 ug/mL. Between-run precision at the low calibrator (2.5 ug/mL) and high calibrator (75 ug/mL) was 5% and 7% respectively. The between run accuracy at the low calibrator (2.5 ug/mL) and high calibrator (75 ug/mL) was 6% and 8% respectively. We conducted controlled ethanol consumption studies on five genotyped individuals. Ethyl glucuronide concentrations were measured at seven time points over a thirty-six hour period. The average Cmax was 52 ug/mL with an average time to peak of 4.65 hours.

Conclusion: Ongoing efforts to increase our sample population to 50 individuals, which have been grouped as ultra, extensive, moderate, or poor metabolizers based on their genotypes, will allow us to determine whether the *2 and *28 polymorphisms in the UGT1A1 and UGT2B7 genes significantly affect ethyl glucuronide formation in humans.

B-46

Positive Propylene Glycol in a Patient with Ethylene Glycol Toxicity

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Background: Both ethylene glycol and propylene glycol can be used as antifreeze. Propylene glycol is also used as a solvent in many intravenous, oral, and topical pharmaceutical preparations. Ingestion of ethylene glycol or propylene glycol can cause increased serum osmolality and anion gap acidosis, but ethylene glycol is much more toxic than propylene glycol. However, large doses of propylene glycol can be toxic; particularly when it is given over a short period of time. It is clinically important to differentiate these two compounds if glycol poisoning is suspected. We report an interesting case in which the patient initially presented with ethylene glycol poisoning, but later showed a high level of propylene glycol. The objective of this study is to identify the origin of propylene glycol.

Methods: The patient was a 61 years old African American female who presented to an outside hospital after being found unconscious with an overdose of unknown etiology. Upon arrival to our hospital, the patient was in respiratory failure with evidence of an increased anion gap metabolic acidosis, increased serum osmolal gap, and negative volatiles. Ethylene glycol and propylene glycol in the patient's serum were simultaneously analyzed with a laboratory developed capillary column gas chromatography assay when the patient was admitted. Then the patient was treated with emergent hemodialysis followed by continuous veno-venous hemofiltration and fomepizole. The patient also received phenytoin and a high dose of lorazepam overnight for a witnessed seizure. Ethylene glycol and propylene glycol were subsequently measured 13 hours and 38 hours later.

Results: Upon arrival to the hospital, gas chromatography revealed ethylene glycol to be significantly elevated at 22 mg/dL and propylene glycol was negative. Thirteen hours later, the ethylene glycol level was undetectable. However, a new peak identified as propylene glycol was at a level of 27 mg/dL. The medication list review revealed that the patient had been given phenytoin and a high dose lorazepam drip which contain propylene glycol at 40% (v/v) and 80% (v/v), respectively. The lorazepam drip was discontinued and the following day the propylene glycol level decreased to 13 mg/dL and ethylene glycol was still undetectable.

Conclusion: The positive propylene glycol in this patient is caused by medications containing this compound. This case study supports the notion that propylene glycol accumulation is a relatively common phenomenon that is becoming increasingly recognized in the intensive care unit setting. Furthermore, it highlights the importance of identifying and reporting this potentially harmful compound whenever glycols analysis is performed.

B-47

Simultaneous determination of 8 pesticides in plasma using GC/MS with solid phase extraction

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Objective: To develop a safe and sensitive the method of gas chromatography mass spectrometry(GC-MS) using solid phase extraction to simultaneously determine 8 pesticides(including Methamidophos,Acephate,Dimethoate,Parathion methyl,Malathion,Parathion,Fenvalerate, and Decamethrin) in human plasma.

Methods 1mL pH6.0 phosphate buffer was added into 1mL plasma sample containing 8 pesticides. The mixture was processed in ultrasonic oscillator for 5 minutes and then centrifuged at 2500 rpm for 5 minutes. The supernatant was transferred to Oasis HLB(3cc/60mg) cartridges for solid-phase extraction and then eluted by 2ml of dichloromethane. The eluent was vaporized at 40°C to dryness, the residue was dissolved in 200 uL of dichloromethane, then the 8 pesticides were identified and quantified by GC-MS with selected ion monitoring (SIM) mode.

Results The good linearity of the method was ranged from 10 ng/L to 1000ng/L(for all the pesticides, r>0.9932); The intra-assay and inter-assay variations were lower than 9.58% and 8.86%, respectively. The overall accuracy of this method was 90.1% to 112.4% and the lower limit of detection was 2ng/L.

Conclusions This method can sensitively, accurately, simply and rapidly detect 8 pesticides in plasma, and would be useful for the diagnosis, treatment and monitoring of pesticides intoxication.

Table 1 Ions monitored in the analysis of 8 pesticides

No	Pesticide	Retention (min)	Qualitative ions	Quantitative ion
1	Methamidophos	4.47	94, 141, 64	94
2	Acephate	6.05	136, 94, 95	136
3	Dimethoate	8.45	87, 125	87
4	Parathionm ethyl	9.9	125, 109, 263	125
5	Malathion	10.6	127, 173, 125	127
6	Parathion	10.8	109, 291, 127	109
7	Fenvalerate	18.25	125, 167, 225	125
8	Decamethrin	19.13	181, 253, 255	181

B-48

CEDIA Cyclosporine Applications for the Ortho Clinical Diagnostics VITROS Systems

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Background. Cyclosporine is a cyclic undecapeptide with potent immunosuppressive function in reducing the incidence of tissue rejection following organ transplantation. Cyclosporine therapy has optimum safety and efficacy over a narrow range of concentration. It is essential for monitor cyclosporine in organ transplantation to achieve optimal immunosuppressive effects on patients. The Ortho Clinical Diagnostics VITROS™ 5600 Integrated System and VITROS™ 5,1 FS Chemistry System are new applications for the CEDIA Cyclosporine Assay. The CEDIA Cyclosporine assay uses the bacterial enzyme -Galactosidase that has been genetically engineered into two fragments. The assay is based on the competition of cyclosporine in the sample with cyclosporine conjugated to the Enzyme Donor (ED) fragment of -Galactosidase for antibody binding sites. In the presence of cyclosporine, the drug binds to the antibody, leaving the ED fragment free to form active enzyme with the Enzyme Acceptor (EA) fragment. In the absence of cyclosporine, the antibody binds to cyclosporine conjugated on the ED fragment, inhibiting the re-association of EA and ED. The amount of active enzyme formed and resultant absorbance change are directly proportional to the amount of drug present in the sample.

Methods. The performance of the CEDIA Cyclosporine application on the VITROS 5600 and 5,1 FS Systems was determined for precision, linearity and accuracy against the Hitachi 911 System. Two assay ranges were developed, a Low Range of 25 to 450 ng/mL and a High Range of 450 to 2000 ng/mL

Results. Tests for within-run and total precision (N=80 per level) were run over 20 days. Within run CVs were 7.0, 1.3, 1.1, 1., and 1.7% and total CVs were 9.7, 2.8, 2.5, 3.2 and 3.8% at 49, 188, 302, 793 and 1602 ng/mL on the VITROS 5600 Integrated System. Within run CVs were 9.4, 2.5, 1.3, 2.4 and 1.6% and total CVs were 13.0, 3.9, 3.2, 4.3 and 4.5% at 52, 201, 323, 816 and 1649 ng/mL on the VITROS 5,1 FS Chemistry System. Linearity was good across the reportable range of 25 ng/mL to 450 ng/mL for the low assay and 450 to 2000 ng/mL for the high range assay. Agreement with the predicate Hitachi 911(also running the CEDIA Tacrolimus Assay) was good using patient samples spanning the reportable range:

Low Range assay

VITROS 5600 = 1.07 (Hitachi 911) + 3.8 with a correlation coefficient of 0.994
VITROS 5,1 FS = 1.08 (Hitachi 911) - 1.4 with a correlation coefficient of 0.995
High Range assay
VITROS 5600 = 1.05 (Hitachi 911) + 26 with a correlation coefficient of 0.995
VITROS 5,1 FS = 1.05 (Hitachi 911) +28 with a correlation coefficient of 0.996

Conclusions: We conclude that the performance of the CEDIA Cyclosporine Assay on the VITROS 5600 Integrated System and the VITROS 5,1 FS Chemistry System warrants their introduction in clinical practice.

B-49

Development and Validation of Voriconazole Serum level Using Ultra Performance Liquid Chromatography (UPLC)

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Background: The determination of serum levels of voriconazole (VRCZ) is of essential importance for the treatment of fungal infections caused by *Aspergillus ssp* and other emerging filamentous fungi . The risk of toxicity for this drug is increased in patients with hepatic and / or renal failure.

Methods: For the control of serum VRCZ a quantification method was developed directly using Ultra Performance Liquid Chromatography (UPLC) in samples from patients taking the medication . We used a *Hewlett-Packard* model HP1290 Infinity®, Zorbax Eclipse Plus C18® column (2.1 x 50 mm, 1.8 µm) obtained from *Agilent* and a mobile phase consisting of water: acetonitrile (63:37, v / v) with flow of 0.450 ml / min. The determination of VRCZ occurred at 256 nm and had retention time of 1.20 min and total analysis time of 3.50 min. We used standards purchased from *Sigma-Aldrich* and the pooled serum-free VRCZ was used to perform the calibration curve. One aliquot of 0.50 ml of serum with added 0.20 ml of sodium hydroxide (0.1 M) was extracted with 2.5 ml of ether p.a. (*Merck*) under stirring on a vortex mixer for one minute. The ether layer was separated under centrifugation at 4000 rpm for 5 min at 15 ° C. The extracts were subjected to evaporation under a flow of N_2 in dry bath at 50 ° C. The residues were reconstituted with 100µl of mobile phase.

Results: The linearity was observed ($r^2 = 0.99968$) in the concentration range expected. In this study samples were used VRCZ added between 0.25 to 10.00 µg/ml and evaluated in quintuplicate. From these results it was determined the limit of quantification (LQ), which was defined as the lowest concentration and doesn't exceed 20%. The concentration was determined of 0.10 µg/ml as limit of detection (LOD) by the results obtained. The method was effective, efficient and sensitive with linearity in the concentration range studied. The intra-assay and inter-assay average accuracy was 102.8% and 103.3% respectively. The average intra-assay coefficient of variation (CV) was 2.17% and 3.55% was inter-assay. The extraction recovery was 82.7%. For the specificity test we used Fluconazole and Busulfan. No interference was observed.

Conclusion: Considering the robustness coupled with the efficiency and selectivity of UPLC, this assay can be used for measuring VRCZ aiming to control serum level for attaining therapeutic levels or prevent toxicity. The method was fast and efficient. The use of ether as solvent extraction ensures simplicity and low cost to carry out this analysis by UPLC and can be used in routine clinical laboratory use, specially in hospitals that care for immune compromised patients.

B-51

Evaluation of ARK Diagnostics Immunoassays for Gabapentin, Lamotrigine, Levetiracetam, Topiramate, and Zonisamide on the Beckman AU400e Chemistry Analyzer

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Therapeutic drug monitoring for antiepileptic drugs is important to optimize dose for efficacy, assess toxicity and drug-drug interactions, and to monitor patient compliance.

Objective: The performance of Gabapentin, Lamotrigine, Levetiracetam, Topiramate, and Zonisamide immunoassays obtained from ARK Diagnostics were evaluated with an open-channel automated chemistry analyzer. Methodology: The five assays studied (homogeneous enzyme immunoassays) were designed for the quantification of antiepileptic drugs in human serum or plasma. The assays were performed using

a Beckman AU400e instrument. Assay precision, accuracy, analytical measurement range, and method comparison studies were conducted using samples prepared at known concentrations, quality control materials, and patient specimens. Results were compared to previously validated immunoassays (TDx) or LC-MS/MS. **Results:** Assay performance was consistent with manufacture claims and clinical needs for all five assays. Method validation results are summarized in the table.

Conclusions: ARK Diagnostics antiepileptic drug assays can be performed with the Beckman AU400e to support therapeutic drug monitoring needs and are comparable to chromatographic methods.

Method Validation Results

			Concentrations (ug/mL)		
	Gabapentin	Lamotrigine	Levetiracetam	Topiramate	Zonisamide
Analytical Measurement Range	1.5 - 40.0	1.0 - 40.0	5.0 - 100.0	2.0 - 60.0	2.5 - 80.0
	Mean=2.5 (100%) %CV=8.1 N=5	Mean=2.1 (105%) %CV=5.9 N=5	Mean=7.4 (98.7%) %CV=3.5 N=5	Mean=2.4 (96.0%) %CV=6.5 N=5	Mean=5.0 (100%) %CV=4.1 N=5
Accuracy (% of Target) Total Imprecision (%CV)4 days	Mean=8.1 (101.3%) %CV=4.0 N=5	Mean=11.1 (92.5%) %CV=3.6 N=5	Mean=29.2 (97.3%) %CV=3.1 N=5	Mean=10.2 (102.0%) %CV=3.8 N=5	Mean=23.6 (94.4%) %CV=4.8 N=5
	Mean=25.3 (101.2%) %CV=2.7 N=5	Mean=23.0 (92.0%) %CV=7.5 N=5	Mean=75.6 (100.8%) %CV=3.6 N=5	Mean=40.3 (100.8%) %CV=3.8 N=5	Mean=51.4 (102.8%) %CV=2.1 N=5
	Slope = 1.01	Slope = 0.9798	Slope = 1.0897	Slope = 1.0005	Slope = 1.0187
	r = 0.9987	r = 0.9933	r = 0.9947	r = 0.9869	r = 0.9873
Method Comparison	y-intercept = 0.2440	y-intercept = -0.2285	y-intercept = 0.1649	y-intercept = 0.5808	y-intercept = -1.830
	Method: LC-MS/MS	Method: LC-MS/MS	Method: LC-MS/MS	Method: Immunoassay-TDx	Method: Immunoassay-TDx
	N = 45	N = 29	N = 93	N = 21	N = 22

B-52

Improved Method for Abbott ARCHITECT Tacrolimus Sample Pretreatment

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Background: Sample pretreatment is required to extract tacrolimus from whole blood to release tacrolimus and remove interfering substances. The aim of this study was to evaluate the performance of the ARCHITECT Tacrolimus assay using a modified batch sample pretreatment procedure with a novel test tube rack system.

Methods: Sample pretreatment with extraction buffer was performed in batch mode using microcentrifuge extraction tubes and a novel test tube rack system. This system ensures linkage of the extraction tubes to the primary patient sample tubes through a Locked Sample Identity System (LSIS) to prevent confusion of patient samples during processing. The LSIS safeguards sample identity as the extraction tubes remain in the test tube racks during the entire sample pipetting, vortex and centrifugation steps, thus minimizing technician error. Passing-Bablok statistics and the CLSI EP9-A2 and EP10-A3 guidelines were used for analysis of data.

Results: ARCHITECT Tacrolimus assay imprecision was evaluated using three multi-constituent controls. The total %CVs were 4.8% at 5 ng/mL, 3.4% at 10.6 ng/mL, and 4.3% at 21.5 ng/mL. The functional sensitivity (20%CV) of the ARCHITECT assay was 0.6 ng/mL (upper 95% CI). A total of 100 samples from renal allograft recipients were tested during method comparison studies with the current extraction method with the following **Results:** Spearman r = 0.99, y = 1.05x - 0.17 and an average bias of 0.36 ng/mL.

Conclusion: The performance of the ARCHITECT Tacrolimus assay with the batch method for sample pretreatment and LSIS was comparable to the current method with improved laboratory workflow.

B-53

Development and validation of a simplified method for determining azathioprine metabolites using HPLC (UV-Vis) and determination of their values in a population of renal transplant recipients

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Background Azathioprine (AZA) is a immunosuppressant with broad clinical use in autoimmune pathologies and against rejection in organ transplant. AZA has a complex metabolism that is not fully understood. Three enzyme systems compete to metabolize 6-MP: xanthine oxidase (XO), thiopurine methyltransferase (TPMT) and hypoxanthine phosphoribosyl transferase (HPRT). The reaction of 6-MP to 6-thiouric acid (6-TU) and 6-methylmercaptopurine (6-MMP) are catabolic routes. TPMT exhibits codominant genetic polymorphism and the distribution of these variant alleles differs significantly among populations. High TPMT activity probably causes diminished production of 6-TGN and worse transplantation outcome.

Our objective was develop and validate a simplified analytical methodology for determining of azathioprine metabolites 6-TGN and 6-MMP using high performance liquid chromatography with an ultraviolet-visible detector (HPLC UV-Vis) and determinate the values of these metabolites in a population of renal transplant recipients.

Methods To adequate the process to legislation Clinical and Laboratory Standards Institute guidelines was followed. The method was validated for linearity, selectivity, specificity, recovery, repeatability and reproducibility.

Washed erythrocytes (400 µL) were transferred to a tube and 100 µL of 3 mg/mL DTT solution was added. The totality was deproteinized by 100 µL of 70% perchloric acid and centrifuged. The supernatants were removed and then heated for 45 min at 100 °C. After cooling to room temperature and filtration 100 µL aliquot was injected and separation was performed on a reversed-phase column, mobile phase A was potassium phosphate and mobile phase B was methanol. Detection of 6-TGN and 6-MMP was performed at 342 nm (UV-Vis).

Approval was obtained from the Commission of Ethics for Analysis of Research Projects and patients provided free informed consent. Blood samples

were collected from 124 stable renal transplant recipients at the outpatient unit of the Nephrology Service at the Clinics Hospital (Sao Paulo, Brazil).

Results Assay linearity for 6-TGN ranged from 0.30 to 89.71 µmol/L and from 0.30 to 93.86 µmol/L for 6-MMP. Repeatability CV were 3.50, 5.06, 1.09 and 0.04, 0.35, 1.58%, while reproducibility CV were 8.65, 7.18, 8.44 and 12.73, 6.40, 4.88% for 6-TGN and 6-MMP, respectively.

6-TGN and 6-MMP patient analysis values ranged from non detectable to 1569 pmol/8 x 10^8 RBC (median of 200.50) and non detectable to 113057 pmol/8 x 10^8 RBC (median of 5166), respectively.

Conclusion The method proposed is specific and selective. The gradient elution mode was optimal to elute 6-TGN and separate 6-MMP from DTT. Determination of the concentrations of both metabolites at a single wavelength 342 nm (UV-Vis) permitted the analysis using an equipment with a simple UV-Vis detector, avoiding the use of a photodiode array detector (PDA) to analyze both metabolites in a single run.

The results of patients are in agreement with others studies, thus certifying the usefulness of this analytical tool in monitoring of patients taking AZA. Is important to note that 29% of patients had no quantifiable levels of 6-TGN and probably worse transplantation outcome.

B-54

Modeling and simulation of positive and negative interference in a two-stage immunoassay

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Background: The potential for either positive or negative interference by a single interferent in certain immunoassay designs is well-known, as has been known to occur for some two-stage digoxin assays. The phenomenon is attributed to the difference in off-rates for different antibody (receptor) ligands: after initial incubation (first stage), receptor occupancy by different ligands can change unequally during a wash step prior to the second stage measurement of unoccupied receptors, such that different combinations of analyte vs. interferent concentrations can produce either positive

or negative assay interference. We are not aware, however, of a formal presentation or analysis of the underlying kinetic mass balance model equations for such assay conditions. Our objective was to document an analytical model solution for such a two-stage assay in the presence of a second unintended ligand (interferent), and to demonstrate conditions (relative concentrations, receptor affinities of analyte and interferent, and wash times) in which negative and positive interference may be observed in model simulations.

Methods: Receptor occupancy states were modeled as probabilities (0 to 1; sum of states=1) of being bound to the intended analyte (A), unbound (C), or bound to unintended interferent (B) according to reversible binding: A↔C↔B. In the first stage, kinetics of state transitions in presence of analyte and interferent concentrations [*a*] and [*b*], respectively, are given by: dA/dt=k1[*a*]C-k2A, dB/dt=k3[*b*]C-k4B, dC/dt=-(dA/dt+dB/dt), where k2/k1 is the ligand-receptor dissociation constant for *a* (K*a*), and k4/k3 is the ligand-receptor dissociation constant for *b* (K*b*), and where initial conditions were A(0)=B(0)=0, C(0)=1. In the second stage (wash step, [*a*]=[*b*]=0), state transitions are given by dA/dt=-k2A, dB/dt=-k4B, dC/dt=-(dA/dt+dB/dt), with initial conditions given by results of the first stage incubation. Analytical solutions were obtained for each of the above differential equations. Simulated assay results were obtained for conditions of variation of dimensionless parameters [*b*]/K*b*, K*b*/K*a* and wash time *w*/k2, with assumption that K*b*/K*a* corresponds to the ratio k4/k2 (that is, that differing receptor affinities of ligand and interferent reflect differing off-rate constants). Interference was evaluated by results for C([*a*]) in the presence of a given [*b*] compared to a standard curve for C([*a*]) when [*b*]=0.

Results: Only positive interference is observed for (K*b*/K*a*)≤1. For (K*b*/K*a*)>1, both positive and negative interference may be observed depending on combinations of [*b*]/K*b*, K*b*/K*a* and *w*/k2. For example, with first-stage incubation to steady-state (A=[*a*]/K*a*/d, B=[*b*]/K*b*/d, where d = (1+[*a*]/K*a*+[*b*]/K*b*)), followed by wash stage of duration *w*=0.1/k2, the conditions of K*b*/K*a* = 20 and [*b*]/K*b* = 0.2 produce interferences ranging from +10% to -15% across the measurement range of [*a*]. This is simply one example of model simulation results that are parallel in pattern and scale to those that have been observed experimentally for interferences observed in two-stage digoxin assays.

Conclusions: Kinetic mass balance model simulations for a two-stage immunoassay can demonstrate positive and negative interference, and can demonstrate extents to which negative and positive assay interference by a single interferent can occur under various conditions of relative concentrations of analyte and interferent.

B-55

Interference of Prescriptive and Over-the-Counter Medications with DAU Immunoassays on the Vitros 5600

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Background: Clinical laboratories favor immunoassay-based methods to screen for drugs of abuse in urine (DAU). These methods can be adapted to existing instrumentation and facilitate achievement of turn-around-times desired by the emergency department and the pain management clinics. To be useful in these settings, assays for the amphetamine, benzodiazepine, barbiturate, and opiate classes should cross-react with a number of drugs within the given class of agents. Unfortunately, this often leads to one of the major limitations of these assays, i.e., undesired cross-reactivity with other, unrelated drugs. The false-positive results that follow confound the use of these assays when the need for rapid response does not allow for confirmatory testing. Our laboratory monitors urine drug screening results which do not confirm using LC/MS so that we can investigate the cross-reactivity of medications determined to be common to such samples. Buprenorphine, tapentadol, tramadol, cyclobenzaprine, meprobamate, dextromethorphan, and carisoprodol were common to multiple unconfirmed samples for several DAU screening immunoassays. Of these compounds, only dextromethorphan was reported to cross-react with the methadone and opiate assays.

Methods: Solutions of the aforementioned drugs, and when possible metabolites (all from Cerriliant Inc), were prepared by adding the compounds individually to drug free urine to obtain final concentrations of 10,000 or 100,000 ng/mL. Each solution was tested for cross-reactivity with the amphetamines, barbiturates, benzodiazepines, cannabinoids, cocaine, methadone, opiates (all Ortho-Clinical Diagnostics), and propoxyphene (Siemens Healthcare Diagnostics, Inc) assays on the Vitros 5600 (Ortho-Clinical Diagnostics). Cross-reactivity was calculated as a percentage of the response observed for the unrelated drug compared to the designated cut-off of the assay.

Results: The broadest spectrum of cross-reactivity was observed between these compounds and the propoxyphene assay with the least cross-reactivity observed for norbuprenorphend (5%) and the greatest for cyclobenzaprine (81%). Tapentadol, desmethyltapentadol, n-desmethyltramadol, and dextromethorphan were also detected using the propoxyphene assay at 10-20%. Cyclobenzaprine cross-reacted with both the opiate (43%) and methadone (55%) assays, as did dextromethorphan (60 and 21%, respectively). The tapentadol metabolites, desmethyltapentadol and tapentadol sulfate, exhibited cross-reactivity with the amphetamine, opiate, and methadone assays (20-30% each).

Conclusions: With one exception (meprobamate), each of the drugs, or a related metabolite, demonstrated some degree of cross-reactivity with at least one DAU screening immunoassay. Recently introduced and used in pain management, the cross-reactivity of tapentadol with the opiate assay could be desirable; however, the potential of the drug and its metabolites to cause positive screening results with the amphetamines, methadone, and propoxyphene assays is concerning. As the drug gains use in pain management, clinical laboratories must be aware of the potential cross-reaction with the various assays. These studies have proven useful in our laboratory's handling of inquiries regarding screening results using the Vitros 5600's when these results did not confirm. We caution that our data cannot be universally applied to other DAU immunoassay systems (or with future generations of these reagents) as it is likely that different cross-reactivity will be observed.

Tuesday PM, July 26

Poster Session: 2:00 pm - 4:30 pm
Molecular Pathology/Probes

B-56

Identification of Host Transcriptomic Differences between Black and White Individuals with Community Acquired Pneumonia and Severe Sepsis

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Background: Severe sepsis, an aberrant systemic host response to infection, is responsible for ~250,000 American deaths, annually. Not only is severe sepsis a major public health issue, but the incidence is disproportionally higher in black Americans compared to white and Hispanic Americans. We have previously reported that the disparate incidence of severe sepsis persists after adjusting for social and healthcare delivery factors, raising the possibility that there are underlying differences in the biologic response. However, the role that biology plays in potentiating racial disparity in infection and sepsis is a poorly understood ongoing controversial debate. Understanding how individuals of differing ancestry respond to infection is key in developing appropriate therapeutic interventions. The aim of this study was to test the hypothesis that host transcriptomic responses to infection differ across racial groups.

Methods: Racial differences in response to infection may involve the interaction of multiple gene products, so we used a genome-wide mRNA expression platform to identify genes underlying these differences. mRNA was extracted from whole blood samples (n=46) obtained from self-identified black (n=23) and white (n=23) subjects who were enrolled in the Genetic and Inflammatory Markers of Sepsis (GenIMS) study. GenIMS is a large prospective observational cohort study (N=2,320) of subjects with community-acquired pneumonia aged 18 to 70. These subjects represent a clinically relevant model because CAP is the most common cause of sepsis. Since blacks in GenIMS who were hospitalized with infection have a different clinical profile compared to whites.We conducted a nested case-control study within the prospective cohort study, GenIMS. Controls (whites) were matched to cases (blacks) using a propensity model that included baseline sociodemographic and clinical characteristics. All subjects had CAP. Each patient sample was analyzed twice using the Illumina Human RefSeq8 Expression BeadChips®, which target approximately 24,500 well-annotated transcripts per sample. The expression array raw data was analyzed using RMAExpress and BRB array tools to perform normalization, probe analysis, quality assessment and data preprocessing. To identify transcriptomic differences, we used the R and Significance Analysis of Microarrays ('samr" package) pair analysis.

Results: After adjusting for false discovery (FDR<0.05), 21 transcriptomic differences were detected between blacks and whites. Seven genes were up-regulated and 14 genes were down-regulated in blacks compared to whites. These genes are involved in transcription, RNA binding and zinc/ iron homeostasis. Pathway analysis identified 6 pathways that were differentially expressed between blacks and whites (identified using pathway data [MSigDB] from Gene Set Enrichment Analysis). These pathways are mostly associated with G-protein, zinc transport, transcriptional regulation, inflammation, and apoptosis signaling. Regardless of race, 11 genes were differentially expressed between individuals with severe sepsis and individuals without severe sepsis (FDR <0.20;unpaired SAM).

Conclusion: Our preliminary data suggest that in the setting of CAP and severe sepsis, host transcriptomic responses to infection are different among self-identified black and white individuals. Further investigation is warranted in a larger cohort of subjects to validate the functional importance of the identified genes as potential biomarkers of susceptibility to infection and sepsis. Developing population-based molecular 'signatures' may be a simplistic first step to personalized medicine.

B-57

Prevalence and Characterisation of Integrons among Antibiotic resistant Gram-negative Pathogens

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Background: The multidrug resistant Gram-negative obligate aerobe *Acinetobacter baumannii* is a major health hazard with increased morbidity and mortality among intensive care and burns units' patients. Recent association of *A. baumannii* with injured civilian and military expatriates involved in the Afghanistan and Iraq conflicts has raised concerns for immediate attention as far as treatment strategies are concerned. The epidemic behaviour and multiple antibiotic resistance of *A. baumannii* have been linked with the carriage and expression of mobile genetic elements called integrons. Integrons assemble antibiotic resistant genes from the environment, integrate them by site-specific recombination into the genome of their host bacteria and express them. In this study two classes (class 1 and 2) of integrons were characterized to determine their resistant patterns both phenotypically and genotypically.

Methods: 17 clinical strains of *Acinetobacter baumannii* and 4 control strains of *E. coli* designated as NCTC 10418, 8/CTX, 9/TEM10 and 10/TEM3 were investigated using British Society of Antimicrobial Chemotherapy (BSAC) guidelines. Pure colonies of the bacterial strains were subcultured in LB broth and the DNA extracted using Qiagen Blood and Tissue Kit and also by boil extraction. Six different sets of primers (IDT® Oligonucleotide DNA Technologies) were used to identify and amplify specific target sequences. All PCR products were resolved by agarose (Fisher Scientific UK Ltd, Loughborough, Leicester, UK) gel electrophoresis at 100 volts and visualised under ultra violet light using a transilluminator. The bacterial strains were tested against 12 antimicrobial agents. The conserved segment (CS) amplicon was purified and sequenced using the 5'-CS primer by GATC Biotech Ltd, London, UK. Sequences obtained were aligned using the National Center for Biotechnology Information (NCBI) database basic local alignment search tool (BLAST).

Results: All primers except *Intl 2* and $bla_{\text{OXA-24-like}}$ successfully amplified all the target sequences which were present in the PCR reaction mix. Conserved segment PCR was positive for all test strains except one strain representing 94% prevalence rate and gave partial product length of 662bp. Multiple sequence alignment and BLAST revealed a complete sequence length of 2217 bp for *A. baumannii* (GenBank accession number HM175868.1) with 99% identity with 4 antibiotic resistant Gram-negative bacteria; *Escherichia coli, Enterobacter cloacae, Enterobacter aerogenes* and *Klebsiella pneumonia* harbouring 3 antibiotic resistant genes namely: class I integron aminoglycoside 6'-N-acetyltransferase (aacA4), chloramphenicol acetyltransferase (catB8), and aminoglycoside 3'-adenyltransferase (aadA1). Integrase gene PCR for the identification of class 1 integrons was positive for all strains except one strain representing 94% of cases.

The $bla_{\text{OXA-23-like}}$ gene of approximately 1062 bp was amplified in all the strains. As expected specific primers targeted against the $bla_{\text{OXA-51-like}}$ gene intrinsic to almost all strains of *A. baumannii* yielded a positive PCR result.

The antibiotic susceptibility testing including AmpC and extended-spectrum beta-lactams was negative for all clinical strains.

Conclusion: The PCR result for Class 1 integrase gene, multiple sequence alignment and the antibiotic susceptibility testing confirm that *A. baumannii* is intrinsically resistant to most antibiotics used. Class 1 integrons were also found to be highly predominant (94%) in antibiotic resistant strains of *A. baumannii*.

B-58

A Novel Monoclonal Antibody for Non-small Cell Lung Cancer and Its Biological Characteristics

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Background: Lung cancer is the leading cause of cancer death worldwide. Antibody based immunotherapy that targets tumor antigens or cell surface markers has achieved some success as a cancer therapy.

Methods: BALB/c mice were immunized with human lung adenocarcinoma cells SPC-A1 and the spleen cells of the immunized mice were conventionally fused with myeloma cells SP2/0. The positive clone, which produced McAb against SPC-A1,

was obtained by means of indirect cell ELISA screening, and the specificity of the McAb was indicated through ELISA and an indirect immunofluorescence assay. The relative molecular mass (Mr) of the antigen recognized by the McAb was measured by western blot and immunohistochemical analysis was performed to detect the tissue specificity of the McAb. SPC-A1 cells were plated on a soft agar matrix and treated with various concentrations of McAb, the colony formation efficiency and the inhibition ratio of colonies was then calculated. The xenograft was established upon injection of SPC-A1 cells and McAb was administered at three different doses. The mice were monitored for tumor size. Tumors were removed and weighed three weeks after the initiation of treatment, and tumor growth inhibition was calculated. SPC-A1 cells were cultured with or without McAb for 24 h and 48 h, then cell morphology changes were observed and the apoptosis rates were measured by flow cytometry.

Results: We obtained a cell clone that secreted McAb specific to NSCLC. The McAb, named NJ488-1, belongs to the IgG1 subclass and the titer of purified McAb was 1: 2×10^6. The McAb NJ488-1 could specifically identify human lung cancer antigen as well as antigen located in the cytoplasm of SPC-A1. The antigen recognized by McAb NJ488-1 proved to be a protein with a Mr of 70 kDa by western blot. The immunohistochemical staining results indicated that McAb NJ488-1 could react positively to NSCLC tissues, but negatively or weakly to SCLC, pulmonary pseudotumor, and breast cancer tissues. The colony formation efficiency in soft agar assay in McAb groups was reduced in a dose-dependent manner. The McAb caused varying degrees of reduction in tumor volume compared to control mice. In the 200 μg/mL, 400 μg/mL and 800 μg/mL McAb groups, tumor growth inhibition was found to be 10.44%, 37.29%, and 44.04%. The difference in average tumor weight between the 400 μg and 800 μg McAb group and the control group ($p = 0.032$ and $p = 0.015$, respectively), and between the 200 μg and 800 μg McAb group ($p = 0.048$) was statistically significant. McAb NJ488-1 led to obvious cyto-morphology changes and significantly induced the apoptosis of SPC-A1 cells in a time-dependent manner ($p = 0.000$).

Conclusion: We successfully obtained and characterized McAb NJ488-1, which exhibited selective reactivity to NSCLC and exhibited anti-tumor activity both in-vitro and in-vivo. This is potentially of great value concerning immunodiagnostics and immunotherapy of NSCLC and holds promise for further research regarding the mechanism underlying tumor progression.

B-59

Detection of a new mutation in SRY gene in patients with disorder of sex development using HRM with unlabelled probe

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Background: Mutations in the High Mobility Group box (HMG box) are the main SRY-associated reasons for disorder of sex development (DSD). Although direct DNA sequencing is considered as a "gold standard" for mutation detection, the extremely labor and time-consuming procedures involving limit its uses. High Resolution Melting (HRM) analysis with unlabeled prove is a simple, rapid and low-cost mutation scanning method for both known and unknown mutations. This study surveyed mutations in the HMG box in SRY gene in patient with DSD using the same techonology.

Methods: 21 patients with DSD (15 with 46XY,DSD, 3 with 46XX,testicular DSD and 3 with 46,XY complete gonadal dysgenesis) were enrolled in this study at diagnosis, as well as a control group of 28 healthy males. EDTA-anticoagulated venous blood specimens were collected from the patients and controls. DNA was extracted using the QIAamp DNA Mini Kit (QIAGEN, Germany). Asymmetric PCR was performed to enrich the target strand of SRY gene in the the SRY HMG-box. 4 unlabelled probes were designed to cover the whole length range of the HMG-box. Melting curve analysis was performed on the LightCycler® 480 (Roche Diagnostics, Germany). Meanwhile, the SRY gene was sequenced to verify the scanning results.

Results: Among 21 patients, SRY gene was positive in 19 patients, while in the control group, SRY gene was positive in all specimens. For a patient with SRY positive 46 XY CGD a mutation was first identified with HRM technology with unlabelled probe and then confirmed as Lys123 Glu with sequencing. This patient is a 17-year-old girl, with the chromosome karyotype 46XY. Her clinical manifestations were normal female external genitalia, congenital gonadal dysgenesis and primary amenorrhea. By retrieving HMG box-associated SRY mutations in Human Genome mutation database, it was confirmed to be a new mutation. No mutation was detected among the 28 SRY gene positive control specimens.

Conclusion: Using HRM with unlabelled probe, the mutation in the HMG-box of SRY gene can be detected quickly. It is a simple, cost effective and high throughput method for scanning genetic mutation in SRY HMG-box. There is no need for further sequencing if no mutation was scanned by the unlabelled probe HRM technology. Otherwise, the mutation should be verified by sequencing.

B-60

miRNA profiling in pulmonary sarcoidosis

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Background: Small non-coding RNAs (miRNAs) are involved in the posttranscriptional regulation of numerous human genes, mainly via degradation of target mRNAs. There is evidence that upregulated or downregulated miRNAs expressions play an active role in the pathogenesis of pulmonary diseases. To date, there is no information about the miRNA expression profile(s) in pulmonary sarcoidosis.

Methods: We, therefore, performed screening of 380 miRNAs (TaqMan® Array Human MicroRNA A Cards v2.0) in bronchoalveolar cells (BAL) obtained from six patients with sarcoidosis (S; chest X-ray stage II, disease persistence after 2 years follow-up, non-smokers) and from six control subjects (C; normal BAL profile, non-smokers). The expression of upregulated or downregulated miRNA identified during the screening phase has been further investigated in a larger group of sarcoidosis patients (n=40) and control subjects (n=12) by quantitative RT-PCR. Various computational algorithms were applied to predict corresponding miRNA targets and molecular pathways that may be perturbed in sarcoidosis.

Results: MiRNA profiling screening revealed several differentially expressed miRNAs between sarcoidosis and controls. Of these, *let-7c* and *miR-381* were upregulated and *miR-146a, miR-150, miR-186, miR-212, miR-222, miR-223, miR-424 and miR-885-5p* downregulated in sarcoidosis when compared to controls. To exclude the influence of smoking on miRNA expression, all subjects enrolled into this study were non-smokers. Investigations are ongoing to confirm expression patterns in a larger group of patients and controls. Furthermore, we applied various computer algorithms to predict the miRNA targets, thus to reveal candidates for experimental validation in biological system.

Conclusion: In the screening phase, we detected ten candidate miRNAs with deregulated expression in bronchoalveolar lavage cells obtained from patients with pulmonary sarcoidosis when compared to control subjects. Studies in a larger patient group, miRNA target prediction and experimental validation analyses are ongoing.

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Erythropoietin Promoter Gene Polymorphism Is Independently Associated With Anemia in Patients With Type 2 Diabetes Mellitus

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Background: Anemia is a prevalent but often unrecognized condition in patients with type 2 diabetes mellitus (DM). In patients with chronic kidney disease (CKD), anemia occurs earlier and is more severe in those with DM compared to those with non-diabetic CKD. Anemia aggravates the development and progression of both microvascular (nephropathy, retinopathy, neuropathy) and macrovascular (ischemic heart disease, cerebrovascular disease, peripheral vascular disease) complications of diabetes, leading to increased hospitalization and mortality. The etiology of anemia in diabetes may be multifactorial and includes kidney diseases, nutritional deficiency, inflammation, drugs and hormonal changes. However, whether genetic predisposition affects a DM patient's risk of developing anemia has not been well explored. Sequence variation in erythropoietin, angiotensin converting enzyme and tumor necrosis factor-alpha are reportedly associated with the risk of diabetic retinopathy and diabetic nephropathy. In this report, we aim to investigate the associations of genotype variations in erythropoietin, angiotensin converting enzyme and tumor necrosis factor-alpha with anemia in patients with type 2 DM.

Patients and Methods: We performed a cross-sectional study between April 2008 and November 2008 in 1,715 diabetic patients. Exclusion criteria are regular dialysis or erythropoietin use, malignancy, liver cirrhosis, gastrointestinal bleeding, abnormal mean corpuscular volume, white blood cell (WBC) or platelet count. The genomic DNA was extracted from peripheral blood samples. Genotyping for the tumor necrosis factor-alpha G-308A, angiotensin converting enzyme I/D and erythropoietin polymorphism was performed using a polymerase chain reaction-restriction fragment length polymorphism method. Single nucleotide polymorphism genotyping was in good compliance with Hardy-Weinberg equilibrium. Statistical analysis was

performed using SPSS software version 17.0. Results are expressed as mean ±standard deviation for normally distributed data and as median for nonparametric data. Student t-test was used for comparison of means between two groups.

Results: Of the 1142 patients enrolled, 335 (29%) have normocytic anemia. Patients with anemia are older, have longer duration of DM, worse renal function, more severe albuminuria, higher systolic blood pressures (BP), lower diastolic BP, slightly lower glycosylated hemoglobin (HbA1c) and WBC. T allele (vs. G: odds ratio [OR] 1.415, P = 0.005) and TT genotype (vs. GG: OR 5.695, P = 0.002) in the promoter region of erythropoietin gene (rs1617640) are independently associated with increased risk of anemia after adjusting for subject age, sex, duration of DM, WBC, HbA1c, eGFR, albuminuria, and BP. Insertion and deletions in angiotensin converting enzyme and tumor necrosis factor-alpha gene polymorphisms do not have significant associations with risks of anemia.

Conclusions: The strong independent association of this erythropoietin SNP with anemia in DM suggests that its potential regulatory function warrants further investigation. A better understanding about the role of erythropoietin genetic variation in anemia will help identify patients at risk and develop innovative therapy for this and other complications of DM.

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Expressions of EBV-encoded small RNA1 and Toll-like receptor 3 in non-resolving inflammation of rheumatoid arthritis

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Background: Rheumatoid arthritis (RA) is a chronic autoimmune disease characterized with perpetuated inflammation over multiple joints, which usually causing deformity and disability in cases without adequate treatment. To date, there are only 30% of RA patients can get complete remission by various kinds of disease-modifying anti-rheumatic drugs (DMARD). In previous reports pointed out a very impressive concept that non-resolving inflammation is a major driver of many chronic inflammatory diseases, and rheumatoid arthritis might be a typical example. They emphasized that to cure, not to palliate rheumatoid arthritis, current anti-inflammatory therapy might be necessary to synergize with other therapy targeting causal factors. Recently, we carefully reviewed pathologic findings upon our patients receiving synovectomy due to refractory synovitis in knee joint. Lots of lymphocytes and plasma cell infiltration were abundant in their synovial tissue. As we know, plasma cells are responsible with autoantibody (such as rheumatoid factor and anti-CCP) and derived from B cells. B cells were well known to carry CR2 molecule, the EBV receptor. In pervious report pointed that EBV induces signaling from the Toll-like receptor 3 (TLR3), which is a sensor of viral double-stranded RNA (dsRNA) and induces type I IFN and proinflammatory cytokines. In the current study, we investigated the relationship of EBV-encoded small RNA1, Toll-like receptor 3 and clinic-pathological findings.

Patients and Methods: All patients (42 women and 7 man, age 38-79 years old) were diagnosed with RA according to the American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis and followed up at Chang Gung Memorial Hospital-Kaohsiung Medical Center. Though general condition got some improvement by DMARDs, but they all suffered from a big joint disability due to persistent joint inflammation. Therefore, joint replacement was performed. After surgery intervention, removed synovial tissue was examined by standard HE stain. Then, EBV-encoded small RNA1 *in situ* hybridization and Toll-like receptor 3 immunohistochemical analysis were performed to study on the paraffin-embedded tissue of 49 non-resolving inflammation of rheumatoid arthritis. In addition, 32 specimens from cases with osteoarthritis receiving TKA were enrolled as control.

Results: All these 49 synovial tissues were found to be present with strong inflammation including hypertrophy of synovial lining with plasma cell and lymphocytes infiltration, as well as neovascularization. By *in situ* hybridization, all non-resolving inflammation of rheumatoid arthritis (49/49, 100%) showed expression of EBER1 in synovial lining cells, plasma cells, endothelial cells or infiltration lymphocytes. There was a significant statistical difference of EBER1 expression among non-resolving inflammation of rheumatoid arthritis and osteoarthritis (p<0.001, by Fisher's exact test). When EBER1 expression was strong, the expressions of Toll-like receptor 3 were also strong expression (p<0.001). Higher expression of TLR3 immunostaining also correlated significantly with non-resolving inflammation of rheumatoid arthritis (p<0.001).

Conclusions: Putting the above findings together with our observation, non-resolving inflammation of rheumatoid arthritis is strongly associated with EBV infection, and strongly expression of EBER1 and TLR3 might be one of the key.

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Evaluation of SNPs of microRNA-biogenesis genes and risk of schizophrenia

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Background: Schizophrenia, one of the most serious, disabling diseases, has been associated with a sizeable proportion of genetic risk, but findings have been inconsistent. MicroRNAs (miRNAs) are small regulatory RNAs that modulate the expression of approximately one-third of all human genes. Small changes in miRNA expression have been associated with several psychiatric and neurological disorders, but whether polymorphisms in genes involved in processing of miRNAs to maturity influence susceptibility to schizophrenia has not been elucidated.The present study investigated the association between schizophrenia risk and single-nucleotide polymorphisms (SNPs) in miRNA processing genes.

Methods: we assessed the associations between schizophrenia risk and 6 potentially functional SNPs from 5 miRNA processing genes (*DROSHA, DGCR8, DICER1, AGO1, GEMIN4*) in a case-control study of 256 Chinese schizophrenia patients and 252 frequency matched (age, gender, and ethnicity) controls. All the SNPs (rs10719, rs3757, rs3742330, rs636832, rs7813, rs3744741) were genotyped by high resolution melting (HRM).

Results: we found that two SNPs in the *DGCR8* and *DICER1* gene were significantly associated with altered schizophrenia risk. The recessive model of rs3757 in *DGCR8* (TT vs. TC+CC) exhibited a significantly increased risk with an odds ratio [OR] of 1.58 (95% confidence interval [CI], 1.19-2.11); the recessive model of rs3742330 in *DICER1* (AA vs. AG+GG) exhibited a significantly increased risk with OR of 1.49 (95%CI, 1.04-2.13). Other SNPs and the haplotype of GEMIN4 (rs3744741 and rs7813) did not show any association with schizophrenia. We also compared with the low-risk reference group within none unfavorable allele of the two significantly SNPs, the median-risk and high-risk group. The median-risk group show no significantly difference with the reference group; and the high-risk group exhibited a 1.31-fold (95%CI, 1.08-1.59) increased risk of schizophrenia.

Conclusion: The present study provides the first evidence that specific genetic variants in miRNA-related genes may affect schizophrenia susceptibility.

B-64

Stabilization of Cellular RNA in Blood Samples for Non-Invasive Diagnosis and Prognosis

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Background: Messenger RNA molecules (mRNA) in blood cells are indicators of the activity of their genomes, illustrating what genes are expressed and to what extent. Profiling of cellular mRNA expression patterns has been demonstrated using microarrays, quantitative reverse transcription real-time PCR (RT-qPCR) and molecular beacons (1, 2, 3). Such profiling methods have become increasingly important for both disease characterization and biomarker discovery, but also carry a risk of data misinterpretation (3). A potential issue relates to the handling of blood samples *ex vivo* prior to the extraction of mRNA. Microarray data has shown that clusters of genes were strongly up- and down-regulated in as little as 2 hours post-phlebotomy. Profiles of fresh samples compared to samples shipped overnight were strikingly different, particularly with genes that are known to participate in stress-induced pathways (3). This evidence emphasizes the importance of developing blood collection devices that are capable of stabilizing mRNA expression immediately after a blood draw.

Objective: To develop a standardized blood collection device capable of stabilizing mRNA expression patterns immediately after a blood draw and prevent blood cell lysis for an extended period of time at ambient temperature.

Methods: Blood samples were drawn from healthy donors into both K_3EDTA(BD Vacutainer®) and Cell-Free RNA™ BCT collection tubes developed by Streck Inc. White blood cells (WBCs) were separated, total cellular RNA was extracted and mRNAs for RASSF1A, c-fos, glyceraldehyde-3-phosphate dehydrogenase (G3PD), and 18S rRNA were quantified by RT-qPCR at different time points post-collection. Molecular beacon technology was used to detect G3PD mRNA within intact cells using flow cytometry. All blood samples were kept at ambient temperature until

further processed.

Results: While blood drawn into K_3EDTA tubes showed significant changes in cellular mRNA copy numbers for c-fos, G3PD, RASSF1A and 18S rRNA, copy number of these biomarkers were unchanged in blood stabilized with Streck's Cell-Free RNA BCT. Moreover, stabilization was also illustrated using a molecular beacon detected by flow cytometry. While an increase in G3PD mRNA levels is found in WBCs stored in K_3EDTA tubes, no change in mRNA levels is detected in samples stored in Cell-Free RNA BCT.

Conclusion: Cell-Free RNA BCT blood collection tubes developed by Streck Inc. provide preservation and stabilization of cellular mRNAs for at least for three days at ambient temperature. This technology preserves the genetic expression patterns of specific genes allowing for the use and development of non-invasive diagnosis and prognosis methodologies based on cellular RNA in blood.

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B-65

Frequency of rs12979860 and rs8099917 Il28B polymorphisms in a control group and in hepatitis C patients followed in a Brazilian Hospital

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Background: Genetic variation in the *IL28B* gene region on chromosome 19 was recently identified for predicting response of patients chronically infected with genotype 1 HCV infection. This finding has been confirmed in several independent cohorts. The most strongly associated single nucleotide polymorphism (SNP) for treatment response, rs12979860, has also been shown to be significantly associated with spontaneous hepatitis C clearance. Other SNPs in close proximity to *IL28B* associated with treatment response and spontaneous clearance were described, such as rs8099917. Frequency of different alleles is different around the world and still there are few data about the distribution in our country.

Methods: For this study genotyping at two sites of interest, rs12979860 and rs8099917, were tested using TaqMan custom designed probes (Applied Biosystems) on an ABI7500 instrument and the ABI TaqMan allelic discrimination kit from Applied Biosystems. Analytical performance characteristics of the assay were performed and the method was validated for whole blood and plasma samples.

We analyzed SNP rs12979860 and rs8099917 in 56 samples from a private hospital in São Paulo, Brazil. Two different groups were studied: 1) 37 control samples from healthy donors; 2) 19 chronically HCV infected patients. Frequency of different alleles and genotypes were accessed and statistical analysis was performed using chi-square test with Yates'correction (BioStat 5.0 Software).

Results: Frequency of different genotypes in both groups was accessed. For rs12979860, genotype TT was present in 5.4% in the control group and in 21.1% in the HCV infected patients group. For rs8099917, genotype GG was not present in the control group, but in 10.5% in the HCV infected patients group. The distribution of different alleles in both groups was statistically significant ($p = 0.0058$ for rs12979860 and $p = 0.0162$ for rs8099917).

Conclusion: We validated an assay that can be performed in whole blood or plasma samples. Frequency of genotypes for these SNPs was determined in a population from São Paulo, Brazil. Although we still have a small number of patients, it was possible to verify that alleles associated with worse treatment response (T allele of rs1297960 and G allele of rs8099917) have a higher frequency among chronically HCV infected patients than in control samples. Statistical significance was reached for both tested SNPs, but it was more evident for rs12979860. Our results corroborate studies performed in other countries, especially in the Western hemisphere, where rs8099917 is not statistically associated with HCV infection.

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Characterization of CYP2C19 Allele Frequencies in an US Pan-ethnic Population

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Background: Cytochrome P450 2C19 (CYP2C19) is involved with the metabolism and elimination of many commonly prescribed drugs. Genetic polymorphisms in CYP2C19 are common and can affect therapeutic response to a variety of drugs. Detecting genetic variations in drug-metabolizing enzymes is useful for identifying individuals who may experience adverse drug reactions with conventional doses of certain medications. The reported allele frequencies in ethnic populations vary. Many published studies on allelic frequency only test limited variants. We undertook a study to determine the allele frequencies for CYP2C19*2, *3, *4, *5, and *17 on de-identified DNAs whose only descriptor was self-identified ethnicity.

Methods: 1548 patient samples were tested of which 942 had an ethnicity specified. Genomic DNA was amplified by PCR and subjected to allele-specific primer extension using SNaPshot® reagents (Applied Biosystems, Inc., Foster City, CA) for five variants (*2, *3, *4, *5, *17). The extension products were size-fractionated by capillary electrophoresis. Automated allele calling used Genotyper software and associated macros (Applied Biosystems, Inc.).

Results: See Table.

Conclusion: The ethnic data and CYP2C19 genotyping results will help update allelic frequencies in various populations.

CYP2C19 Allele frequencies

Genotype	Caucasian	African American	Hispanic	European Caucasian	Asian	Jewish	Other	Total
*1/*1 (assumed; no variant identified)	194(20.6%)	71(7.5%)	58(6.2%)	11(1.2%)	6(0.6%)	0	17(1.8%)	357(38%)
*1/*2	97(10.3%)	45(4.8%)	29(3.1%)	6(0.6%)	8(0.9%)	0	10(1.1%)	195(21%)
*1/*3	0	0	0	0	1(0.1%)	0	0	1(0.1%)
*1/*4	2(0.2%)	0	1(0.1%)	0	0	0	0	3(0.3%)
*1/*17	162(17.2%)	43(4.6%)	42(4.5%)	8(0.9%)	3(0.3%)	2(0.2%)	8(0.9%)	268(28%)
*2/*2	19(2.1%)	4(0.4%)	1(0.1%)	0	2(0.2%)	0	1(0.1%)	27(3%)
*2/*17	31(3.3%)	17(1.8%)	5(0.5%)	1(0.1%)	3(0.3%)	0	1(0.1%)	58(6%)
*17/*17	21(2.2%)	9(1.0%)	138(14.7%)	1(0.1%)	0	0	0	33(4%)
Total	526(55.8%)	189(20.1%)	138(14.7%)	27(2.8%)	23(2.4%)	2(0.2%)	37(3.9%)	942(100%)

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Rapid and reliable testing of interleukin (IL) 28B genotype in different biological specimens

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Background: Several recent studies reported a close correlation between the presence of polymorphisms (SNPs) in the IL28B gene and the rates of spontaneous and treatment-induced HCV clearance. However, the diagnostic utility of IL28B genotype is not yet completely understood and requires large-scale epidemiological and clinical studies. For rapid data gathering on the natural history of HCV infection in subjects with different IL28B genotype, simple, sensitive and rapid methods suitable for processing non-invasive and archival biological samples are urgently needed.

Methods: We developed a real-time PCR method suitable for very small DNA quantities derived from different biological specimens. The PCR target was rs12979860, the best characterized IL28B SNP in Western populations. PCR was carried out by a Taqman assay (Applied Biosystems) using a Rotor Gene 3000 thermal cycler (Corbett). The reaction was performed in 10 microliter volume containing 5 ng of DNA.

Results: Reliable IL28B genotyping of 58 HCV-positive subjects was obtained from different sources of starting material: whole blood (#58), buccal swab (#33), serum samples (#40), formalin fixed paraffin-embedded (FFPE) liver tissue (#26). At least two different specimens were analyzed for each subject with consistent results. A preliminary survey of IL28B genotype prevalence in subjects with chronic HCV

infection from our area was consistent with data reported in Caucasian populations (TT: 15.3%, TC: 50%; CC: 34.7%).

Conclusion: Real-time PCR was used to develop a simple, rapid and reliable method for IL28B genotyping suitable for non invasive samples as buccal swab or archival materials as serum or paraffin-embedded tissue samples. This method may be useful for large-scale epidemiological studies or retrospective cohort studies of the natural history of HCV infection in relation to IL28B genotype.

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Characterization of the INFINITI® Platform for Custom-Built CYP2D6 Based Genotyping Applications

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Genetic variations in key enzymes involved in drug metabolism pathways as well as variations in drug transporters and targeted receptors should all be considered in the design of a pharmacogenomics study aimed at characterizing the inherited basis of variation in drug efficacy or toxicity. AutoGenomics INFINITI® Platform offers a variety of applications to genotype CYP2D6 which is one of the most functionally polymorphic drug metabolizing enzymes. A universal core CYP2D6 panel can be custom-modified by adding or replacing analytes for other gene variants relevant for each study protocol. Based on the study results, the custom panel can be further developed to include only the clinically relevant genetic variants. INFINITI® CYP450 2D6I is the core CYP2D6 assay panel combining two multiplex-PCR reactions into a BioFilmChip® for genotyping the following variants: [*2, *3, *4, *5(deletion), *6, *7, *8, *9, *10, *12, *14, *17, *29, *41, and *XN(duplication)]. In contrast, INFINITI® CYP2D6T is a single multiplex-PCR reaction panel for genotyping most of the above variants except the following: [*10, *12, *17, and *XN (duplication)]. AGPGX01 and AGPGX02 represent custom-built applications developed from the core panels CYP2D6I and CYP2D6T, respectively. The AGPGX01 is designed for a codeine pharmacogenomics study by adding the primers/probes for an additional UGT2B7 [*2] genetic variant. Similarly, AGPGX02 is designed for a tamoxifen-related study by adding the primers/probes for two additional genetic variants, CYP3A5 [*3] and SULT1A1 [*2]. All these applications have been optimized for testing DNA samples extracted from blood as well as from saliva samples. AutoGenomics INFINITI® Platform represents an effective and flexible tool in conducting research and clinical studies. Ultimately, a well-defined application panel targeting the clinically relevant genetic variants can be developed to achieve the goal of personalized therapeutics.

B-69

Misclassification of an Apparent Alpha 1-antitrypsin "Z" Deficiency Variant by LightCycler Melting Analysis

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Background: Alpha-1 antitrypsin (AAT) deficiency is one of the most common potentially lethal genetic diseases among Caucasian adults. The *SERPINA1* gene (which codes for the AAT protein) is highly polymorphic, with over 100 variants documented in the literature. Many of these variants produce normal proteins, the most common being the M variant. Of the deficiency alleles, S and Z are the most common and are caused by single nucleotide substitutions. As a ubiquitously expressed member of the SERPINA family of protease inhibitors, AAT accomplishes its most important function by coating the lungs to inhibit neutrophil elastase. Thus, a deficiency in AAT results in the aberrant proteolysis of the connective tissue matrix of the lungs, leading to progressive pulmonary damage and eventually emphysema. In addition to the loss of anti-protease activity, certain AAT variants, such as the Z variant, have a propensity to polymerize in hepatocytes and may progress to liver dysfunction in childhood. AAT deficiency is treated using recombinant protein therapy, but severe liver disease may require transplantation.

Methods: AAT deficiency is diagnosed using a combination of genetic and biochemical tests. In our laboratory, total AAT is measured using an immunoturbidimetric assay, AAT phenotype is determined using isoelectricfocusing electrophoresis, and genotypic identification of Z and S AAT alleles is performed by melt curve analysis using a LightCycler. Published algorithms have been proposed which integrate serum AAT concentration with S and Z genotyping. Phenotyping is performed for wild-type or heterozygous individuals with an AAT concentration <100 mg/dL. Sequencing of the *SERPINA1* gene can be used to resolve discrepancies.

Results: Here we describe a patient that had a total AAT of 100mg/dL that was determined to be a Z heterozygote using the LightCycler genotyping assay. Phenotype testing was performed due to an AAT concentration near the decision threshold and was determined to be Pi MP. Sequencing results revealed that the discrepancy was caused by a previously reported polymorphism corresponding to a rare, non deficiency, P allele ($P_{St\ Albans}$). Closer analysis of the mutations revealed that similar melting temperatures could be expected, as both mutations are G to A and located two base pairs apart from each other. The probe that caused the discrepancy was designed to match the wild-type sequence. A new probe was designed to specifically detect the Z allele. The Z-specific probe showed a distinct melting pattern from the $P_{St\ Albans}$, eliminating the possibility of future discordance.

Conclusion: This study illustrates the importance of understanding the limitations of the probes utilized for genotype analysis. The specificity of the probe for the wild-type sequence allowed for a rare variant to incorrectly suggest the presence of the Z allele. Laboratories utilizing melt-curve analysis to diagnose patients should be aware of the potential for false positive results caused by polymorphisms located in the binding region of the genotyping probes. Designing probes to match the targeted mutation could reduce false positive results. In general, when designing genotype probes, the surrounding bases should be thoroughly examined for similar mismatches that may affect the melting temperature.

B-70

Gene promoter sequence variants in the genes for chemokines CCL19 and CCL21 in Czech patients with myocardial infarction

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Background: Migration and activation of inflammatory cells is an important pathogenetic mechanism of coronary artery disease and myocardial infarction (MI). Chemotactic cytokines expressed in atherosclerotic plaques (e.g. CCL2, CCL5, and more recently also CCL19 a CCL21) contain functional sequence gene variants, which may affect accumulation of macrophages, extent of inflammation and thus may contribute to genetic susceptibility to MI and its manifestation. We, therefore, investigated the association between myocardial infarction and selected polymorphisms in the regulatory regions of genes for the chemokines CCL19 and CCL21.

Methods: Based on a pilot re-sequencing study we selected and, using PCR-SSP, determined three polymorphisms of *CCL19 gene* (GenBank ID rs2233872) and *CCL21* gene (GenBank ID rs11574914 and rs11574915); the study group comprised 211 Czech patients with myocardial infarction and 150 healthy control subjects.

Results: There was no difference in allelic frequencies of the investigated SNPs between patients and controls ($p>0.05$). However, the proportion of homozygotes for the minor *G allele of the gene promoter variant *CCL21* (rs11574915 GG) was lower among the MI patients (1%) in comparison with the control subjects (5%). Protective effect of the GG genotype reached nominal significance ($p=0.03$), after correction for multiple comparisons value of p(corr) was 0.09.

Conclusion: Though rare in the Czech population, *CCL21* (rs11574915) GG genotype may confer protection from myocardial infarction. Our preliminary data have to be independently replicated in the second set of Czech MI patients and preferably also in other centres/populations. Further, analysis of functional effects of *CCL21* rs11574915 genotypes is desirable.

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B-71

Short tandem repeat analysis detection indicating acute lymphoblastic leukemia (ALL) recurrence prior to clinical and cytogenetic evidence

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Background: Sequential monitoring of chimerism after bone marrow transplant (BMT) has been shown to be predictive for graft failure and relapse. Short tandem repeat (STR) markers are routinely monitored to quantitatively determine engraftment status post-BMT.

Objective: Describe the detection of a non-donor, non-recipient STR allele preceding

clinical or cytogenetic evidence of pediatric acute lymphoblastic leukemia (ALL) recurrence.

Methods: DNA was isolated from peripheral blood lymphocytes. STR genotyping was performed via multiplex fluorescent PCR amplification using the AmpFISTR Profiler Plus ID amplification kit (Applied Biosystems). Products were separated by capillary electrophoresis on a 3130XL Genetic Analyzer (Applied Biosystems).

Results: The patient is a 3-year-old girl with a diagnosis of ALL who received a BMT. Eight and sixteen weeks post-BMT, recipient STR patterns were consistent with complete bone marrow engraftment. Forty weeks post-BMT, eight of nine informative loci showed donor alleles; however, one additional unknown allele was present at D21S11 (23.2). This allele was not consistent with either donor or recipient, and persisted with repeat analysis. The patient lacked clinical or cytogenetic findings of ALL relapse at this time. To exclude the possibility of molecular contamination, a new sample was requested (sample D). In this sample, the same additional allele was present at D21S11, and another non-donor, non-recipient allele was found at D8S1179. When sample D was collected, a routine WBC showed an elevated count consistent with ALL recurrence. (Table 1)

Conclusions: This case illustrates a potential source of complexity in the interpretation of BMT analyses-genomic instability, which is not uncommonly encountered in patients with hematopoietic malignancies. Laboratories might ignore the development of a non-donor, non-recipient allele, assume contamination, and not report this finding. However, our data indicate this finding should be reported to alert clinicians to the possibility of imminent disease recurrence prior to other clinical or cytogenetic indications.

Samples with STR, cytogenetic, and clinical results

Sample	Weeks Post-BMT	Cytogenetics/ FISH	STR results (D21S11)	STR results (D8S1179)	Clinical/Cytogenetic Recurrence
Pre-BMT recipient	N/A	Hyperdiploidy (trisomy for chromosomes 4, 17, ABL1, BCR locus, and tetrasomy for AML1 locus)	29,30	13,14	N/A
Donor	N/A	N/A	27,29	10,15	N/A
Post-BMT A	8 weeks	Not performed	Engrafted	Engrafted	No
Post-BMT B	16 weeks	Not performed	Engrafted	Engrafted	No
Post-BMT C	40 weeks	100% donor cells, no clonal aberrations	Additional allele 23.2	Engrafted	No
Post-BMT D	48 weeks	Complex abnormal karyotype (trisomy 4, 17, BCR locus and tetrasomy AML1 locus)	Additional allele 23.2	Additional allele 8	Yes

B-72

Association of the genetic marker for abacavir hypersensitivity *HLA-B*5701* with *HCP5* rs2395029 in Mexican Mestizos

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Background: Prospective screening for *HLA-B*5701* decreases or abolishes abacavir hypersensitivity reaction (AHR). In Caucasians, the *HCP5* rs2395029(G) allele is in complete linkage disequilibrium (LD) with *HLA-B*5701* (r^2=1). Our aim was to assess the frequency of *HLA-B*5701* and its LD with *HCP5* rs2395029(G) allele to extend our knowledge of genetic variants of critical relevance for the development of pharmacogenetics in Mexico.

Methods: We genotyped 300 Mexican Mestizos from the Mexican Genome Diversity Project (MGDP). *HLA-B*5701* genotyping was performed using a DNA sequencing method. *HCP5* rs2395029 was genotyped using a custom TaqMan SNP Genotyping Assay and confirmed by direct sequencing. Genotypes for 14 SNPs in the *HCP5* region were retrieved from the MGDP database for LD analysis.

Results: *HLA-B*5701* carrier frequency was 2% and the allelic frequency was 0.010. Haplotype analysis revealed that *HLA-B*5701* and the *HCP5* rs2395029(G) allele are in complete LD (r^2=1) in this Mexican Mestizos sample.

Conclusion: It is feasible to have a pharmacogenetic program based on *HCP5* rs2395029 genotyping as a screening tool with confirmation of *HLA-B*5701* carriage by sequenciation, to prevent AHR in Mexican patients before initiating abacavir therapy.

B-73

Evaluation of the Cepheid GeneXpert MTB/RIF assay for rapid detection of M. tuberculosis and rifampin resistance in clinical specimens

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Background: Accurate and rapid detection of Mycobacterium tuberculosis (MTB) is essential for the diagnosis of pulmonary tuberculosis. And also accurate and rapid detection of anti-tuberculosis drug-resistant strain is important for adequate treatment of MTB. The Cepheid GeneXpert MTB/RIF assay is an automated kits consisted of hands-free sputum-processing system and a real-time nested PCR. It extracts mycobacterial DNA, detects the presence of MTB, and simultaneously identifies the rifampin resistance within two hours. We evaluated diagnostic performance and clinical usefulness of the GeneXpert MTB/RIF assay.

Methods: We performed the GeneXpert MTB/RIF assay in 71 fresh sputum specimens (71 patients) which were positive for MTB by TB/NTM real time PCR kits (LG Life Science Diagnostics, Korea). Acid-fast bacilli stain and solid/liquid media culture were performed in parallel by standard laboratory procedures. Rifampin resistance detected by the GeneXpert MTB/RIF assay were compared with phenotypic tests by culture and with genotypic analysis of 81-bp rifampin resistance-determining region (RRDR) in the *rpo*B gene by sequencing. In vitro detection limit of MTB was assessed by known number cells of MTB spiked in sputums.

Results: Clinical specimens were from 53 known and 18 newly diagnosed tuberculosis patients, and consisted of 39 smear-positive and 32 smear-negative ones. The GeneXpert MTB/RIF assay detected MTB in all 71 specimens and identified rifampin-resistant mutations in 21/71 samples (29.6%). In rifampin resistance, the GeneXpert MTB/RIF assay was concordant with phenotypic rifampin susceptibility test by culture in 100% (61/61), and with genotypic analysis of RRDR region in 98.3% (57/58). One sample showed mixed sequence of wild and mutant strains. Limit of detection of MTB was 1.3 X 10 ^4 cells in sputum per test. Detection limit ratio of rifampin resistant MTB in susceptible ones was 1/5.

Conclusions: The Cepheid GeneXpert MTB/RIF assay sensitively and reproducibly detected the presence of MTB and its rifampin resistance in clinical specimens. It will be an easy, safe, fast, and reliable routine on-demand method for simultaneous detection of MTB and rifampin resistance.

B-75

Evaluation of a *CYP2C19* genotype panel on the GenMark eSensor® platform and the comparison to the Autogenomics INFINITI™ and Luminex *CYP2C19* panels

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Background: Given as dual antiplatelet therapy with aspirin, clopidogrel inhibits platelet function, improving the condition of those with acute coronary syndromes (ACS) and those undergoing percutaneous coronary interventions (PCI). Differences in drug efficacy are linked to clopidogrel pharmacokinetics, specifically to the *CYP2C19* genotype for certain patient populations. ACS patients who are carriers of the loss-of-function alleles of *CYP2C19,* e.g. the *2 or *3 alleles, have higher risk of adverse cardiovascular events, especially after PCI. The FDA issued a boxed warning for clopidogrel in 2010, stating that 2-14% of the US population are poor metabolizers, and that *CYP2C19* genotyping tests are available to determine if a patient is a poor metabolizer, allowing health care professionals to consider alternative antiplatelet medications. Thus, it is important that clinical laboratories have access to analytical platforms that are validated for such a genotyping service.

Objective: We compared the GenMark eSensor® platform for *CYP2C19* genotyping with other methods designed to detect variant alleles of this gene. We also assessed the reproducibility of the method.

Methods: In this method comparison study, *CYP2C19* genotypes were determined for 111 samples with the eSensor (*1 to *10, *13, and *17) and at least one other platform. Other methodologies included the expanded *CYP2C19* panel offered by Autogenomics (*1 to *10 and *17) and Luminex reagents, which detect *CYP2C19*

alleles *1 to *8, and bi-directional sequencing. Samples included DNA extracted from blood collected at the University of Chicago (n=27) and ARUP Laboratories (n=41), as well as 43 DNA samples obtained from the Coriell repositories. Several DNA extraction methods were used, including the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany), the Maxwell® 16 Blood DNA Purification Kit (Promega, Madison, Wisconsin), and the MagNAPure Kit (Roche, Indianapolis, Indiana). Within-day and between-day reproducibility studies were performed on several DNA samples with known *CYP2C19* genotypes (*1/*9, *2/*17, *2/*2, *2/*10 and *2/*4). **Results:** Complete (100%) concordance was observed for all samples for the genotypes available on each respective platform. The analytical sensitivity study demonstrates that concentrations of DNA as low as 0.05 ng/µl may be used on GenMark's eSensor platform. In addition, a compound genotype of a *CYP2C19* *2/*4/*17 was detected which was confirmed across two different platforms. Between-day and within-day replicate testing showed 100% expected *CYP2C19* genotype reproducibility, with a 97.5% yield of valid runs.

Conclusions: We showed that the GenMark eSensor *CYP2C19* genotyping assay is accurate and compares well with 2 current commercial platforms in clinical use. With a relatively rapid turn-around time of ~4 hours and a high rate (97.5%) of valid runs, the eSensor platform can be easily translated in helping physicians identify slow and rapid metabolizers of clopidogrel who may benefit from alternate therapy or unconventional dosing of clopidogrel. Extended panels with additional SNPs may be advantageous should future studies demonstrate the utility of expanded SNP panels in different patient populations, particularly in those that are not yet well-described (including Asian and African-American populations).

B-76

Rapid and Cost Effective Flow-through Based Assay for High Risk Human Papillomavirus (HR-HPV) Screening in Cervical Samples

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Background: Persistent infection of high-risk human papillomavirus (HR-HPV) has been widely associated with cervical intraepithelial neoplasia (CIN) and cervical cancer, 99.7% of cervical carcinoma with HR HPV infection (Walboomers *et al.* 1999). In addition, HR-HPV detection assay correlates well with cytogenetic data on premalignant lesions (i.e. CIN) and cervical cancer studies (Melchers *et al.* 1999), suggesting that early diagnosis and continuously monitoring of HPV infection is most efficient for cervical cancer prevention. For the reason, most healthcare authorities, including WHO, recommend screening the most prevalent 14 High-Risk types of HPV with special emphasis on Type 16 and 18. Hence an efficiency, fast and affordable screening assay is on these HR-HPV capable of distinctly identifying HPV types 16 and 18 would be most beneficial especially for low resource setting populations.

The GenoFlow HR-HPV screening assay is designed to detect the 14 different HR-HPV types that are vital for cervical cancer screening. In addition, GenoFlow HR-HPV assay can distinguish HPV16 and HPV 18 genotypes which are useful for prognosis purpose and for vaccination program. Furthermore, the Flow-through platform provides an efficiency hybridization and high throughput assay (i.e. 48 reactions in a single run) that save manpower and shorten turnaround time.

Methods: The studied cohort comprised archive samples of 119 women whose cervical scrapes were collected in liquid based cytology medium (ThinPrep) and sent to DiagCor Bioscience, Hong Kong, for HPV genotyping testing in November 2010 to January 2011. The DNA of cervical samples were extracted by QiAamp Blood mini extraction kit (Qiagen), and eluted with 75ml of elution buffer. The GenoFlow test uses biotin-labeled primers and specific probes to detect 16 HR-HPV types. The extracted DNA was mixed with PCR reagent mix and DNA Taq Polymerase provided with the GenoFlow test kit and PCR-amplified using the thermocycling condition stated in the manual. The amplicons were genotyped using Flow-through hybridization according to manufacturer's instructions.

Results: All cervical samples were firstly genotyped by GenoFlow HPV genotyping assay. In these samples, 80 samples were HPV positive and 39 samples were HPV negative. The HR-HPV assay can specifically identify 16 high risk HPVs without cross-reacting with low risk HPVs DNA, the concordance rate of HR-HPV is 90% to the GenoFlow HPV genotyping assay. By comparing to GenoFlow genotyping assay, the specificity and sensitivity of HR-HPV assay is 87% and 91%. In addition, the overall hand-on time was dramatically reduced from 107 minutes to 35 minutes comparing to conventional hybridization method which is almost 70% reduction.

Conclusion: HR-HPV screening assay can specifically identify the high risk human papillomavirus from low-risk types, and the improvement of cost, throughput and turnaround time of this assay can help to promote HPV DNA screening in developed and, especially, in developing countries in order to reduce the incidence of cervical cancer globally.

B-77

Efficiency of COLD-PCR and High Resolution Melting for detecting K-ras mutation

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Background: Molecular diagnostic tests can detect the target molecule with high sensitivity by use of nucleic acid amplification. However, in cancer, low level mutations cannot easily detected because of the much more DNA amounts of a wild-type background. Therefore, mutation enrichment technique is necessary, and one of the novel technique is COLD-PCR. COLD-PCR is able to amplify minority alleles selectively from mixtures of wild-type and mutation-containing sequences, by PCR at lower denaturation temperature. We reported previously the comparison of the detection sensitivity of K-ras mutations by COLD-PCR and conventional PCR, by use of the following single strand DNA conformation polymorphism (SSCP) analysis (Clinical Chemistry 55(6), Suppl. A219, 2009). In this study, we studied an efficiency of the combination of COLD-PCR and high resolution melting (HRM), in comparison with COLD-PCR-SSCP analysis.

Methods: Genomic DNA purified from cancer cell lines with K-ras mutation was diluted by wild-type DNA from other cell lines. The mutant-type DNA used was purified from Lu65 (codon 12: TGT), NEDATE (codon 12: GGT/GAT) and colorectal cancer tissue (codon 13: GGC/GAC). The DNA mixtures were amplified by full COLD-PCR and conventional nested PCR, followed by HRM analysis on a 7500 Fast Real Time PCR System (Applied Biosystems, Life Technologies).

Results: DNA from NEDATE were 1:10, 1:20, 1:50, 1:100 and 1:200 diluted in wild-type DNA, and amplified by full COLD-PCR and conventional nested PCR. The K-ras mutation was detected in 1:50 using conventional nested PCR and HRM analysis, while not detected in 1:100. However it was clearly visible following full COLD-PCR and HRM even in 1:100 diluted DNA. Other mutant DNA showed similar result that COLD-PCR and HRM analysis had better sensitivity in detecting low-level mutations. HRM analysis can be performed by using automated analyzer, thusfar COLD-PCR-HRM analysis could be a convenient screening system for detecting K-ras mutation. When some mutation is suspected, the amplified products could be confirmed by DNA sequencing.

Conclusion: We revealed that the combination of COLD-PCR and HRM analysis is useful for mutation enrichment and mutation screening using clinical specimens with low-level mutations.

B-78

Limiting Factors of Fast SYBR Green Real-Time PCR

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Background: There is an increasing need for real-time PCR assays that can be performed quickly and cheaply in settings such as developing-world point-of-care locations. Recently, the advent of various probe-based chemistries has dramatically improved real-time PCR specificity and multiplexing capability. However, intercalating dye chemistries, most commonly SYBR® Green I, in conjunction with melt-curve analysis remain more cost-effective for reactions requiring no or limited multiplexing. We sought to test the cycling-speed limits of a commercially available SYBR Green I master mix (Quanta® PerfeCta® SYBR Green Fastmix®) using a fast-ramping real-time PCR instrument (the Cepheid® Smartcycler® II) as a proof-of-concept, to assess the use of intercalating dye master mixes in fast real-time PCR applications.

Methods: Human genomic DNA was extracted in triplicate from a single whole-blood sample using a Qiagen EZ1 robot. DNA quantity and purity was then assessed via UV spectroscopy. Each of the three extractions were normalized with respect to concentration, and then run in parallel in five separate 25µl PCR reactions, each using two different primer sets and three different annealing/extension times, for a total of 30 reactions per extraction. One no-template control was included with each set of samples run on the Smartcycler. Primer sets were selected to amplify 100bp and 300bp regions of genomic DNA. PCR cycling protocols consisted of a 95°C, 1 second denature step, and a 60°C annealing/extension step for either 30s, 15s or 6s run for 50 cycles. PCR protocols included a final melt-curve stage, run from 60°C to 95°C at a ramp rate of 0.1°C/sec. Each PCR reaction contained 12.5µl Quanta 2X PerfeCta SYBR Green Fastmix, 10ng gDNA, and 0.3µM each primer. Final reaction volume was adjusted to 25µl with nuclease-free water. Cycling threshold (Ct) was manually

set to a constant value across all runs.

Results: In samples amplified for the 100bp target, Ct values remained relatively low and end-point fluorescence remained high even when anneal/extension times were dropped to 6s. However, when anneal/extension times for the 300bp target reactions were decreased, Ct values rose considerably, with an accompanying increase in between-run variability and decrease in end-point fluorescence. In addition, some 300bp-target reactions with shorter annealing/extension times demonstrated increased primer-dimer formation and non-specific amplification relative to target signal in the subsequent melt-curve analysis. Cycling time was ~50 minutes for the 30 second anneal/extend protocol, ~38 minutes for the 15 second protocol, and ~30 minutes for the 6 second protocol.

Conclusion: These findings suggest that intercalating dyes can be effective in fast PCR protocols if limited or no multiplexing is required. Run times could potentially reach under 20 minutes by reducing the number of cycles. Short target length is critical, as longer targets can lead to incomplete extension, weak amplification and increased non-specific amplification. With proper assay design, intercalating dyes may prove useful in providing inexpensive, robust qualitative real-time PCR assays in settings where fast turnaround and low per-sample cost are critical.

B-79

Treatment Outcome In Ph-Negative Bcr-Abl-Positive Chronic Myeloid Leukemia (CML) Patients

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Background. Approximately 1% of the CML patients do not have t(9;22)(q34;q11) detectable by conventional cytogenetics, but carry *BCR-ABL* fusion revealed by fluorescence in situ hybridization (FISH) and/or reverse-transcriptase PCR (RT-PCR). Imatinib efficacy in this group of patients remains unclear.

Aim. To assess imatinib treatment efficacy in Ph-negative *BCR-ABL*-positive CML patients. Methods. Initial diagnostics including chromosome banding analysis (CBA) and RT-PCR was done in 531 primary CML patients. CBA was performed after 24 hours culture. G-banding was performed by a trypsin-Giemsa method. Karyotypes were described according to ISCN (2005). At least 20 metaphases for each sample were analyzed. In Ph-negative patients who had *BCR-ABL* transcript, FISH assay using Dual-Colour Dual-Fusion BCR-ABL Translocation Probe (Abbott, USA) was applied on at least 200 interphase nuclei (I-FISH) and all available metaphases. CBA and FISH were performed at the time of diagnostics and every 6 months of imatinib treatment. Measurements of *BCR-ABL/ABL* transcripts ratio by real-time quantitative PCR were done every 3-6 months. Point mutations in the *BCR-ABL* tyrosine kinase domain were detected by direct sequencing of RT-PCR products. Compete cytogenetics response (CCgR) was assumed as less than 1% of FISH-positive nuclei (N. Testoni, Blood,2009) Event-free survival (EFS) was calculated in respect of intention-to-treat and defined as the time from imatinib beginning until any of the following events occurred: any sign of treatment failure (according to the European LeukemiaNet criteria (M. Baccarani, JCO,2009)), progression to AP/BC or death of any reason.

Results. Normal karyotype was detected in 12 newly diagnosed CML patients (2.3%). However, all of them harboured *BCR-ABL* fusion revealed by FISH and RT-PCR. Ph-negative group included 2 males and 10 females with median age of 51 years. 4 patients had e13a2 transcript variant, 8 patients - e14a2. Three FISH patterns were identified. 9 patients had cryptic insertion of *ABL* into *BCR* gene on chromosome 22. In 2 patients insertion of *BCR* into *ABL* gene on chromosome 9 was found. 1 patient had loss of *ASS*, 5'*ABL* and 3'*BCR* genes with fusion gene formation on der(22). 7 out of 12 patients received imatinib treatment. 6 of 7 patients achieved complete hematological response achievement at 3 months. Median number of *BCR-ABL*-positive nuclei at 12 months was 22% (range 1-50%), at 18 months 40% (range 0-92%). Only 1 patient achieved CCgR (assessed by I-FISH). One patient progressed to AP and died subsequently. None of patients had BCR-ABL mutations. EFS in Ph-negative CML patients treated by imatinib was significantly lower than in 244 Ph-positive ones, for whom long-term follow-up data was available: 0.14±0.13 vs 0.62±0.03 (p=0.007), while overall survival was comparable in both groups: 0.83±0.15 vs 0.85±0.02% (p=0.88).

Conclusions. Our data suggested that main mechanisms of *BCR-ABL* formation in Ph-negative CML are cryptic insertions of *ABL* into *BCR*, or vice versa. In our series treatment outcomes in this group were significantly worse in comparison with Ph-positive CML patients. Resistance in the observed group seems to have BCR-ABL-independent mechanisms, because of lack of BCR-ABL mutations, duplication or amplification.

B-80

Method Comparison for MGMT Hypermethylation Analysis

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Introduction: Molecular diagnostic laboratories are facing an increasing demand for molecular oncology testing. This testing often involves the detection of specific acquired mutations in DNA extracted from formalin-fixed, paraffin-embedded (FFPE) tissue blocks for prognostic or predictive purposes. In addition to the growing list of clinically significant tumor mutations, promoter methylation status of specific genes is growing in importance in the clinical laboratory, specifically for methyl-guanine methyl transferase (MGMT). Glioblastoma patients with MGMT promoter methylation detected in their tumor DNA were found to respond more favorably when treated with the drug temozolomide. The quality of DNA used in methylation studies is less than ideal due to the source of tumor DNA (FFPE tissue) and the harsh bisulfite treatment that often precedes the actual methylation testing. Two methods for analyzing the methylation status of tumor DNA are evaluated and compared.

Methods: Thirteen glioblastoma DNA samples were isolated from archived FFPE tissue blocks. MGMT methylation status was determined by a nested PCR approach in which primers specific for the methylated or unmethylated MGMT promoter were used in separate PCR reactions. Gel electrophoresis was performed to assess MGMT methylation status. Additionally, the PyroMark Q24 pyrosequencing platform (QIAGEN) was used to sequence a region of the MGMT promoter to determine its methylation status.

Results: Of the thirteen samples tested, the nested PCR analysis identified MGMT promoter methylation in nine samples (69%) while the remaining four samples (31%) tested negative for methylation. When the same samples were tested for MGMT methylation by the pyrosequencing assay, eleven produced results concordant with the nested PCR results and two samples (positive for methylation by nested PCR) failed to produce any results, presumably due to poor DNA quality and/or quantity. For the seven samples positive for methylation by pyrosequencing the percent of DNA with MGMT methylation in the samples analyzed ranged from 14% to 53%.

Conclusions: DNA methylation testing will likely grow in importance for clinical molecular laboratories, yet there is a lack of consensus concerning the ideal testing methodology. The majority of published studies demonstrating correlations between MGMT methylation status and clinical response used a nested PCR approach similar to the one used here. This technique, however, may not be suitable for a clinical laboratory due to the increased risk of contamination and false positive results caused by the post-amplification manipulation of each PCR in the nested PCR protocol. Alternate methods such as the pyrosequencing method may be less robust than nested PCR but can offer a more quantitative analysis of methylation status and a lower risk of PCR contamination and laboratory error.

B-83

To screen mutations in candidate genes of the hereditary cataract with special morphous

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Background: Congenital cataract is the main cause of the visual impairment in childhood. According to the different forms and sites of lens opacity under slit lamp, congenital cataract can be divided into different categories which include the nuclear cataract, zonular cataract, blue dot cataract, sutural cataract and cortical cataract ect. The objective of this study is to investigate the mutation pattern of commonly morbigenous gene in sporadic special morphous cataract.

Methods: 5 patients with the hereditary special morphous cataract were recruited in Zhongnan hospital. DNA was extracted by proteinase K method from peripheral blood of patients. Mutations screening was carried out in the crystallin (CRY) CRYAs,CRYBs,CRYGs and gap junction protein, alpha 8(GJA8) gene by polymerase chain reaction and DNAsequencing.

Results: We failed to find any mutations in CRYAs,CRYBs,CRYGs and GJA8 genes. But we find a singe-base substitution in intron2(5'+75 T→G) of CRYBA1 in two patients with blue dot cataract.

Conclusion: The intron2 (5'+75 T→G) of CRYBA1 in blue dot cataract patients was a new single nucleotide polymorphism which could be used as a genetic marker of these two patients. CRYAs, CRYBs, CRYGs and GJA8 could be exclude for the defect genes of the patients. We needed to carry further study to reveal the causative genes of the five patients.

B-84

Rapid and Specific Detection and Differentiation of *Mycobacterium tuberculosis* (MTB) and Nontuberculosis Mycobacteria (NTM) by Real-Time PCR and Reverse Blot Hybridization Assay

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Background: Rapid and specific techniques to detect and differentiates both *Mycobacterium tuberculosis* (MTB) and Nontuberculosis Mycobacterium (NTM) directly in clinical patients are important for the diagnosis and treatment. Molecular method using DNA probes and amplification products offer detection of MTB and NTM directly from clinical specimens. Recently, the real-time PCR assay using specific detection of the targets by fluorescent probes that is getting the spotlight in these the diagnosis and management of patients with tuberculosis promotes the specificity of assays. In addition, another benefit of the real-time PCR assay is the ability to perform multiplex amplification and detection of targets.

Methods: In this study, we rapid and accurate differentiate both MTB and NTM at DNA level based on region of difference 9 (RD9), IS*6110* and RNA polymerase ß-subunit (*rpoB*) genetic region by real-time PCR, and then directly identify the NTM by reverse blot hybridization assay. Moreover, by including internal control (I.C.), any PCR blocking elements within the sample can be checked.

Results: Of the 161 clinical samples from patients with tuberculosis, 103 samples were NTM and 58 samples were MTB, the sensitivity of this real-time PCR assay for the detection and differentiation of MTB and NTM was 100 and 88.3 %, and the specificity of MTB and NTM showed the same, respectively. Also, it was shown that the identification of these clinical samples using reverse blot hybridization assay corresponded with conventional methods. In addition, direct use of real-time PCR products at reverse blot hybridization assay was successful in identification of MTB and *M. intracellulare*.

Conclusion: These results indicate that the assay using real-time PCR assay is comparable with commercial kits based on rRNA sequences, notably that of 16S rRNA (e.g. AccuProbe, Gen-Probe Inc., LiPA; Innogenetics N. V.). The method using real-time PCR and reverse blot hybridization at diagnisis and treatment can allow for the accurate and rapid identification of MTB and NTM.

B-85

Development of amplicon-based targeted resequencing for large-sized genes using next generation sequencing technology: an example of *BRCA1/BRCA2*

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Backgrounds: Next generation sequencing (NGS) technology has a potential to change current practice in molecular diagnostics. Targeted resequencing of large genes such as *BRCA1/BRCA2 or DMD* is an example among various clinical applications of NGS. Here, we developed an amplicon-based NGS testing for *BRCA1/BRCA2* using the Roche GS Junior platform and evaluated its performance against conventional Sanger sequencing.

Methods: The *BRCA1/BRCA2* coding regions were amplified using the same protocol as used in Sanger sequencing. After adapter ligation to index three different samples with known pathogenic or neutral sequence changes, PCR amplicons were pooled in equimolar concentrations and sequenced with the Roche GS Junior system.

Results: Four different pathogenic sequence changes including small insertions/deletions as well as nucleotide substitutions, and fifteen neutral nucleotide substitutions were successfully identified in each of two separate runs. However, unlike nucleotide substitutions, insertions/deletions were falsely detected in homopolymer regions under analysis criteria to ensure high sensitivity. In addition, read coverage across exons showed great variability of more than hundred times.

Conclusions: Our results demonstrate that NGS outperforms conventional Sanger sequencing for targeted resequencing of large gene in analysis times and costs, although some issues such as coverage variability and homopolymer error remain to be solved.

B-86

Mycophenolic acid modulation of epithelial tight junctions - a mechanism of gastrointestinal toxicity?

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Background: Gastrointestinal toxicity is a common adverse effect of mycophenolic acid (MPA) treatment in solid organ transplantation patients with a little understood mechanism. Using a proteomics approach, we observed a regulation in the myosin light chain 2 (MLC2) expression upon MPA treatment. Myosin light chain (MLC) phosphorylation and epithelial tight junction modulation lead to defective epithelial barrier function, a mechanism which is described to be important in some gastrointestinal diseases. The aim of this study was to investigate whether the MPA is able to disrupt colonic epithelial integrity via modulating epithelial barrier permeability.

Methods: Human colonic cells were exposed to therapeutic dose of MPA and expression of MLC and myosin light chain kinase (MLCK) was analysed using PCR and immunoblotting. Permeability was assessed by measuring transepithelial resistance (TER) and the flux of paracellular permeability markers (FITC-dextran) across epithelial monolayers.

Results: MPA increased expression of MLC and MLCK both at transcriptional and protein level. In addition MLC phosphorylation was also increased after MPA treatment. Exposure of MPA resulted in increased epithelial permeability in cells without significant change in cells viability which indicates that tight junction modulation was not due to cell death.

Conclusion: Our results suggest a modulating role of MPA on intestinal epithelial barrier permeability through alteration in MLC phosphorylation which leads to the pathophysiology of intestinal epithelial barrier. Further studies can help to understand the precise molecular mechanisms of MPA induced tight junction disruption.

B-87

Association between two single nucleotide polymorphisms at corresponding microRNA and schizophrenia in a Chinese population

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Background: Numerous linkage and association studies have been performed to identify genetic predispositions to the disease in different population, but its genetic basis remains unclear. Some findings may provide a clue in understanding the association with abnormal innate immunity in schizophrenic patients. MiRNAs involve in regulating both schizophrenic and immunity. And single nucleotide polymorphisms (SNPs) within miRNAs can change their characteristics, resulting in functional and/or phenotypic changes. In this study, two SNPs (hsa-pre-mir-146a rs2910164 G>C and hsa-mir-499 rs3746444 T>C) at two miRNAs were genotyped to demonstrate their association with susceptibility to schizophrenia.

Methods: Two SNPs were analyzed among 268 Chinese schizophrenia patients and 232 healthy controls by PCR-RFLP and sequencing.

Results: No association was found between the two polymorphisms and schizophrenia either in cases or in controls. Schizophrenia patients with family history showed significant increase of the G allele frequency in rs2910164 in comparison to those without (p=0.018). And the CC genotype frequency of rs3746444 was also higher in the patients having hallucinations than those without (p=0.012). In addition, patients carrying CC genotype of rs3746444 were found to be more lack of motivation in comparison to normal controls (p=0.042). Allele and genotype frequency of rs3851179 showed no significant difference between patients and normal subjects or between patients with and without clinical variables.

Conclusion: Although patients carrying CC genotype of rs3746444 were found to be easier to develop hallucination and individuals carrying C allele more lack motivation, there is lacking association between Schizophrenia and the two SNPs at miRNAs, which may regulate immune response.

Table 1 Comparison of rs2910164 and rs3746444 polymorphisms among patients with different clinical features and controls

	patients									Controls
	Hallucinations (n=139)	Delusional (n=177)	Psychotic thought disorder (n=93)	Affective disorders (n=90)	Indifference (n=73)	Behavior disorder (n=110)	Lack of motivation (n=63)	Social dysfunction (n=229)	Family history (n=49)	
rs2910164										
GG	24(49.0)	29(59.2)	22(47.8)	17(37.0)	9(19.6)	23(50.0)	13(26.5)	42(85.7)	5(10.2)	27
CC	65(52.8)	80(65.0)	34(30.4)	33(29.5)	29(25.9)	53(47.3)	31(25.2)	102(82.9)	20(40.8)	123
CC)	50(52.1)	68(70.8)	35(36.8)	40(42.1)	35(28.9)	34(35.8)	19(19.8)	85(88.5)	24(49.0)	82
p	0.261	0.207	0.006	0.024	0.117	0.076	0.175	0.072	0.197	/
G	113(40.6)	136(39.0)	78(42.9)	67(37.2)	47(32.2)	99(45.0)	57(36.5)	186(40.6)	30(30.6)	177(38.1)
C	165(59.4)	216(61.0)	104(57.1)	113(62.8)	99(67.8)	121(55.0)	99(63.5)	272(59.4)	68(69.4)	281(61.9)
P	0.499	0.887	0.271	0.828	0.193	0.088	0.720	0.444	0.160	/
rs3746444										
TT	112(55.7)	134(66.7%)	74(38.1)	73(37.6	56(28.9)	82(42.3)	43(21.4)	167(83.1)	40(81.6)	180
TC	19(34.5)	34(61.8)	16(32.0)	14(28.0)	15(30.0)	22(44.0)	15(27.3)	52(94.5)	7(14.3)	45
CC	8(66.7)	9(75.0)	1(11.1%)	3(33.3)	3(22.2)	6(66.7)	5(41.7)	10(83.3)	2(4.08)	7
p	0.186	0.564	0.504	0.719	0.894	0.530	0.170	0.472	0.671	/
T	243(87.4)	302(85.3)	164(90.1)	160(88.9)	127(85.8)	186(85.5)	101(80.2)	386(84.2)	87(88.8)	405(87.3)
C	34(12.6)	52(14.7)	18(9.9)	20(11.1)	21(14.2)	34(15.5)	25(19.8)	72(15.7)	11(11.2)	59(12.7)
P	0.861	0.414	0.319	0.578	0.643	0.329	0.042	0.191	0.685	/

B-88

Frequency Distribution of Single Nucleotide Polymorphisms in the promoter of p-selectin Gene in Chinese Tibetan and Han population

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Background: Chinese Tibetan population have lived in high altitudes for years and evidence show that they possess heritable adaptations to tolerate environmental hypoxia, such as high level of RBCs and hemoglobin, which increase the risk of coronary artery disease (CAD). However, there are no evidences show that Tibetan have higher morbidity of atherosclerosis or AHD than other ethnic group. Single nucleotide polymorphisms (SNPs) in P-selectin gene has been proved to be involved in coronary artery disease,stroke, and other diseases. Difference of frequency distribution of SNPs in p-selectin gene between Tibetan and Han population is still unkown. In study,SNPs in promoter region of P- selectin in the Chinese Tibetan and Han population for the first time to analyze their hereditary difference.

Methods: 303 Chinese Tibetan population and 267 Chinese Han population were involved in this study and 3 SNPs , -1969 G/A (rs1800805), -2123 C/G rs1800807 and -1817 T/C rs1800808 were analyzed. The high-resolution melting (HRM) was used to genotyping samples for the three single nucleotide polymorphisms (SNPs). The HRM method was optimized on a LightCycler 480 machine, and genotyping was performed by GeneScan software.

Results: Chinese Tibetan population showed a significant increase of the allele G frequency in *rs1800805 in comparison to* Chinese Han people (p=0.03).There was no significant differences in the genotype and allele distribution of rs1800807 and rs1800808 for the P-selectin gene between Chinese Tibetan and Han population ($P > 0.05$) . Compared with England and American, the distribution had significantly differences among ethnics ($P < 0.001$) .

Conclusion: This study identifies that the genotype and allele of *rs1800805* showed significant difference between Tibetans and Han population. This frequency difference may be the result of different ethnics.

Tab1. Gene type and Alleles frequency for promoter region of P- selectin SNPs for Chinese Tibetan and Han population

303 Chinese Tibetan population and 267 Chinese Han population								
Rs1800805			Rs1800807			Rs1800808		
Gene type	Tibetan	Han	Gene type	Tibetan	Han	Gene type	Tibetan	Han
AA	8	14	CC	9	5	CC	13	13
AG	71	81	CG	111	109	CT	79	81
GG	224	172	GG	183	153	TT	211	173
allele								
A	87	109	C	129	119	C	105	107
G	519	425	G	477	415	T	501	427
A/G	0.167	0.256	C/G	0.270	0.286	C/T	0.209	0.250

B-89

A novel mutation in APC gene in a Chinese familial adenomatous polyposis pedigree

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Background: Familial adenomatous polyposis is an autosomal dominant disorder characterized by predisposition to colorectal carcinoma. It is reported that mutations in the adenomatous polyposis coli (APC, Gene ID: 324) gene are related to autosomal dominant adenomatous polyposis. The objective of our study is to identify the causative defects associated with autosomal dominant adenomatous polyposis in a Chinese family.

Methods: Families were ascertained and patients underwent comprehensive colonoscopic and other clinical examinations. Blood samples were collected and genomic DNA was extracted. The mutation screening of candidate gene (APC) was conducted by polymerase chain reaction (PCR) analyses and sequencing.

Results: According to the colonoscopy, we detected numerous of colorectal adenomas (range 100-1,000) in the patients. The results of direct DNA sequencing revealed a insertion mutation at codon 610 in exon 15 of APC gene(c.1828_1829insG), which resulted in frameshift change (p.Asp610GlyfsX23) in all 4 patients, but was absent in the unaffected persons and 200 normal controls. This mutation produces a truncating protein, which has only 633 amino acid. APC protein lost biological activities due to its truncated change.

Conclusion: The heterozygous insertion mutation in APC gene cosegregated with the FAP disease. Therefore, we could regard it as the pathogenic gene of this Chinese FAP pedigree. We identified a novel insertion mutation in Chinese population, which can enrich the germline mutation spectrum of APC gene.

B-91

Multiplex fluorescent analysis of five STR for the indirect DMD/BMD diagnosis

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Background: DMD[MIM 310200]and BMD[MIM 310376] are allelic, X-linked recessive disorders caused by mutations of the DMD gene [MIM 300377] located at Xp21.2. DMD/BMD is an X-linked recessive disorder affecting 1 in 3500 (DMD) and 1 in 30,000 (BMD) male births. DMD is rapidly progressive , with affected boys being wheelchair-bound by age 12 years and and die early in their third decade of life from respiratory or cardiac failure. While BMD [MIM 300376] is a milder form of the disease. Partial deletions and duplications of the DMD gene exons account for 70% of cases of DMD and 85% of BMD,respectively, while the remaining cases are caused by single point mutations or small rearrangements. Now, it can be directly detected almost all the partial exons deletions and duplications of the DMD gene by Multiple ligation-dependent probe amplification (MLPA) developed recently. But, the rest mutations can not be detected routinely. Here, we report a home-brew linkage analysis method for the indirect diagnosis of DMD/BMD carrier and patient, which can be an alternative option while the MLPA failed to detect the mutations directly.

Methods: Four intronic microsatellites(located from the beginning to the end of the DMD gene) and one marker downstream to the DMD gene were selected, and primers were designedfor multiplex PCR amplification.

Results: We tested 61 unrelated females, and found that the HF of each loci was similar to the online Leiden muscular dystrophy database(www.dmd.nl),and the accumulated heterozygosity of the five maker is 0.99.

Conclusion: This assay provides a rapid method for linkage diagnosis of DMD/BMD in families where mutations can not be identified .However, considering that approximately one-third DMD/BMD patients originate through new mutations, it should be sufficiently aware of the risk before running a linkage analysis in a DMD/BMD family.

Primers design details

Primer name	Repeat	Localization in DMD gene	Primer	
			Forward	Reverse
Int2	(TG)23	Intro2	Fam-5'-CCTGATGTCGATTTGGTTTTT-3'	5'-TTAGCTTGCTCTGAGTAAACC-3'
Int45	(CA)28	Intro45	Fam-5'-TCAAGAGATTTCAAAACCAA-3'	5'-AGGAAGTAGACAACTCATTGT-3'
Int49	(AC)24	Intro49	Fam-5'-ACTGAAGGCTTTGGCCATAT-3'	5'-GGCAAGTTTCTCTTCGTCAA-3'
Int62	(GAA) 27	Intro62	Fam-5'-ATCACTGCCATGGTGAATGAA-3'	5'-CACTTTGGGAGGCTGAGCT-3'
Ext79	(TG)18(AT)14	>200kb 3' of exon79	Fam-5'-CTGGGACAGAAAGGGTTGAA-3'	5'-GGCGGATCACAAGGTAAA-3'

B-93

Rapid UDP-glucuronosyltransferase1A1 (UGT1A1) Gene Mutations screening by High-Resolution Melting Analysis

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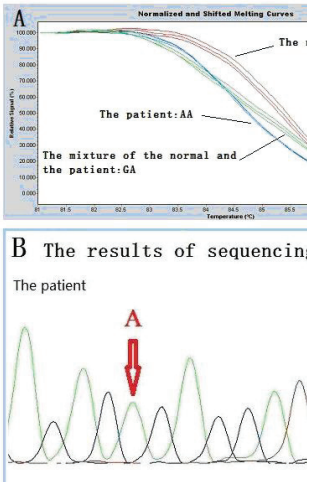
Objective To design a method to screen the coding regions of UGT1A1 gene by High-Resolution Melting(HRM) Analysis and confirm the mutation of UGT1A1 gene of a Crigler-Najjar Syndrome Type2 patient.

Method A male neonatus with Crigler-Najjar Syndrome Type 2 and a normal control were recruited in this study. We collected their genomic DNA from peripheral blood leukocytes, designed six PCR primer pairs to amplify coding regions and promotor of UGT1A1 gene, performed HRM analysis for the amplification products of the patient, the normal as well as the mixture of the two to screen these regions for mutations, and sequenced the fragment screened out to confirm the mutation.

Results The melting curves showed a significant difference between the patient and the normal control in exon1 (Fig.1A) while there is no difference in the other regions; Sequencing analysis revealed that a homozygous c211G> A(Fig.1B) located in the patient 's exon1.

Conclusion Point mutations of UGT1A1 gene can be screened accurately and rapidly by HRM analysis and the homozygous G> A mutation at nucleotide211 (Gly71Arg) in Exon1 causes the patient Crigler-Najjar Syndrome Type 2.



B-94

Relative Frequencies Of Polymorphisms Associated With Thrombophilia In Northern Italy

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Background: polymorphisms in specific genes have been reported as genetic risk factors for thrombophilic disorders and they appear to have different prevalence in the general population. The aim of this work is to evaluate, in our geographical area in northern Italy, the frequencies of specific polymorphisms associated to thrombophilia in a not selected population, sent to our laboratory for the evaluation of genetic predisposition to cardiovascular accidents.

Methods: from June 2009 to January 2011, 653 patients with an age range of 16-81 years (mean age 40.3 years) were screened. For each patient all the following genetic variants were analysed: Factor II G20210A, Factor V Leiden G1691A, Factor V A5279G (Y1702C), 5,10-methylenetetrahydrofolate reductase (MTHFR) C677T, MTHFR A1298C, by means of TaqMan real-time PCR with the commercial kit "BioDect Pannello Coagulazione", Biodiversity, Italy. For each patient five targets, each corresponding to a genetic polymorphism, were analysed in parallel in five separate tubes. In each amplification mixture both tagged probes for the variant and normal alleles were present.

Results: 44/653 (6.7%) subjects were homozygotes for normal alleles. Heterozygosis for prothrombin G20210A polymorphism was found in 34 subjects (in 30 of them it was associated with other polymorphisms, only in 4 it was present alone). Homozygosis was never found. The allele frequency was 2.6%. Factor V G1691A Leiden polymorphism was found in 40 subjects (2 homozygotes and 38 heterozygotes), associated with other polymorphisms in 37 cases and present alone in 3 cases. The allele frequency was 3.2%. Factor V A5279G (Y1702C) variant was never found. We observed a very high frequency for C677T and A1298C MTHFR: C677T homozygosis in 124 cases (19.0%), C677T heterozygosis in 118 (18.1%), A1298C homozygosis in 51 (7.8%), A1298C heterozygosis in 84 (12.9%), double heterozygosis for both polymorphisms in 159 cases (24.3%). So, in the analysed population the frequency of C677T, A1298C and normal allele were respectively 45.3%, 29.7% and 25.0%. As expected, no association was found between homozygosis for a variant allele and heterozygosis or homozygosis for the other one. MTHFR polymorphisms were observed in association with FII G20210A in 29 cases and with FV Leiden in 36 cases. An association between FII G20210A and FV Leiden was found in 2 cases.

Conclusion: the observed allele frequencies for FV Leiden and for FII G20210A polymorphisms are consistent with those reported by other authors in Europe (1.4-4.2% and 1.0-3.5%, respectively). Also the genotype prevalence and allele frequency of MTHFR polymorphisms are compatible with some other reports. We observed that in the tested population the two MTHFR gene variants C677T and A1298C were more frequent than the normal alleles. These data suggest that MTHFR genotyping, in order to assess the thrombotic risk, is of questionable clinical utility and might be more effectively substituted by the analysis of homocysteinemia. In all subjects analysed we only detected C677T and A1298C in trans configuration and never observed the same variants occurring in cis. We conclude that this configuration must be at least very rare.

B-95

The relationship between ChREBP gene polymorphisms and coronary artery disease in Han population

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Background: ChREBP regulates lipogenesis and glucose utilization in the liver. Reports suggested a contradictive association between rs3812316 in this gene and Triglyceride level. We hypothesized ChREBP gene polymorphisms be associated with CAD.

Methods: The ChREBP gene polymorphisms were analyzed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) methods in 200 controls and 310 CAD patients in Chinese Han population. Serum lipids and glucose concentrations were measured in all subjects.

Results: The rare allele G at the rs3812316 site was significantly lower in the CAD group after adjusting (OR^a =0.589, 95% CI=0.361-0.961, P =0.034). No significant difference between cases and controls was found in genotype or allele distributions of rs7798357, rs17145750 and rs1051921 between controls and patients. Haplotype CGC was significant higher in CAD group (P<0.01, OR=2.364, 95%CI=1.608~3.474), haplotypes GGC, CGT, CCC were significant lower in CAD group (P<0.05). And we did not find any statistically signicant associations between SNPs and lipids and glucose levels in the control and CAD group.

Conclusion: rs3812316 and the haplotypes constructed based on the SNPs in ChREBP gene appeared to be related to susceptibility to CAD in Chinese Han population.

Tuesday PM, July 26

Poster Session: 2:00 pm - 4:30 pm
Mass Spectrometry Applications

B-96

Characterization of reference material NMIJ 6201-a, C-reactive protein (CRP) in solution and recombinant ^{15}N-labeled CRP as reagents in isotopic-dilution mass spectrometric quantification

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Background: This study sought to determine the fitness of two proposed reagents for use in the development of an isotopic-dilution mass spectrometric (IDMS) approach to the quantification of C-reactive protein (CRP) in serum. IDMS quantification requires both a standard protein of the analyte in question, with sufficient purity and known concentration, as well as an isotopically-labeled form. It is proposed to use the certified reference material produced by the National Metrology Institute of Japan, CRM6201-a, C-reactive protein in solution, as the standard material. CRM6201-a is a recombinant form of CRP expressed in bacteria. As part of the study, an isotopically-labeled form of CRP was generated by recombinant expression in yeast under conditions of enriched isotopic nitrogen (^{15}N) using standard techniques and purified by phosphorylcholine binding. As both of these reagents are of recombinant origin, it is necessary to demonstrate that these forms respond similarly to CRP of human origin in the steps of the analytical process. The successful characterization of these reagents will allow for the development of a method of higher order for CRP in serum, for which none currently exists, and would serve to provide greater harmonization within the *in vitro* diagnostic industry.

Methods: CRM6201-a was compared against two native forms of purified human CRP, of either human serum or pleural fluid origin. A concentration curve was generated for each material in turn with an appropriate amount of internal standard added to all samples. The material was affinity purified, digested and analyzed by liquid chromatography tandem mass spectrometry (LC-MS/MS) using up to 5 distinct peptides with at least 2 products per peptide. The percentage of ^{15}N incorporation was determined by comparison of the matrix-assisted laser desorption ionization MS spectra of four tryptic peptides from the labeled internal standard with a calculated isotopic distribution using custom software.

Results: : Regression parameters of the three sources of CRP (CRM6201-a, serum, pleural) were not significantly different among three replicates with average values of: slope 5.6, 5.7, 5.1 ; intercept 0.008, 0.053, 0.051 ; r^2 0.9967, 0.9906, 0.9975, respectively for each source. A similar experiment performing only digestion likewise produced parameters which were very similar between the CRP types and also exhibiting a high degree of linearity ($r^2 > 0.99$). Analysis of the percent ^{15}N incorporation was determined to be 98.2 % (%CV= 0.3) with a Pearson correlation coefficient of 0.9986 (%CV=0.14) based on using four tryptic peptides. Software was validated by confirming that the expected value of ^{15}N incorporation was obtained using the spectra of a peptide having a natural isotopic distribution.

Conclusion: NMIJ 6201-a, CRP in solution responds in a similar manner as two native forms of CRP in both digestion and affinity purification/digestion methods. This indicates the reference material is suitable for use in CRP metrology. The labeled internal standard in all cases yielded a high degree of linearity indicating that the internal standard is proportionally equivalent among all of the CRP sources and is likewise suitable for CRP metrology, particularly with such a high percentage of labeled nitrogen.

B-97

Clinical Mass Spectrometry to Quantify Therapeutic and Endogenous Cardenolides with Anti-cancer Properties

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Background. Digoxin is a plant-derived cardenolide used to treat cardiac arrhythmias, yet several studies indicate that some cancer patients treated with digoxin respond more favorably to cancer therapies. Our lab has shown that the endogenous mammalian cardenolide, digoxin-like immunoreactive factor (DLIF), found in blood can selectively induce apoptosis in cancer cell lines, but not in untransformed cells. This suggests that DLIFs may be novel functional indicators of the endogenous anti-tumor response. These endogenous compounds are very difficult to purify and are detected using anti-digoxin antibodies which are inherently neither specific nor sufficiently sensitive for measuring DLIF in serum. Mass spectrometry could provide a reliable, sensitive, and specific alternative. *The ultimate objective of this project is to develop a rapid and accurate clinical LC-MS/MS method for measuring DLIF and dihydro-DLIF concentrations in blood.* This abstract reports the initial stage of the project - the development of a clinically validated mass spectrometry method to quantify the plant-derived cardenolides digoxin and dihydrodigoxin as surrogates for DLIFs.

Methods. A Thermo LTQ FT Fourier-transform ion cyclotron resonance mass spectrometer (FTICR-MS) with nano-ESI source was used to analyze and quantify digoxin and dihydrodigoxin in methanol in FT-MS mode. To increase analytical sensitivity, a Thermo TSQ triple quadrupole tandem mass spectrometer was used for detection. Digoxin standards were directly infused for tuning and selection of fragmentation conditions for selective reaction monitoring. Digoxin standards (with deuterated digoxin as internal standard) were injected over a C18 reversed-phase column into the AP-ESI source of the Thermo TSQ using an Agilent HPLC binary pump. Digoxin retention time was 1.7 min during isocratic elution with 40% acetonitrile, 10 mM NH_4OAc. Instrument response to digoxin was calibrated by calculating the ratio of digoxin and digoxin-D_3 peak areas.

Results. The FTICR-MS system detected digoxin as the sodium adduct (803.4 *m/z*) with digoxin-D_3 (806.4 *m/z*) as internal standard. The calibration was linear (r^2 = 0.995) over a range of 1-10 ng/mL. Dihydrodigoxin was also detected as the sodium adduct (805.4 *m/z*) using digitoxin (787.4 *m/z*) as internal standard with linearity (r^2 = 0.988) from 1-10 ng/mL. The LC-MS/MS system using the TSQ gave greater sensitivity (lower limit of detection ~0.1 ng/mL) with selective reaction monitoring transitions of *m/z* 798/651 for digoxin and *m/z* 801/654 for digoxin-D_3. Calibration of digoxin on this system was linear (r^2 = 0.9828) from 0.4-10 ng/mL, and the coefficient of variation at the lower end of the dynamic range was <15%.

Conclusions. We have optimized parameters for measurement of both digoxin and dihydrodigoxin in methanol using the FTICR-MS system. The improved sensitivity of approximately 0.1 ng/mL digoxin observed using the TSQ system, as well as the capacity for separating serum components effectively by HPLC, now sets the stage for optimization of a sensitive and specific method for measurement of the endogenous DLIFs in serum using LC-MS/MS technology. Supported by NIEHS P30ES014443, KSEF Grant #148-502-10-263, and NSF/EPSCoR Grant #EPS-044749

B-98

Measurement of 25-hydroxyvitamin D3 and C3-epi-25-hydroxyvitamin D3 using UPLC/MS/MS in paediatric and adult populations

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Background: The C3-epimer of 25OHD has been identified as a potential interference in the assessment of vitamin D sufficiency, although the clinical significance remains unclear. In 2006, Singh *et al* described a chiral chromatography method to partially separate these compounds. The study concluded that the C3-epimer was primarily detected in infants and not adults. More recently, NIST described a candidate reference procedure for the measurement of 25OHD in serum using extended reversed-phase HPLC tandem mass spectrometry that demonstrated baseline resolution of the C3-epimer from 25OHD3. In this study we developed a UPLC reversed-phase chromatographic separation of 25OHD3 from C3-epi-25OHD3 to determine the C3-epi-25OHD3 concentrations in paediatric and adult populations.

Methods: 156 anonymized adult and 62 paediatric (age 0-9years) serum samples from the North West of England were analysed using an established semi-automated SPE-UPLC/MS/MS. Serum samples and calibrators were placed on a robotic liquid-handling system and identified by bar code to be tracked throughout the extraction procedure. Tri-deuterated 25OHD2 was used as an internal standard for C3-epi-25OHD3, as it eluted closer to the retention time for the C3-epimer compared to the 25OHD3 internal standard, to compensate for any matrix effects upon the ionisation of the molecule. The internal standard was added to the dispensed samples prior to protein precipitation. Following centrifugation (off-line), the supernatant was transferred to a conditioned Oasis® µElution SPE plate and washed. The retained analytes were eluted by the liquid-handling system and the eluant was chromatographed using a Waters ACQUITY UPLC® with a Zorbax SB-CN column (2.1x50mm, 1.8µm) with a water/methanol/ammonium acetate gradient. A Waters TQD mass spectrometer was used to quantify 25OHD3 and C3-epi-25OHD3, monitoring two transitions for each analyte.

Levels of 25OHD2 were quantified separately to provide a total 25OHD concentration for each sample. All determined concentrations were traceable to NIST SRM 972.

Results: The assay was linear over the range 0.76 - 37.7ng/mL with coefficient of determination (r^2) >0.997. The calculated mean total 25OHD concentrations for the adult and paediatric samples were 14.7ng/mL and 19.6ng/mL respectively. The calculated C3-epi-25-OHD concentrations ranged from 0-3.48ng/mL (median 0.49ng/mL, mean 0.66ng/mL) in this adult population and from 0-4.98ng/mL (median 1.09ng/mL, mean 1.31ng/mL) for the paediatric population. The C3-epimer contributed 0-16.24% to the total 25-OHD concentration (median 4.23%; mean 4.34%) for the adults and 0-26.52% (median 5.77%; mean 7.46%) for the paediatric samples. The paediatric mean was lowered to 4.89% when individuals <1 year olds were excluding from the study. Using non-parametric statistics (CLSI C28-A) a reference range was established for this adult population at the 95% interval the lower and upper limits were 0.073ng/mL and 2.851ng/mL respectively.

Conclusion: C3-epimer-25OHD3 was detected in 90% and 93% all adults and paediatric samples tested respectively. Using the method presented here, 78% of the adult and 42% of the paediatric population would be considered insufficient (Total 25OHD <20ng/mL).

B-99

Development and validation of an isotope dilution tandem mass spectrometry method for the simultaneous quantification of 3-iodothyronamine, thyroxine, triiodothyronine, reverse T3 and 3,3'-diiodo-L-thyronine in Human serum

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Background: The thyroid hormones, thyroxine and triiodothyronine, their metabolites reverse T_3 and diiodothyronines, are important in regulating a number of biological processes. The thyronamines are decarboxylated and deiodinated metabolites of the thyroid hormones. At high concentrations 3-Iodothyronamine (T_1AM) has been shown to decrease heart rate and lower blood pressure in rats and at lower concentrations it opposes the effects of T_3. A sensitive and selective analytical method is required to fully understand the physiology and pharmacology of T_1AM, especially in humans.

Objective: The goal of the study was to develop an isotope dilution tandem mass spectrometry method to simultaneously measure T_1AM , T_4, T_3, rT_3 and 3,3'-T_2 in human serum, which would be the first to enable the quantification of endogenous T1AM in human serum.

Methods: An API-5000 tandem mass spectrometer equipped with TurboIonSpray source and Shimadzu HPLC system was employed. 100 µL of human serum was deproteinized by adding 150 µL of acetonitrile containing labeled internal standards. The supernatant was diluted with 500 µL de-ionized water and a 300 µL aliquot was injected onto an Agilent ZORBAX SB- C-18 column (2.1x30mm 1.8 Micron). After washing the column for 4.5 minutes with mobile phase A (.01% formic acid in 98 % water, 2% methanol) at the flow rate of .25 mL/ minute, the switching valve was activated and the analytes of interest were eluted into the mass spectrometer with a gradient of 35% mobile phase B (methanol with .01% formic acid) to 64% B in 2.5 minutes then 80 % B in 2.4 minutes. Finally the column was washed with 100 %B for 1.5 minutes before equilibration for the next injection. Quantification by multiple reaction-monitoring (MRM) analysis was performed in the positive mode. The ESI source was operated with ionspray voltage at 5500 V and heated temperature at 650°C. Gas settings were as follows: curtain gas 35, collision gas 4, nebulizer and heated gas 50. Retention time and ion pair for each analyte, its internal standard and compound dependent parameters was listed below:

T_1AM (7.2, m/z 356.2/212.1), T_1AM- d_4 (7.2, m/z 360.1/216.1) DP=86, CE=24 CXP=19

3,3'-T_2 (8.56, m/z 525.9/382.4), 3,3'-T_2-$^{13}c_6$ (8.56, m/z 531.9/388.4) DP=80 CE=26 CXP=15

T_3, rT_3 (8.83, 9.2, m/z 651.9/606.1), T_3-$^{13}C_6$, rT_3-$^{13}C_6$ (8.83, 9.2, m/z 657.9/612.1) DP=120 CE=29 CXP=13

T_4 (9.4, m/z 777.9/634.3), T_4-$^{13}C_6$ (9.4, m/z 783.8/640.2) DP=120 CE=37 CXP=21

Results: The within-day CVs were < 8.9 % and between-day CVs were between 1.6% and 7.6 % for all analytes. Recovery ranged from 92.8% to 95.4%. Good linearity was obtained within the concentration range of 5-300 pg/mL for T_1AM, 5-300 pg/mL for 3, 3'-T_2, 2.5-300 ng/dL for T_3 and rT_3 and 0.6-18 µg/dL for T_4. The lower limits of detection (LLOD) were 2.5 pg/mL for T_1AM and 3, 3'-T_2, 1.0 ng/dL for T_3, rT_3 and 0.15 µg/dL for T_4.

Conclusions: A sensitive, simple, accurate, and specific isotope dilution tandem mass spectrometry method was developed and validated for the simultaneous measurement of T_1AM , T_4, T_3, rT_3 and 3,3'-T_2 in human serum. Reference intervals for each of the analytes were determined

B-100

A streamlined method for methylmalonic acid (MMA) quantitation in serum using automated supported liquid extraction and LC-MSMS and correlation of MMA and vitamin B12 Results

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Background: Methylmalonic acid (MMA) is a biochemical marker for an inborn error of metabolism or vitamin B12 deficiency. It is a sensitive test of the functional adequacy of vitamin B12 since it is often the first analyte to be elevated in sub-clinical deficiency. Many currently used methods include manual sample processing and chemical derivatization. We developed a highly-automated method that does not require derivatization using supported liquid extraction and liquid chromatography tandem mass spectrometry (LC-MSMS). Our lab performs approximately 10,000 vitamin B12 tests per month, of which less than 2% are abnormal (reference range >200 pg/mL) and less than 10% are borderline (200-300 pg/mL).

Methods: Using a liquid handling robot, patient serum samples were diluted with deuterated internal standard (MMA-d3) in water and 5% HCl then extracted using supported liquid extraction in a 96-well format. After evaporation and reconstitution in 10% methanol and 0.4% formic acid, samples were injected onto the LCMSMS. A 5 um 150x3.2 mm organic acids column was used to separate MMA from succinic acid, an isobaric interferent. Isocratic separation was used with 10% methanol and 0.4% formic acid. The run time was 4.5 minutes. Electrospray ionization in the negative ion mode was used. The B12 assay is by chemiluminescent immunoassay (SIEMENS Immulite 2000). Results from patients where both MMA and B12 tests were ordered were compared.

Results: Daily calibrations between 0.1 and 40.0 umol/L were reliably linear and reproducible. Intra- and inter-assay CVs for at least 20 measurements of three concentrations (0.1, 2.0 and 10.0 umol/L) were 1.4-5.7% and 6.4-9.9%, respectively. There was no interference from succinic acid. Recovery was 87% and the limit of quantitation was 0.1 umol/L. Preliminary results indicate that only one third of abnormal or borderline B12 results were also abnormal for MMA (reference range </=0.3 umol/L) and two thirds of abnormal MMA results were normal (>300 pg/mL) for B12. For abnormal MMA results (median 0.5 umol/L), the median B12 result was 379 pg/mL.

Conclusions: This is a simple and robust method for MMA quantitation that requires minimal hands-on processing and no derivatization. A similar LC-MSMS method in production in our laboratory that uses manual sample processing requires approximately seven hours of hands-on time. The adoption of liquid handling automation and 96-well format for this method has reduced hands-on time to less than three hours. Having recently internalized MMA, B12 and MMA results will continue to be monitored to evaluate order utilization and the reference range and borderline region for vitamin B12.

B-101

Metabolomic Analysis of Cushing's Syndrome by QTOF LC-MS

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Background: Cortisol, which is increased in Cushing's Syndrome, plays a key role in several different metabolic pathways and is known to regulate hundreds of genes. To characterize the biochemical changes that occur in Cushing's Syndrome, we developed a metabolomic screen of urine, using an Agilent 6540 QTOF LC-MS.

Methods: Urine from 5 subjects with Cushing's syndrome (urine free cortisol range 59-1604 ug/24hr) and 6 healthy control subjects (urine free cortisol less than 45 ug/24hr) were deproteinized with 50% acetonitrile, diluted 20 fold and analyzed in triplicate by 4 different methods. To optimize measurement of hydrophobic analytes a C-18 reverse phase column was run in both positive and negative ion mode. To optimize measurement of hydrophilic analytes an aqueous normal phase type C silica column (Diamond Hydride) was run in both positive and negative ion mode. Data was collected in MS mode from 50-1700 m/z over a 10-20 minute gradient and analyzed

by Mass Hunter and Mass Profiler Professional software (Agilent).

Results: By principal component analysis, all 5 subjects with Cushing's Syndrome were found to cluster in a different position compared to normal subjects for each of the four different methods. Using the automated feature finding in Mass Hunter Qualitative analysis generated a range from 7,500 to 35,000 compounds during the first pass analysis. These lists were pared down by filtering to remove background noise (compounds present in the blanks) and any compounds not present in all 3 replicates of the samples. After further filtering for compounds that consistently differed by more than 10-fold and that had a significant t-test p value <0.05 between the normal and Cushing's, between 400-1200 different compounds were found with each procedure. Approximately one third of the peaks were identified based on their molecular mass using the Metlin database. Over 25 differentially expressed metabolites of cortisol , steroid hormones, bile acids, and other glucocorticoids were identified. In addition, the compounds ubiquinone, aldosterone, ceramide, dihydrosphingosine, and N-methylhistamine which have previously been described to be altered with Cushing's Syndrome were significantly elevated in the patient group. The following differentially expressed metabolites were also tentatively identified but will require confirmation by MS/MS analysis and the testing of additional samples: Homolanthionine, Dihydrodipicolinic acid, L-Cysteinylglycine, Niacinamide, Homocarnosine.

Conclusion: For those patients treated surgically, the monitoring of the normalization of the differentially expressed metabolites by the various types of cortisol replacement therapy may be useful for determining the adequacy of treatment. In summary, a relatively simple procedure for the accurate mass QTOF LC-MS analysis of urine that detects more than 1000 low molecular weight analytes has been developed. The characterization of the metabolon of various diseases, including Cushing's Syndrome, may provide new insights into the pathogenesis, diagnosis and treatment of disease.

B-102

Analysis of a Panel of Steroids Using Micro-Flow Chromatography Tandem Mass Spectrometry

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Background: An analytical method has been developed to perform quantification of a group of steroids - dehydroepiandrosterone sulfate (DHEAS), Dehydroepiandrosterone (DHEA), Cortisol, Corticosterone, 11-Deoxycortisol, Androstenedione, Testosterone, 17-OH-Progesterone, Progesterone, and Estradiol - using micro-flow chromatography interfaced to a tandem mass spectrometer. The use of micro-flow chromatography offers several key advantages: reduced solvent consumption, increased sensitivity of the method compared to conventional HPLC, and reduced chromatographic run-time.

Methods: A rapid and sensitive analytical method has been developed to leverage the benefits of micro-flow chromatography tandem mass spectrometry for the analysis of a panel of steroids. For each of the 10 steroid analytes monitored, a deuterated internal standard was employed to account for matrix and ionization effects. The compounds were extracted from 200uL of human serum by protein precipitation extraction. After dilution the extracts were analyzed by micro-flow chromatography-API/MS/MS. Total run time was less than 10 minutes, which is significantly shorter than the equivalent analysis performed under conventional HPLC conditions.

Results: The quantification of a group of steroid analytes - dehydroepiandrosterone sulfate (DHEAS), Dehydroepiandrosterone (DHEA), Cortisol, Corticosterone, 11-Deoxycortisol, Androstenedione, Testosterone, 17-OH-Progesterone, Progesterone, and Estradiol - using micro-flow chromatography interfaced to a tandem mass spectrometer, has proven to be both robust and reproducible. Measured accuracies and precisions were acceptable over a linear range of approximately 3 orders of magnitude. This method offers the additional benefits of reduced solvent consumption, increased sensitivity, and reduced chromatographic run-times. Furthermore, the use of micro-flow chromatography permitted the use of significantly smaller injection volumes - an important consideration for analyses where sample volume may be limited.

B-104

Evaluation of Two Immunoaffinity Extraction Products for LC-MS/MS Analysis of Serum 1α,25-dihydroxyvitamin D

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Background: Vitamin D plays important roles in bone health and a variety of other pathophysiological conditions, and it has two common forms (D2 and D3). 1α,25-dihydroxyvitamin D [1,25-$(OH)_2$D] is the active metabolite of vitamin D, and its measurement is very challenging due to its low circulating concentration and presence of interfering substances in serum. In this report, two immunoaffinity extraction products were evaluated to purify 1,25-$(OH)_2$D in serum prior to LC-MS/MS analysis.

Methods: Immunoaffinity extraction products were from Immundiagnostik AG (ImmunoTubes, designed for LC-MS assay) and Immunodiagnostic Systems Limited (IDS antibody beads, designed for immunoassay). In the ImmunoTube method, 500 µL serum samples and 25 µL hexadeuterated internal standards were added directly to the immobilized antibody, and incubated for 60 min. After three washes with water, 1,25-$(OH)_2$D was eluted with 400 µL of reagent alcohol. Samples were dried and resuspended in 100 µL of 70/30 methanol/water. In the IDS antibody method, serum samples were protein precipitated with equal volume of acetonitrile and further purified with SPE. Eluate from SPE was dried, resuspended in PBS buffer containing 5% methanol, and incubated with 400 µL IDS antibody beads slurry in a spin column for 90 minutes. The subsequent washing and elution steps were the same as the other method. It is noteworthy that modified extraction procedures were used for both products compared to the manufactures' recommendations. Forty µL of sample prepared with either method was subjected to LC-MS/MS analysis operated in the MRM mode. Chromatographic separation was achieved on an Onyx Monolithic C18 column with a gradient mobile phase buffers consisted of methanol and water with 0.5 mM lithium acetate. The lithium-adducts of 1,25-$(OH)_2$D were monitored because of its improved sensitivity in the positive ESI mode.

Results: Both immunoaffinity methods completely removed co-eluting isobaric interference peaks. Significant ion suppression was observed only with the IDS antibody method. Using the ImmunoTube method, the analytical measurement range (AMR) was 3.4-206.2 pg/mL for 1,25-$(OH)_2D_3$ and 3.9-212.6 pg/mL for 1,25-$(OH)_2D_2$ with an accuracy of 89.8-98.4% and 97.5-115.7%, respectively. With the IDS antibody, the AMR was 15.8-277.9 pg/mL for 1,25-$(OH)_2D_3$ and 13.9-238.2 pg/mL for 1,25-$(OH)_2D_2$ with an accuracy of 95.2-113.6% and 95.7-120.2%, respectively. Inter-assay and intra-assay CVs were 2.5%-7.0% for the ImmunoTube method and 6.0-13.8% for the IDS antibody method. Further evaluation of the ImmunoTube method by comparing with a radio immunoassay using 40 patient samples showed a linear Deming regression slope of 0.751, a y-intercept of 0.84 pg/mL, an r value of 0.7909, and a mean percentage bias of -27.1%. Comparison of the ImmunoTube method with a reference LC-MS/MS assay (n=20) showed a slope of 1.020, a y-intercept of 1.32, an r value of 0.9797, and mean percentage bias of -2.9%.

Conclusion: Both immunoextraction products removed interferences present in the human serum. Immunoaffinity extraction with the ImmunoTubes was the method of choice because of its simplicity and superior performance. With this sample preparation technique, a simple LC-MS/MS method was developed to quantify serum 1,25-$(OH)_2$D with the highest selectivity and sensitivity reported so far.

B-105

Separation of Isobaric Steroids Using Differential Mobility Spectrometry Tandem Mass Spectrometry

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Background: Tandem mass spectrometry has become increasingly popular as an analytical technique primarily due to the additional selectivity that is provided compared to single-stage MS and other techniques. Despite this increased selectivity, there are nevertheless many application areas where still greater selectivity is desired, primarily due to the structural similarities of target compounds. The analysis of steroids is just such an application, as many of these compounds are very similar in structure and may have identical chemical composition, making it virtually impossible to separate these species even by accurate-mass measurements. In the work presented here an orthogonal separation technique has been utilized to differentiate between isobaric steroids, using Differential Mobility Spectrometry (DMS) prior to analysis by LC-MS/MS.

Methods: A fast LC-MS/MS method was developed for the analysis of a group of steroids using the AB SCIEX TripleQuad™ 5500 mass spectrometer coupled to a Shimadzu Prominence HPLC system. The method employed an MRM scan for the detection of target compounds. A Differential Mobility Spectrometry (DMS) cell with a planar geometry was interfaced to the front end of the mass spectrometer, to enable the separation of isobaric steroids. Separation was accomplished by leveraging differences in the high-field and low-field mobility of the analytes. Since it was possible to completely separate compounds having identical MRM transitions by utilizing the DMS cell, chromatographic separation of isobaric steroids was not required, which enabled the use of a very rapid chromatographic method.

Results: The use of a planar geometry Differential Mobility Spectrometry (DMS) device, interfaced to a tandem mass spectrometer, has enabled the complete separation of isobaric steroids prior to analysis by LC-MS/MS. An example of a pair of isobaric steroids that were successfully separated using this novel technology

are testosterone, m/z 289, and dehydroepiandrosterone (DHEA), m/z 289. In the absence of the DMS device, when both compounds are present in a sample the MRM transitions used to monitor DHEA (289/271 and 289/253) display chromatographic peaks corresponding to both testosterone and DHEA. Thus, in order to avoid the false detection of DHEA in the presence of testosterone, it is absolutely essential to achieve baseline chromatographic separation of these two compounds. In contrast, when the DMS cell is turned on, the MRM transitions used to monitor DHEA are completely free of interference from testosterone. As a result, it is no longer necessary to chromatographically separate these two compounds when the DMS device is employed, and the chromatographic run-time may be shortened significantly. We have compared the performance of the DMS device under conditions of various relative concentrations for a variety of different isobaric steroids.

Conclusion: The use of a planar geometry Differential Mobility Spectrometry (DMS) device, interfaced to a tandem mass spectrometer, has allowed the separation of isobaric steroids by introducing an orthogonal mode of selectivity. This configuration has permitted significant increases in sample throughput, as chromatographic separation of isobaric compounds is no longer a requirement.

B-106

A simple and fast liquid chromatography-tandem mass spectrometry method for the measurement of plasma arginine, symmetric dimethylarginine and asymmetric dimethylarginine

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Background: Symmetric dimethylarginine (SDMA) and asymmetric dimethylarginine (ADMA) are methytransferase modified arginines liberated during protein catabolism. SDMA is a competitive inhibitor of arginine uptake and ADMA inhibits nitric oxide synthase. Both have been shown to be related to cardiovascular disease, while SDMA has also been identified as a biomarker for renal insufficiency.

Objective: To develop a short liquid chromatography tandem mass spectrometry method for measuring plasma arginine, SDMA and ADMA with a simple sample preparation.

Methods: EDTA plasma (75µL) and internal standard (75µL; L-Arginine-$^{13}C_6$ and ADMA-d_7 in water) were vortexed then protein precipitated with methanol (0.5mL). After centrifugation at 13,000 g for 10 minutes, the supernatant (0.2mL) was transferred to a sample vial and vortexed with 0.7mL of methanol:25mM ammonium formate with 1 % formic acid (68:32v/v). The resulting solution (25µL) was injected onto a Polaris Si-A analytical column (Varian; 5µ, 100 x 4.6mm) in a TLX2 system coupled to a Quantum Access mass spectrometer. The mass spectrometer was set at positive electrospray ionization. Quantification was based on peak area ratios of arginine (m/z 175.1->70.5) to Arg-$^{13}C_6$ (m/z 181.1->74.5) and SDMA (m/z 203.1->172.2) and ADMA (m/z 203.1->46.7) to ADMA-d_7 (m/z 210.2->77.5).

Results: The analytical run time was 5 minutes per injection. No significant ion suppression was observed. The method was linear over the ranges of 0.90-80.0 µg/mL, 10.5-994.9 ng/mL, and 30.7-981.7 ng/mL for arginine, SDMA and ADMA, respectively. Analytical recovery ranged between 88.4-111.4% for all analytes over the linear ranges. Precision was evaluated using EP10-A3 protocol by running 30 replicates of low, medium and high concentrations over 5 days (Table 1). No significant carryover was observed up to 80 µg/mL, 1,258 ng/mL and 1,059 ng/mL of arginine, SDMA and ADMA, respectively.

Conclusions: This validated LC-MS/MS method involved a simple sample preparation and a short chromatography time.

Total and intra-assay precision (EP10-A3)

Precision	Arginine (µg/mL)			SDMA (ng/mL)			ADMA (ng/mL)		
Level	4.03	10.51	17.01	73.58	161.74	252.70	52.04	122.12	190.98
Total %CV	3.6	3.56	3.98	5.96	6.04	4.63	13.49	10.99	7.22
Intra-assay %CV	2.73	2.64	2.36	4.32	3.43	2.99	10.56	8.31	5.29

B-107

A routine method for simultaneous measurement of blood immunosuppresive drugs and serum 25-hydroxyvitamin D concentration employing a single LC-MSMS system

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Background: Sirolimus, tacrolimus and cyclosporine are commonly used immunosuppressive drugs (ISD) after transplantation, and total 25-hydroxylvitamin D (D) is a high volume test. It is our desire to combine these two different methods to be run routinely onto one mass spectrometer instrument.

Methods: For ISD, 50ul of whole blood sample, standard or control is mixed with 50ul of 25mM ZnSO4 briefly and then mixed vigorously with 200ul methanol:acetonitrile=70:30 containing internal standards of 5ng/ml 32-desmethoxyrapamycin and 20ng/ml ascomycin. Samples were centrifuged for 10 minutes at 13000 rpm, and 30ul of the supernatant was injected into the Aria TLX-2 (Thermo Fisher Scientific) for online cleanup (Thermo: Cyclone-P 0.5x50mm column) at flow rate 3ml per min with 15mM ammonium acetate/0.1% formic acid:methanol=80:20 and then eluted (Thermo: Hypersil GOLD 50 x 3 mm column) at flow rate 0.75ml per min with 100% methanol/15mM ammonium acetate to API-5000 (AB Sciex) mass spectrometer for analysis. For D, 50ul of serum sample, standard or control is mixed vigorously with 200ul acetonitrile containing internal standard of 10ng/ml d6D3. Samples were centrifuged for 5 minutes at 13000rpm, and 50ul of the supernatant was injected into the TLX-2 for online cleanup (Thermo: Cyclone-P 0.5x50mm column) at flow rate 1.5ml per min with 0.05% formic acid:methanol=80:20 and then eluted (Phenomenex: Kinetex 50x 4.6mm, 2.6um column) at flow rate 0.7ml per min 0.05% formic acid:methanol=7:93 to the API-5000. Total time is 5 min for simultaneous results in each method sharing the same mass spectrometer employing atmospheric pressure chemical ionization.

Results: The assay is linear up to 100ng/ml and sensitivity to 0.5ng/ml for sirolimus and tacrolimus. For cyclosprine, the assay is linear up to 2000ng/ml, and sensitivity to 10ng/ml. For Vitamin D2 and D3 the assay is linear up to 100ng/ml, and sensitivity to 1ng/ml. Day-to-day precision (%CV) ranged from 5.6 % to 8.5 %. Patient correlation studies using this method and established solid phase extraction for ISD and liquid-liquid extraction for D revealed a slope(S)=0.96, intercept(I)=0.2 and r=0.95 for sirolimus, S=1.01, I=0.2 and r=0.98 for tacrolimus, S=0.90. I=12 and r=0.98 for cyclosporine, S=0.99, I=0.9 and r=0.95 for D3 and S=0.91, I=0.6 and r=0.98 for D2.

Conclusion: We concluded that the method described here is ideally suited for a small to medium sized institution wishing to run therapeutic monitoring of blood sirolimus, tacrolimus and cyclosporine concentrations, and serum 25-hydroxylvitamin D simultaneously with a single LC-MSMS system.

B-108

High-Throughput Analysis of Levetiracetam in Serum Using Ultra-fast SPE-MS/MS

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Background: Levetiracetam is an anticonvulsant medication used to treat seizures. Fast, sensitive and accurate therapeutic drug monitoring of levetiracetam is useful for patient care. Mass spectrometry-based assays have emerged as a viable analytical method due to their sensitivity, specificity, and robustness. However, LC-MS assays are limited due to slower turnaround times as compared to immunoassays. We evaluated the ability of an ultra-fast SPE-MS system to analyze levetiracetam in human serum with much faster sample cycle times and similar analytical results compared to HPLC or LC-MS/MS assays.

Methods: Calibration standards were prepared by spiking bovine serum with levetiracetam to final concentrations ranging from 1ug/ml to 100ug/ml. Commercially available quality control standards made in human serum were also analyzed. The serum samples were precipitated with 3:1 acetonitrile containing d3-levetiracetam. The precipitated samples were centrifuged, subsequently, the supernatant was removed and diluted with methanol prior to injection. Sample analysis was performed at a rate of 8.5 seconds per sample using a RapidFire RF300 system coupled to a QQQ mass spectrometer. The SPE method consisted of a C18 column and elution with 100% methanol. Data analysis was performed using RF*Integrator* software. This methodology is capable of throughputs >400 samples per hour.

Results: Feasibility was assessed using prepared calibration standards and commercially available quality control standards that were run in triplicate over a series of days to establish both intra- and inter-day precision and accuracy. Levetiracetam had both intra- and inter-day accuracies within 15% and coefficient of variation values less than 10% for all concentrations. A quadratic fit was applied to the range of 1-100ug/ml and had an R^2 value greater than 0.999. Blank serum was treated and analyzed in the absence of internal standard in the same manner as the other samples to establish signal windows which were found to be greater than 10 to 1. These analytical results are comparable to those using LC-MS/MS, however the analysis time for SPE-MS/MS was approximately 20 times faster. Blinded patient samples will be evaluated to further validate this method.

Conclusion: Based on these results, levetiracetam can be accurately and precisely

measured in serum using ultra-fast SPE-MS/MS at rates of 8.5 seconds per sample. While the analytical results were comparable to LC-MS/MS, the analysis time was approximately 20 times faster. SPE-MS/MS may be useful for the fast and efficient analysis of similar clinical research assays.

B-109

Can 2,3-butanediol levels help distinguish chronic alcoholics from occasional drinkers?

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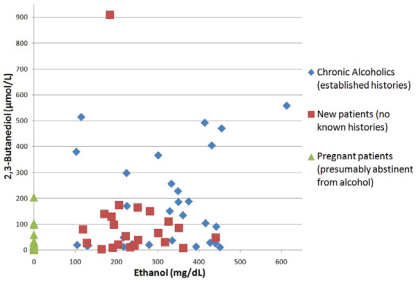
Background: Alcohol dependence affects about 12% of American adults and has significant medical and social consequences. Currently, there are no specific clinical assays that can distinguish between chronic alcoholics and occasional drinkers. 2,3-butanediol has been reported to be elevated in the blood of chronic alcoholics, but the utility of 2,3-butanediol as a marker of chronic alcoholism has not been thoroughly evaluated.

Methods: We developed an LC-MS assay for measuring 2,3-butanediol concentration in serum. We selected samples from patients with blood ethanol concentrations above 80 mg/dl and compared their 2,3-butanediol levels to those from third trimester pregnant women who were presumably abstinent. Cases with blood ethanol levels above 80 mg/dL were further segregated into those who had established histories of alcohol abuse and/or multiple hospitalizations for intoxication versus those who were visiting our emergency department for the first time with no known histories. Serum gamma glutamyl transferase (GGT) concentrations were also recorded for these patients.

Results: 14/29 chronic alcoholics, 4/22 first-time patients, and 1/38 pregnant controls had 2,3-butanediol levels above 150 micromoles/L. The diagnostic sensitivities of 2,3-butanediol (>150 micromoles/L) and GGT (>61 mIU) in detecting alcohol abuse were 49% and 66%, respectively, and 89% when used in combination. While 26/29 chronic alcoholics displayed elevated 2,3-butanediol and/or GGT levels, only 8/22 first-time patients had elevations of either of these markers.

Conclusions: 2,3-butanediol was more frequently elevated in chronic alcoholics than in new patients admitted to our hospital for intoxication, and was only elevated in one of our pregnant controls. Although we were only privy to partial medical histories, and a subset of subjects admitted for the first time for intoxication may have been chronic alcoholics, these trends suggest that 2,3-butanediol may be a useful indicator of chronic alcoholism that can improve diagnostic sensitivity and specificity when used in combination with serum GGT.



B-110

An Analytical Method for the Determination of Testosterone and Epi-Testosterone by Liquid Chromatography-Tandem Mass Spectrometry

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Background: The measured ratio of testosterone to epi-testosterone has been useful for detecting the exogenous administration of testosterone, often used to enhance athletic performance. Epitestosterone, an inactive epimer of the hormone testosterone, is typically present in approximately equal concentrations as testosterone in urine, and the mean testosterone/epitestosterone (T/E) ratios in athletes is generally less than 2.0. The World Anti-Doping Agency has established a standard for T/E ratios of 4.0 as indicative of possible exogenous testosterone administration. In the work presented here, we have developed an LC/MS/MS method to measure urine and serum levels of testosterone and epi-testosterone.

Methods: An analytical method was developed using a QTRAP® 5500 LC/MS/MS system coupled to a Shimadzu Prominence HPLC system. The reversed-phase chromatographic separation of testosterone and epi-testosterone was achieved in a total run time of less than 10 minutes. The MS/MS analysis was performed using the Multiple Reaction Monitoring (MRM) mode of operation, and employed electrospray ionization. Analytical standards were obtained from Cerilliant, as 1.0 mg/mL solutions in acetonitrile, and spiked into blank urine and serum matrices.

Results: Both reversed-phase and normal-phase chromatography approaches have been applied in order to accomplish the chromatographic separation of testosterone and epi-testosterone, with reversed-phase chromatography yielding the best results. Due to their structural similarity, the epimers produce the same MS/MS fragment ions and so it was not possible to distinguish these compounds by tandem mass spectrometry alone. Using an isocratic gradient, these compounds have been completely baseline-separated in a run time of less than 10 minutes. The temperature of the column oven was observed to play an important role in enhancing the chromatographic separation. 'Blank' stripped serum and spiked serum samples have been analyzed to determine LOD and LOQ for the method. Further method optimization is expected to improve the sensitivity of this method, allowing it to be successfully deployed in clinical research laboratories.

Conclusion: An LC-MS/MS method was succesfully developed for the quantitative measurements of testosterone and epi-testosterone in urine and serum matrix.

B-111

Matrix-Associated Laser Desorption Ionization Time of Flight Mass Spectroscopy (MALDI-TOF, MS) for Fast and Reliable Identification of Bacteria in a Pediatric Clinical Laboratory

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Background: The last decade has witnessed advances in technology amenable to use in multiple areas of the clinical laboratory. Most recently, MALDI-TOF MS has been introduced in clinical microbiology to identify (ID) both commonly encountered and fastidious , difficult to identify micro-organisms. We present our experience with MALDI-TOF for routine use in a pediatric clinical laboratory.

Methods: Organisms from agar plates were inoculated in duplicate directly onto a steel template (Bruker Daltonics, Inc, Billerica, MA). After air drying, 1 ul of matrix (α-cyano-4-hydroxycinnamic acid) was placed over each inocula. Samples were evaluated on a MicroFlex LT MALDI TOF MS (Bruker) in linear positive-ion mode; using a 60 Hz nitrogen laser ; with a detection range of 2,000 to 20,000 Da. Spectra were automatically compared to the reference database (Reference Library 3.0 for MALDI Biotyper 2.0) and assigned an identification, the level of accuracy determined by a logarithmic score of 0-3. In general, Genus and species identification were accepted at values of >2.0; Genus at 1.7-2.0, and ; Unreliable at <1.7. Identification was compared to routine laboratory methods or reference methodology including gene sequence analysis.

Results: For this analysis a total of 714 unique determinations were performed among 76 unique Genera and species. Reproducibility studies with 45 isolates demonstrated complete agreement. Twenty one isolates yielded results with known limitations of the system. For the remaining 648 isolates:

588 (90.7%) gave identical Genus species results;

46 (7%) to Genus level, and;

14 (3%) unreliable ID.

Conclusion: The use of same day one-step methodology for identification of micro-organisms from enrichment, selective, and differential media optimizes workflow, eliminates procedural variation, saves both time and cost and is a reliable procedure for routine use. The instrument is also suitable for proteomic research.

B-112

Fast and sensitive amino acid analysis using aTRAQ ™ derivatization and uplc ms/ms

B. Dawson[1], J. Moshin[1], H. Liu[1], S. Daniels[2]. *[1]AB Sciex, Foster City, CA, [2]AB Sciex, Framingham, MA,*

Objective: The objective of this work was to greatly improve upon current amino acid analysis (AAA) methods by shortening the analysis time and lowering the limits of quantitation while maintaining selectivity and sensitivity using Ultra Performance Liquid Chromatography (UPLC) coupled to a state of the art triple quadrupole mass spectrometer and aTRAQ ™ derivatization.

Background: Amino Acid Analysis (AAA) has long been important in determining the presence of inborn metabolic errors. While amino acid analysis based on ion exchange and ninhydrin derivatization has been shown to be accurate and precise, it suffers from extremely long run times and low throughput. HPLC-MS/MS analyses

have greatly increased sample throughput, provided low detection limits, high selectivity in a variety of physiologic fluids with large linear dynamic range.

Methods: Physiological samples were deproteinized and derivatized with an isotopically labeled reagent (Δ8). An unique internal standard for each amino acid was created using an unlabeled reagent (Δ0). Thirty six amino acids were resolved using an Ultra Performance Liquid Chromatography (UPLC) column with an overall runtime of 11 minutes. Detection of the compounds was achieved with a 5500 QTRAP.

Results: Calibration curve data of 36 amino acids in a synthetic urine matrix are presented over a ~ 2.5 orders of magnitude concentration range. Data for controls in synthetic urine matrix are presented as well. Example chromatograms of a human urine sample are shown with resolution of key isobaric interferences. Analyte peaks are easily identified due to the presence of a labeled internal standard for each species.

Conclusions: Sensitivity for the method is excellent with LLOQs 3-80 nM. Accuracy at the 0.5 µM and 10 µM levels is ± 17%. Isobaric components are well resolved in the urine matrix. Over all, the method proves to be accurate, precise and easy to perform for the routine measurement of physiological amino acids.

B-113

Evaluation of LCMS/MS scrambling ratios for deuterium-labeled Vitamin D metabolites, steroids and other compounds of clinical significance

J. Cooper, H. Jian, D. Johnson, I. Dilek, U. Sreenivasan. *Cerilliant, Round Rock, TX,*

Introduction and Objective: A significant clinical challenge with LC-MS/MS is the potential for matrix effects that cause interferences or impact ionization efficiency. Stable isotope-labeled internal standards are frequently used to compensate for matrix effects and to increase the accuracy of quantitation. The use of a labeled internal standard that co-elutes with the drug being monitored can potentially offset patient specific matrix effects (co-eluting concomitant medication, etc.) that may occur at the retention time of the analyte of interest. Complications in the use of deuterium-labeled internal standards can arise from hydrogen-deuterium scrambling in the collision cell at the selected transitions or in the ion source. In this study, we examined deuterium-labeled 25-Hydroxyvitamin D, testosterone, and other compounds of clinical significance by LC-MS/MS at multiple transitions. We investigated reproducibility of the scrambling ratio and influences on scrambling of different LC-MS systems (tandem quadrupole vs. quadrupole time-of-flight), matrix selection, concentration, and deuterium placement in the internal standard.

Methods: LC-MS Systems used were a Waters Alliance UPLC-Xevo G2 Q-Tof system and an Agilent 1290 UHPLC-6460 triple quad system. Various reverse phase columns and mobile phases were used. Samples were analyzed by direct infusion, injection of neat compounds on column and injection of extracted serum samples on column. Serum extraction was conducted using 200µL of serum, adding 200µl of methanol, followed by 1mL of heptane. Samples were then centrifuged, dried down and reconstituted in 100µL of ethanol.

Results: Scrambling was observed on both tandem quad and Q-Tof at select transitions for the deuterium-labeled internal standards studied in both infusion and on column experiments. One example of this scrambling was with D_3-25-Hydroxyvitamin D2, for a specific transition 398->380. The scrambling was consistent and was not influenced by matrix, concentration, column or presence of mobile phase. However, scrambling was able to be mitigated or eliminated by selection of another transition, which was also true for the other compounds investigated. The occurrence and extent of scrambling was consistent for each analyte and was dependent on the transition monitored and the ionization system.

Conclusions: Evaluation of scrambling is important in clinical method development to ensure accurate quantitation and reproducible results for critical decision-making in patient care. Awareness of potential scrambling is important for proper internal standard design and selection. Scrambling can be mitigated or eliminated by altering instrument conditions and transition selection or potentially by selecting a transition with consistent scrambling. Deuterium-labeled internal standards are a viable option for LC-MS/MS analysis with selection of the appropriate transition. They also offer a more cost-effective alternative to carbon-13 or nitrogen-15-labeled analogs with benefits such as ready availability and lower cost per test.

B-115

Method Development for Fast Sensitive LCMS Method for Nicotine and its Metabolites

A. Wang, C. Berg, J. Rivera, B. Dawson, H. Liu. *AB SCIEX, Foster City, CA,*

Background There is a recent increase in demand for nicotine testing to determine tobacco use. During testing, it is important to be able to differentiate active tobacco use from passive exposure. We have developed a fast sensitive method for nicotine and its metabolites as well as the tobacco alkaloid anabasine in plasma and urine. By detecting anabasine, it is possible to differentiate active use of tobacco from the use of nicotine gum or patch.

Methods Standards for nicotine and cotinine were obtained from Cerilliant Corporation and standards for nornicotine, trans-3-OH-cotinine and anabasine were obtained from Toronto Research Chemicals. LCMSMS analysis was performed on a 4000 QTRAP® system in positive ESI mode coupled to a multiplexed Shimadzu UFLC system. Two different chromatographic conditions including HILIC and reverse phase were evaluated. Mobile phase A consisted of H2O with 10 mM ammonium formate buffer at lower pH and mobile phase B consisted of 95% ACN with 10mM ammonium formate in water. For the hydrophilic interaction chromatography, the sample was injected with 100% mobile phase B and rapidly ramping up the aqueous phase to elute the analytes. For the reverse phase chromatography, samples were injected with 95% of the aqueous phase and rapidly ramping up mobile phase B to elute the analytes. Sample preparation involving dilute and shoot for urine and crash, evaporate, reconstitute and shoot for serum and plasma as well as online sample prep were also evaluated.

Results and Discussion Results obtained by using the HILIC or the reverse phase column achieve reproducible method and produce high quality data with a total run time under 5 min. The advantage of each method with different sample preparation process was discussed and compared. Standard curves in urine, plasma and serum matrices were injected in triplicates and showed good linearity (r>0.99) for the desired range of 1ng/mL to 1000ng/mL as well as acceptable %CVs below 15%. The final method reported here also reduces the cost by using online cleaning sample preparation.

B-116

Resolving Testosterone: A Comparison to Clinical Reference Lab Values

W. Woroniecki[1], B. Fernandez[1], R. Huang[1], J. Stone[2], H. Liu[1]. *[1]AB Sciex, Foster City, CA, [2]Kaiser Regional Laboratory, Richmond, CA,*

Background: With publications discussing deficiencies in low level testosterone immunoassay (IA) analysis, LC/MS/MS is becoming a noted alternative technique. A novel method was developed for calculation of total testosterone at low picogram (LOD=5pg/mL @S/N 5) detection limits. Comparisons to an in-house method were made to total calculated testosterone values in human serum generated at three reference labs. Two LC-MS-MS methods and one immunoassay method were compared for total testosterone in human serum. One of the comparisons was to a well characterized data set provided by the CDC.

Methods: A one step liquid-liquid extraction method was developed without derivatization. Samples were dried down and reconstituted in 100 µL. The LOQ of the assay was 20 pg/mL with an approximate S/N of 20. At this standard level the average CV was less than or equal to 10 % with an approximate 5 % deviation in accuracy. The chromatographic method utilized mobile phase modifiers with an optimized gradient (6 min) to reduce background interferences and improve the ionization efficiency.

Results: Comparisons to reference lab calculated concentrations showed good correlation, R2 values ranged from 0.93 to 0.99. The CDC sample set had the highest correlated value of 0.99 and deviation was less than 10% from CDC reported values. Immunoassay values though well correlated (R2 = 0.93) yielded higher calculated concentrations overall. We also noted in our testing an interfering peak when serum separator tubes (SST) with gel were utilized. This peak was separated under the chromatographic conditions employed.

Conclusion: The method was validated for robustness and precision. Limit of Quantitation (LOQ) was well resolved at relevant clinical concentration ranges. At least a S/N of 20 at the LLOQ (20 pg/mL) was established, allowing for lower quantification if desired. R2 values correlated well overall. The strongest correlation (0.99) was seen in the largest and most diverse sample set provided by the CDC. The IA comparison, while yielding a good correlation (0.93), had higher calculated values. Having higher T values when LC-MS methods are compared to IA is not unknown and has been reported previously in the literature.

B-117

Surface activated chemical ionization (SACI) combined with electrospray (ESI) and cation exchange chromatography (CEC) for the analysis of 8-oxodG in urine samples

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Background: Reactive oxygen species may produce DNA alterations whose frequency and mutagenic potential are largely unknown. The reliable detection of oxidative DNA lesions in biological specimens may disclose their role in human disease. 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG) is the most studied biomarker of DNA oxidation, and its direct detection in urine prevents the risk of artefactual oxidation during DNA isolation. LC-MS/MS methods allow the direct analysis of urine with high sensitivity and selectivity, but could be affected by matrix effect. Triple quadrupole was widely employed for detecting 8-oxodG in tandem mass spectrometry (MS/MS) multiple reaction monitoring (MRM) conditions and only few studies used ion trap (IT) analyzer. We identified some limitations in the detection of 8-oxodG by IT analyzer that were bypassed by a newly developed method coupling CEC and SACI-ESI ionization sources to IT analyzer. By this approach we could use lower ionization potential thus reducing chemical noise and matrix effect linked to in-source reactions. Furthermore we could use ion exchange chromatography, that is seldom employed with ESI due to the ion source discharge phenomenon.

Methods: A calibration curve was generated from 0.3 to 100 ng/mL 8-oxodG. Each sample was spiked with 20 ng/mL of [$^{15}N_5$]8-oxodG internal standard. Ultimate 3000 HPLC pump (Dionex, Germany) and Cation Exchange column ThermoElectron CEC, Thermoelectron C18 and HILIC were used. Mass spectra were acquired using HCT ultra ion trap (Bruker Daltonics, Breme, Germany).

Results: Informative fragmentation was obtained from [M+H]+ ion at m/z 284 while [M+Na]+ at m/z 306 led to poor fragmentation and unstable signal. The fragment at m/z 168 was monitored by LC-SACI-ESI-MS/MS based on reverse phase chromatography, with a detection limit, in water solution, of 0.1 ng/mL and a quantitation limit of 0.3 ng/mL. However, when urine matrix was spiked with standard, a loss in sensitivity was observed, with 20-fold lower intensity of the m/z 168 fragment and other distinct m/z signals dominating the spectrum. This possibly resulted from the IT saturation by a contaminant ion present in the matrix and having the same m/z 284 as the analyte parent ion. We developed another LC-SACI-ESI-MS/MS method based of HILIC chromatography with higher affinity towards highly polar compounds, but even in this case the contaminant co-eluted with the analyte. We then used a stronger retention LC protocol, allowing to operate in low ionization voltages. In these conditions two distinct peaks at retention times (RT) 0.8 and 1.3 minutes were achieved and the MS/MS spectrum showed that the most abundant peak at RT 0.8 minutes was due to 8-oxodG while the second at RT 1.3 minutes was due to the contaminant. Under these conditions no difference in signal intensity was observed between water and matrix samples.

Conclusion: A fast and reliable method based on LC-CEC-SACI-ESI-MS/MS approach was developed to detect 8-oxodG in urine. SACI-ESI allowed coupling CEC with MS with better resolution of contaminant(s) having m/z similar to the analyte.

B-118

25-Hydroxyvitamin D3 and 25-Hydroxyvitamin D2 Analysis Using the QTRAP® 5500 Tandem Mass Spectrometer with the MPX™-2 High Throughput Multiplexed HPLC System

J. C. Seegmiller[1], K. J. Goodman[2]. *[1]AB SCIEX, Foster City, CA, [2]AB SCIEX, Framingham, MA,*

Objective: Because of the heightened awareness of vitamin D insufficiency in clinical research, testing volumes for these biomarkers have increased substantially. Therefore, we have developed a simple, robust and high throughput 25-hydroxyvitamin D3 and 25-hydroxyvitamin D2 analysis method suitable for a clinical research setting using a multiplexed liquid chromatography system with tandem mass spectrometry detection (LC/MS/MS).

Methodology: This method was developed with the mindset of a simplistic approach along with high throughput capabilities of the MPX™ High Throughput System synced to a QTRAP® 5500 tandem mass spectrometer (LC/MS/MS). The goal of this method was not to be a burden on either the research laboratory or research staff in terms of the ability to handle high sample volumes along with simplistic sample preparation / method operation respectively.

Samples in this method consisted of serum spiked with 25-hydroxyvitamin D3 and 25-hydroxyvitamin at levels ranging from 0.5 to 100 ng/mL. The method used protein precipitation as sample preparation followed by centrifugation. The resulting supernatant was then injected on to a reversed-phase liquid chromatography column where 25-hydroxyvitamin D3 and 25-hydroxyvitamin D2 eluted using a linear gradient chromatographic method. The chromatographic run time was a total of 2 minutes. With the use of the MPX™ High Throughput System, results were able to be acquired every 1.3 minutes.

This method was found to be precise and accurate from 0.5 to 100 ng/mL with intraassay imprecision (n=8) between 21.71 and 3.68 %CV and accuracy ranging from 93.02 to 111.97 for 25-hydroxyvitamin D3. Analysis over this same concentration range for 25-hydroxyvitamin D2 was found to have intrassay imprecision (n=8) between 20.77 and 5.57 %CV and accuracy between 92.21 to 116.34.

Method robustness was assessed using a serum quality control specimen having a mean concentration of 12.38 ng/mL. Analysis of this control was found to provide intraassay imprecision (n=157) of 5.6 %CV.

Conclusion: The use of a one dimensional multiplexed HPLC system and a MS/MS system has provided a simplistic, robust and high throughput methodology for 25-hydroxyvitamin D3 and 25-Hydroxyvitamin D2 analysis.

Tuesday PM, July 26

Poster Session: 2:00 pm - 4:30 pm
Nutrition/Trace Metals/Vitamins

B-119

Vitamin D laboratory status in a Brazilian sampling: survey and analysis

L. M. Soares, P. F. Tavora, L. Moutinho, H. Garcia, M. Farace. *Lab Rede, Belo Horizonte, Brazil,*

Background: Vitamin D deficiency is a highly prevalent condition, present in approximately 30% to 50% of the general population. There is increasing evidence that, in addition to the well-known effects on musculoskeletal health, vitamin D status may be related to a number of non-skeletal diseases. Beyond defects in bone and calcium metabolism, vitamin D deficiency has been associated with a large number of conditions such as cancer, autoimmune disease, and cardiovascular disease. Vitamin D is mainly synthesized when the skin is exposed to ultraviolet B radiation. Dietary sources of vitamin D3 (cholecalciferol) are few and only significant in oily fish. Vitamin D2 (ergocalciferol), the plant/mushroom form of vitamin D, is almost absent in the diet. Supplementation with vitamin D can be done either with vitamin D2 or vitamin D3 but availability of these two forms greatly differs between countries. Supplemental doses of vitamin D and sensible sun exposure could prevent deficiency in most of the general population. Monitoring serum 25-hydroxyvitamin D levels and correction of vitamin D deficiency is indicated for optimization of musculoskeletal and general health. An assay measuring both $25(OH)D_2$ and $25(OH)D_3$ is recommended. The aim of this study is to analyze the prevalence of vitamin D deficiency in a sampling of the Brazilian population, comparing it to the values found in general population worldwide.

Methods: Serum 25-hydroxyvitamin D [25(OH)D] was measured through Chemiluminescence (Liaison®, Diasorin, US) in 7706 blood samples (women: 6087/men:1619) collected from September 2010 to December 2010 and sent to a Reference Laboratory in Brazil. The samples were derived from five states of the southern and midwest regions. 25(OH)D deficiency was defined as serum concentration below 10.0 ng/mL, values between 10.0 and 30.0 ng/mL were considered insufficiency and those above 30.0 up to 100.0 ng/mL corresponded to the optimal levels. The statistical analysis was based on Chi-square test.

Results: The prevalence of 25(OH)D deficiency found in the population studied was 5.12%. It was similar between sexes in general population, but it was greater in women (12.30%) than in men (7.44%) when only the aged population was considered, being the difference statistically significant ($p<0.01$). The general prevalence was 2.17% in young adults (20-39 years) and 11.26% in the elderly (≥ 65 years). The difference between this two age groups was observed in both sexes and achieved statistical significance ($p<0.01$). Values between 10.0 and 30.0 ng/mL were found in 74.25% of the general population, being greater in women ($p<0.01$) and similar in the age groups analyzed.

Conclusion: Although the prevalence of 25(OH)D deficiency in the population studied was lower than that found around the world, the frequency of 25(OH)D insufficiency observed was very high. Factors such as low sunlight exposure due to contemporary life and use of UVB-blocking sunscreens, age-related decreases in cutaneous synthesis, and diets low in vitamin D may contribute to the high prevalence of vitamin D inadequacy. Even in low latitudes, lab monitoring and correction of 25(OH)D levels are indicated to prevent the potential implications of the deficiency for skeletal and extraskeletal health.

B-120

Underestimation of total plasma 25-OH vitamin D in the presence of high vitamin D2 by immunoassay compared to mass spectrometry

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Background: 25-OH vitamin D2 (ergocalciferol) is frequently used in United States hospitals to treat children with vitamin D deficiency. However in the 2010 report from the Vitamin D External Quality Assessment Scheme (DEQAS), total vitamin D (D2+D3) appeared to be underestimated in samples containing high vitamin D2 by the Diasorin Liason chemiluminescent assay versus Liquid Chromatography-Mass Spectrometry (LC-MSMS). Since this immunoassay is widely used, underestimation may be common in a pediatric population. The objective is therefore to compare recoveries of total vitamin D from a LC-MSMS method and the Diasorin Liason assay in the presence of both high and low vitamin D2.

Methods: A case control methodology was used. Blood samples analyzed at the Children's Hospital Boston core laboratory facility by LC-MSMS were selected for further analysis by Diasorin Liason based on vitamin D2 levels. Cases were defined as having greater than 10 ng/mL vitamin D2, or greater than 50% of total vitamin D. Controls were defined as having less than 1 ng/mL vitamin D2. A total of 25 samples (13 cases and 12 controls) were randomized, de-identified, and then assayed by the Diasorin Liason method. Day to day CVs for total vitamin D were less than 10% for both methods. The Paired Student's T test and Pearson correlations were used to analyze the data.

Results: The mean concentration of vitamin D2 was 21.5 ng/mL (sd=13.1) in cases and 0.5 ng/mL (sd=0.2) in controls by LC-MSMS. Total vitamin D was 35.4 ng/mL (sd=9.3) in cases, and 23.8 ng/mL (sd=11.1) in controls. By the Diasorin Liason method, mean total vitamin D was 22.0 ng/mL (sd=10.6) in cases, and 23.8 ng/mL (sd=11.2) in controls. The mean difference between the two methods for total vitamin D was -13.4 ng/mL (sd=6.0) for cases, and -6.7 (sd=3.9) for controls, which were statistically significant ($p < 0.0001$). The correlation between the two methods among cases was 0.83, and was 0.96 among controls. Total vitamin D deficiency (< 20 ng/mL) was observed in 48% (n=12) of all samples by Diasorin Liason whereas only 12% (n=3) were considered deficient by LC-MSMS. This corresponds to a positive predictive value of 25%. Among cases, the positive predictive value was zero.

Conclusion: The Diasorin Liason method underestimates total vitamin D in the presence of elevated vitamin D2. Relying on this assay in a clinical setting could lead to unnecessary follow-up testing or dosing. Further studies are needed to determine the reason for this discrepancy, and also the degree of misclassification according to intermediate levels of vitamin D2.

B-121

Vitamin D Binding Protein Modifies the Vitamin D-Bone Mineral Density Relationship

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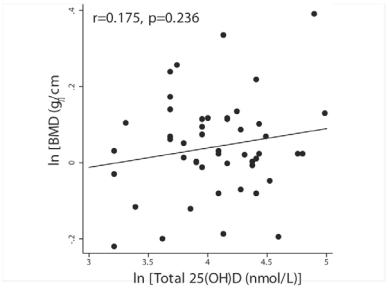
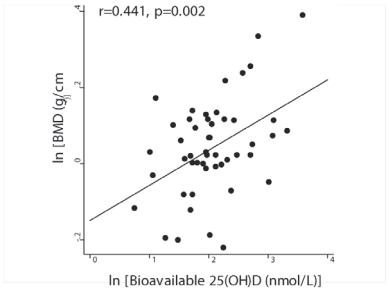
Background: Vitamin D sufficiency is thought to be essential for bone health and the prevention of osteoporosis-related fractures. However studies examining the relationship between total circulating 25-hydroxyvitamin D (25(OH)D) levels and bone mineral density (BMD) have yielded mixed results. Vitamin D binding protein (DBP), the major carrier protein for 25(OH)D, may alter the biologic activity of circulating vitamin D. We hypothesized that free and bioavailable 25(OH)D, calculated from total 25(OH)D, DBP and serum albumin levels, would be more strongly associated with BMD than levels of total 25(OH)D.

Methods: We measured total 25(OH)D, DBP, and serum albumin levels in 49 healthy young adults enrolled in the Metabolic Abnormalities in College-Aged Students (MACS) study. Lumbar spine BMD was measured in all subjects using dual X-ray absorptometry. Clinical, diet, and laboratory information was also gathered at this time. We determined free and bioavailable (free + albumin bound) 25(OH)D (using formulae adapted from analogous methods used to calculate free testosterone) and examined their associations with BMD.

Results: BMD was not associated with total 25(OH)D levels ($r=0.175$ $p=0.236$). In contrast, free and bioavailable 25(OH)D levels were positively correlated with BMD ($r=0.413$ $p=0.003$ for free, $r=0.441$ $p=0.002$ for bioavailable). Bioavailable 25(OH)D levels remained independently associated with BMD in multivariate regression models adjusting for age, sex, body mass index, and race ($p=0.03$).

Conclusion: Free and bioavailable 25(OH)D were more strongly correlated with BMD than total 25(OH)D in this cohort of healthy adults. These findings have important implications for clinical testing for vitamin D deficiency. Future studies should continue to explore the relationship of free and bioavailable 25(OH)D with health outcomes.





B-122

ADVIA Centaur® Vitamin D Total Assay: Performance Evaluations Assessing Method Comparisons and Expected Values

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Background: Vitamin D is a steroid hormone involved in the intestinal absorption of calcium and the regulation of calcium homeostasis; it is essential for strong, healthy bones. Vitamin D deficiency can result from inadequate exposure to the sun, inadequate alimentary intake, decreased absorption, abnormal metabolism, or vitamin D resistance. The most reliable clinical indicator of vitamin D status is 25(OH) vitamin D. Serum and plasma 25(OH) vitamin D levels reflect the body's storage levels of vitamin D and correlate with clinical symptoms of vitamin D deficiency.

Methods: Four vitamin D assays (DiaSorin LIAISON 25 OH Vitamin D TOTAL, IDS 25-Hydroxy Vitamin D EIA, two lots of ADVIA Centaur Vitamin D Total assay* reagents, and liquid chromatography-tandem mass spectroscopy) were used to assay 195 clinical specimens collected in the US across the range of 4 to 150 ng/mL.

An expected value study was performed using the ADVIA Centaur Vitamin D Total assay on serum samples collected from 258 adults not taking supplements containing vitamin D and 286 adults taking supplements containing vitamin D. The samples were collected in different seasons and different geographical regions of the US. Samples were included in this study only if the sample had normal values for PTH, calcium, magnesium, phosphorus, and TSH. The aim of the study was to evaluate method comparisons across four 25(OH) vitamin D assays and to assess expected values on the ADVIA Centaur Vitamin D Total assay.

Results: Deming regression statistics and Pearson coefficients were obtained for all assay combinations (n = 195). For ADVIA Centaur vs. IDS EIA, slope = 1.04, y-intercept = 0.50, r = 0.95; for ADVIA Centaur vs. LC-MS/MS, slope = 1.18, y-intercept = 0.54, r = 0.90; and for ADVIA Centaur vs. LIAISON, slope = 1.35, y-intercept = -9.83, r = 0.69. For LIAISON vs. IDS EIA, slope = 0.79, y-intercept = 7.11, r = 0.69; and for LIAISON vs. LC-MS/MS, slope = 0.92, y-intercept = 5.72, r = 0.66. For IDS EIA vs. LC-MS/MS, slope = 0.99, y-intercept = 0.05, r = 0.88.

The following 25(OH) vitamin D values were obtained for adults not taking supplements containing vitamin D: median, 19.7 ng/mL (49.3 nmol/L); observed range, 2.5th to 97.5th percentile, 10.4-37.4 ng/mL (26.0-93.5 nmol/L); and for adults taking supplements containing vitamin D: median, 21.8 ng/mL (54.4 nmol/L); observed range, 2.5th to 97.5th percentile, 10.4-45.4 ng/mL (26.0-113.5 nmol/L).

Conclusion: In this study, the ADVIA Centaur Vitamin D Total method had good agreement (Pearson coefficients >0.90) with the IDS EIA and LC-MS/MS. The DiaSorin LIAISON assay was not in agreement (Pearson coefficient <0.70) with the IDS EIA, ADVIA Centaur, or LC/MS/MS assays.

* This assay has not been cleared by the FDA and is not available for sale in the US. The assay is CE marked.

B-123

Measurement of ascorbic acid in human plasma by liquid chromatography-tandem mass spectrometry

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Background: Vitamin C (ascorbic acid) is a water soluble vitamin that is essential for the enzymatic amidation of neuropeptides, production of adrenal cortical steroid hormones, promotion of the conversion of tropocollagen to collagen, and for the metabolism of tyrosine and folate. Vitamin C is a powerful reducing agent or antioxidant and plays a role in lipid and vitamin metabolism. Specific examples include: activation of detoxifying enzymes in the liver, anti-oxidation, destruction of free radicals, preservation and restoration of the antioxidant potential of vitamin E, and blockage of the formation of carcinogenic nitrosamines. Prolonged deficiency of vitamin C leads to the development of scurvy, a disease characterized by defects in collagen synthesis, resulting in the failure of wound healing, defects in tooth formation and rupture of the capillaries. Early symptoms of vitamin C deficiency may include weakness, fatigue and listlessness as well as shortness of breath and aching joints, bones and muscles.

Methodology: Deuterated stable isotope (L-ascorbic acid-13C6) was added to 50 uL of heparinized plasma as an internal standard. Protein was then precipitated from the mixture by the addition of 10% trichloroacetic acid. The sample was centrifuged for 10 minutes at 1500 xg. L-ascorbic acid and internal standard were then separated by liquid chromatography (TLX4, Cohesive Technologies, Franklin, Massachusetts) followed by analysis on a tandem mass spectrometer (API 5000, Applied Biosystems, Toronto, Canada) equipped with an electrospray ionization source in positive mode. Ion transitions monitored in the multiple reaction monitoring (MRM) mode were m/z 177.1 → m/z 94.9 for L-ascorbic acid and m/z 183.1 → m/z 100.1 for L-ascorbic acid-13C6. Calibrators consisted of six standard solutions ranging from 0 to 20 mg/dL.

Results: Method performance was assessed using precision, linearity, recovery and specimen stability using lithium and sodium heparinized plasma spiked with L-ascorbic acid solution. Intra-run precision (N=20) coefficients of variation (CVs) ranged from 2.6% to 8.8%. Inter-run precision (N=20) CVs ranged from 5.1% to 15.2%. Linearity studies were performed using lithium heparin and sodium heparin ascorbic acid-depleted plasma spiked with L-ascorbic acid standard. The method demonstrated linearity over the assay range (0.1 to 20 mg/dL), yielding the following equations: lithium heparin plasma observed L-ascorbic acid value = 0.9953*(expected value) - 0.2067, R^2 = 0.9975; sodium heparin plasma observed L-ascorbic acid value = 0.9651*(expected value) - 0.0721, R^2 = 0.9987. Recovery averaged 95% for both lithium heparin and sodium heparin plasma. A stability study demonstrated that specimens are stable at frozen (-80ºC or lower) temperatures for up to 14 days.

Conclusion: This method provides for the reliable, high throughput analysis of L-ascorbic acid in plasma by tandem mass spectrometry.

B-124

Observational study on plasma essential trace elements in very elderly subjects from BELFRAIL cohort

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Background: In coming decades the proportion of very elderly people living in the Western world will dramatically increase leading to an explosion of chronic diseases (CD). Based on experimental studies and clinical findings, there is an emerging body of evidence that essential trace elements (ETE) are related with some CD. Reference values in very elderly subjects and factors affecting these concentrations are not well documented. Therefore, the aims of this study were to: 1- define accurate reference values of ETE in an elderly population, 2- identify common parameters affecting these concentrations.

Methods: 228 subjects from BELFRAIL (BFC80+) cohort were studied. BFC80+ is a prospective, observational, population-based cohort study of subjects aged 80 years and older in three well-circumscribed areas of Belgium. Only three exclusion criteria were used: severe dementia, in palliative care and medical emergency.

ETE (Chromium Cr52, Cobalt Co59, Copper Cu63, Manganese Mn55, Molybdenum Mo95, Selenium Se78, Vanadium V51 and Zinc Zn66) concentrations in plasma were evaluated using Inductively Coupled Plasma Mass Spectrometry (Agilent Technologies, USA). Samples were collected in S-Monovette® for trace metal analysis. Plasma was frozen at -80C° until analysis. Multiple regression analyses

were used to evaluate the influence of: age, gender, body mass index (BMI), smoking status, cystatine, high sensitive CRP (hCRP) and haemoglobin (Hb) on each ETE.

The Protocol was approved by the local Biomedical Ethics Committee.

MedCalc Software (Mariakerke, Belgium) was used for statistical analysis.

Results: Table1 shows multiple regression *p* values for each ETE in relation with each tested independent variables. It shows also reference ETE concentrations intervals after excluding outliers.

Conclusion: The present study establishes reference values for 8 selected ETE and assesses potential influencing factors. It is an important pre-requisite for diagnosis and treatment of ETE deficiencies but also to understand the potential relationships with some CD in the elderly population.

Table1:

Independent variables	Trace Elements							
	Cr52	Co59	Cu63	Mn55	Mo95	Se78	V51	Zn66
Age	0.032**	ns	ns	ns	ns	0.012**	ns	0.057**
Gender	ns	ns	0.011***	ns	ns	ns	ns	ns
BMI	ns	ns	0.008**	ns	ns	0.04**	ns	ns
Smoking status	ns	ns	ns	ns	ns	ns	ns	ns
hCRP	ns	ns	<0.0001*	ns	ns	0.011**	0.019	0.021**
Cystatine	<0.0001*	ns	ns	ns	ns	ns	0.002	ns
Hb	0.039*	ns	ns	ns	ns	<0.0001*	ns	0.001*
Distribution	Log	Log	Normal	Normal	Normal	Normal	Log	Normal
Reference values (median) µg/L	0.01-0.82 (0.32)	0.04– 0.47 (0.12)	754-1575 (1141)	0.37 – 0.96 (0.59)	0.48 – 2.51 (1.09)	46.5 – 102.9 (73.3)	0.74-0.27 (0.13)	559 – 967 (758)

ns: not significant; * increase with the independent variable; ** decrease with the independent variable; *** lower concentrations in females.

B-125

Measurement of all-trans retinol and alpha-tocopherol in human serum by liquid chromatography-tandem mass spectrometry

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Background: Vitamin A (retinol) is a fat-soluble vitamin that plays an essential role in the function of the retina (adaptation to dim light), is necessary for growth and differentiation of epithelial tissue, and is required for growth of bone, reproduction, and embryonic development. Vitamin A must be obtained through the diet and includes retinol, retinyl ester and beta-carotene. Deficiency of vitamin A has profound negative effects on the immune response leading to an increased susceptibility to infection.

Vitamin E (alpha-tocopherol) is a fat-soluble vitamin that contributes to the normal maintenance of biomembranes, along with its requirement for proper neurological and reproductive function as well as its role as an antioxidant and free-radical scavenger protecting the integrity of unsaturated lipids in the biomembranes of all cells. Vitamin E is commonly obtained from dietary oils and fats such as wheat germ oil, sunflower oil, grains and nuts.

Methodology: Deuterated stable isotopes (all-trans retinol-d5 or alpha-tocopherol-d6) were added to 50 uL of human serum as internal standards. Protein was then precipitated from the mixture by the addition of acetonitrile. All-trans retinol, alpha-tocopherol and the internal standards were extracted via an on-line extraction utilizing high-throughput liquid chromatography (TLX4, Cohesive Technologies, Franklin, Massachusetts) followed by conventional liquid chromatography and analysis on a tandem mass spectrometer (API 5000, Applied Biosystems, Toronto, Canada) equipped with a heated nebulizer ion source. Ion transitions monitored in the multiple reaction monitoring (MRM) mode were m/z 269.3 → m/z 93.1 for all-trans retinol, m/z 274.4 → m/z 93.1 for all-trans retinol-d5, m/z 430.4 → m/z 165.2 for alpha-tocopherol and m/z 436.4 → m/z 171.3 for alpha-tocopherol-d6. Calibrators consisted of six standard solutions ranging from 0 to 200 ug/dL for all-trans retinol and 0 to 30 mg/L for alpha-tocopherol.

Results: Method performance was assessed using precision, linearity, recovery and specimen stability. Precision studies were performed using National Institute of Standards (NIST) standard reference material (SRM), and bovine serum spiked with all-trans retinol and alpha-tocopherol standard solutions. Intra-run precision coefficients of variation (CVs) ranged from 1.0% to 1.3% for all-trans retinol and 1.1% to 2.3% for alpha-tocopherol. All-trans retinol inter-run precision CVs ranged from 3.0% to 6.5%, and alpha-tocopherol inter-run precision CVs ranged from 2.0% to 8.2%. Linearity studies were performed using bovine serum spiked with all-trans retinol and alpha-tocopherol standard solutions. Linearity was demonstrated over each assay range (2 to 200 ug/dL for all-trans retinol and 0.5-30 mg/dL for alpha-tocopherol) yielding the following equations: observed all-trans retinol value = 0.9824*(expected value), R^2 = 0.9993; observed alpha-tocopherol value = 0.9826*(expected value) + 0.4294, R^2 = 0.9967. Recovery averaged 101% for all-trans retinol and 103% for alpha-tocopherol across the assay range. A stability study demonstrated that specimens are stable at ambient, refrigerate and frozen (-20ºC or lower) temperatures for up to 14 days.

Conclusion: This method provides for the simultaneous and reliable high throughput analysis of all-trans retinol and alpha-tocopherol in serum.

B-126

Changes in Ionized Calcium Measurements under Aerobic Conditions

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Background: Disturbances in ionized calcium (iCa) are common among critically ill patients and are associated with increased mortality among certain patient populations. While ionized calcium testing provides the most physiologically relevant assessment of calcium homeostasis, it is total calcium testing that usually serves as a surrogate. The overwhelming barrier to the widespread utilization of iCa is the dogma that because iCa concentration varies with pH it must therefore be measured under anaerobic conditions.

Objective: The purpose of this study was to evaluate the extent to which iCa measurement changes under aerobic conditions over time and to determine whether these changes are relevant.

Methods: Twenty-five samples, collected in lithium heparin tubes, were randomly selected from among those that had a physician-ordered basic or comprehensive metabolic profile as part of routine patient care. Samples were manually uncapped and immediately tested for iCa on a Nova 8 analyzer (Nova Biomedical, Waltham, MA). The pH and iCa, corrected and uncorrected, were noted. The tests were repeated every 20 minutes for two hours. The tubes were never placed on ice and once uncapped, never recapped. The data were evaluated using paired t-test analysis for the pH data and Kruskal-Wallis One Way Analysis of Variance on Ranks for the calcium data.

Results: The pH values increased from 7.39 ± 0.045 to 7.59 ± 0.082 (mean ± S.D., p = <0.001), at two hours, with an average increase of 0.09 ± 0.046 per hour. Uncorrected iCa showed a statistically insignificant decrease (1.18 ± 0.11 to 1.11 ± 0.11 mmol/L, p = <0.05) while corrected iCa showed a statistically insignificant increase (1.18 ± 0.10 to 1.20 ± 0.12 mmol/L, p = <0.05). The average rate of change for both uncorrected and corrected iCa was 0.06 ± 0.02 and 0.05 ± 0.03 mmol/L per hour, respectively. In addition to examining our data for statistical significance, we also examined it for relevance. At the two hour window we examined corrected iCa and found that 17 out 25 patients started and remained within the reference interval (1.09 - 1.29 mmol/L). Of the three patients who started below the reference interval, two had values increase to within the reference interval (1.06 to 1.09 and 1.08 to 1.15 mmol/L). One patient's values increased from within the reference interval to exceeding the interval (1.22 to 1.34 mmol/L). Four patients had values that started and remained above the reference interval and one patient sample was lost due to technical difficulties.

Conclusion: There was a statistically insignificant increase in corrected iCa from serum samples left at room temperature and uncapped and this change does not appear to be relevant within a two hour window. The results of this study suggest that under most clinical laboratory settings, iCa testing in an unselected population can be carried out under aerobic conditions. Further studies are necessary to evaluate this change in certain patient populations including acute pancreatitis, extreme vitamin D deficiency, following parathyroidectomy, and those receiving blood products and lacking the ability to metabolize citrate.

B-127

ALOX5AP polymorphism interacts with dietary fatty acids in apparently healthy Caucasians

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Background: Leukotrienes are mediators of inflammation that play an important role in vascular inflammatory diseases such as cardiovascular disease (CVD). 5-lipoxygenase activating protein is encoded by the ALOX5AP gene and is a necessary cofactor for the enzyme 5-lipoxygenase. Single nucleotide polymorphisms (SNPs) in the ALOX5AP gene have been shown to be associated with risk for CVD in several studies; however, not all studies were able to confirm the genetic predisposition of ALOX5AP SNPs to the risk of CVD.

The current study aims to determine the association of ALOX5AP polymorphism with a subclinical marker of CAD—the intimal medial wall thickness of carotid artery (cIMT) and how the plasma phospholipid long chain polyunsaturated fatty acids modulate the genetic influence of ALOX5AP on cIMT.

Methods: In the current study, we selected 720 apparently healthy Caucasian participants aged 45-85 y free of clinical cardiovascular disease from the Multi-Ethnic

Study of Atherosclerosis (MESA). Seven SNPs of the ALOX5AP gene were chosen for genotyping. The internal and common carotid intima-media wall thickness (cIMT) of participants was measured by B-mode ultra-sound, and plasma phospholipid fatty acid composition was determined by gas chromatography.

Results: The minor allele of the SNP rs17222814 was associated with increased common IMT after adjusting for omega-3 fatty acids but not omega-6 fatty acids (EPA p= 0.0077; DHA p= 0.017; ALA p= 0.026). The atherogenic effect of rs17222814 minor allele was attenuated by increased plasma phospholipid omega-3 fatty acids.

Internal cIMT was negatively associated with EPA and DHA, and positively associated with the ratio of omega-6 fatty acids to omega-3 fatty acids in a multi-variant analysis model. Elevated ratio of omega-6 fatty acid to omega-3 fatty acids was associated with increased internal cIMT in individuals who are homozygotes for rs9315050 major allele (p=0.00017) but not carriers of the minor allele.

Conclusion: Previous studies have reported inconsistent results with regards to the genetic influence of ALOX5AP polymorphisms. The current study demonstrates that the lack of consistent findings may in part be due to the modifying effect of intake of long chain omega-3 fatty acids on the association of ALOX5AP gene variants with risk of CVD. Further studies are required to confirm this important gene-nutrient interaction on the risk of CVD.

B-128

Essential trace elements and performance testing score in a Belgian population of 80 years old and more

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Background Heart failure (HF) is a major health problem, associated with high mortality and morbidity and often leads to poor physical functioning. Controversial data about the relationship between essential trace elements (ETE) concentration and cardiovascular disease (especially heart failure) have been published. Therefore, nutritional supplementation with ETE is not yet recommended. The aim of our study was to analyse the following ETE concentrations (Chromium Cr52, Cobalt Co59, Copper Cu63, Manganese Mn55, Molybdenum Mo95, Selenium Se78, Vanadium V51 and Zinc Zn66) to evaluate their potential relationship with performing testing score.

Methods 228 out of the 568 patients from the BELFRAIL (BFC80+) cohort were studied. BFC80+ is a prospective, observational, population-based cohort study of subjects aged 80 years and older in three well-circumscribed areas of Belgium. In total, 29 general practitioner centers were asked to include patients aged 80 and older. The general aim of the BELFRAIL study was to assess the dynamic interaction between health, frailty and disability in a multisystem approach. Only three exclusion criteria were used: severe dementia, in palliative care and medical emergency. To evaluate physical functioning, a performance testing (PT) score was used. It included timed measures of walking speed, rising from a chair, putting on and taking off a cardigan, and maintaining balance in a tandem stand. This score ranges from 0 to 14 (Vaes and al. BMC Geriatrics 2010 10:39).

ETE concentrations in plasma were evaluated using the Inductively Coupled Plasma Mass Spectrometry (ICP-MS) on a 7500 Series ICP-MS system (Agilent Technologies, Santa Clara, USA). Samples were collected in S-Monovette® for trace metal analysis from Sarstetd®, Germany. Plasma was frozen at -80C° until analysis.

Multiple regression analysis was used to evaluate the potential relationship between ETE concentration and PT score.

The protocol was approved by the local Biomedical Ethics Committee.

MedCalc Software (Mariakerke, Belgium) was used for statistical analysis.

Results Multiple regression analysis showed a significant (P<0.0001) relationship between Se concentration and PT score. The same conclusion was observed with Mn (P<0.01). A statistical significant relationship with the PT score was not observed with the others ETE.

Conclusion A significant relationship was observed between plasma Selenium, Manganese and the performance testing score in an elderly population. Further evaluations are needed to: 1- explain the physiopathology of this relationship, 2- study potential benefits of supplementation with those ETE.

B-129

Effects of sample matrices, extreme storage temperatures, and repeated freeze/thaw cycles on integrity of 25-hydroxyvitamin D

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Background: Understanding preanalytical factors such as matrix equivalence and analyte stability is key in obtaining valid laboratory results. Here we compared concentrations of 25-hydroxyvitamin D (25OHD) in its various forms ($25OHD_3$, $25OHD_2$, epi-$25OHD_3$) in several serum/plasma matrices. We tested 25OHD stability in sera stored at -130°C to 37°C for variable times, and subjected sera to 0-4 freeze/thaw cycles.

Methods: For matrix comparison, blood was collected from 27 adults in red-top serum tubes with no additives (SNA), serum separator tubes (SST), or plasma Na-heparin tubes (PH). Paired t-tests identified any statistically significant differences between matrices. To study temperature effects compared to storage at -70°C (reference condition), two vials each of three QC sera containing 7-90 nmol/L per analyte were stored at 4°C for 4 weeks, 23°C for 3 weeks, or 37°C for 8 days, with duplicate measurements taken throughout the test. Freeze/thaw stability was assessed by subjecting two sets of QC sera to four freeze/thaw cycles, with measurements at each cycle. A mixed effects model was used to assess pool-and-time or pool-and-cycle effects. Average percent changes were calculated after testing for interactions. Surplus patient sera (n=35) stored at -70°C and subjected to multiple freeze/thaws were compared with pristine matched sera stored at -130°C; a random effects 1-way ANOVA was used to detect differences. Samples were prepared using liquid-liquid extraction. Concentrations were determined via an isotope dilution UHPLC-MS/MS method with analytical imprecision (CV_A) ≤10% at concentrations ≥20 nmol/L.

Results: There were no statistically significant differences between SNA-SST, SNA-PH, or SST-PH for any 25OHD metabolites (p>0.05). Neither extreme storage temperatures nor four freeze/thaw cycles led to any loss of 25OHD.

Conclusions: Serum or heparinized plasma can be used interchangeably. 25OHD metabolites are extremely stable in serum. The increased concentration observed at times was likely due to desiccation from multiple samplings.

Storage temp/time	Freeze/thaw cycles	Average % change		
		25OHD2	*25OHD3*	*epi-25OHD3*
4°C/4 wk	0	+15	+5	no change*
23°C/3 wk		no change*	no change*	+12
37°C/8 d		+7	no change*	no change*
-70°C	4	+7	+9	no change*
-70°C	1+ (surplus [S])	+5 (S>P)	no change*	+8 (S>P)
-130°C (liquid nitrogen)	0 (pristine [P])			

*p>0.05

B-130

Evaluation of a multi-point calibration curve for HPLC measurement of vitamin K_1

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Background: Vitamin K, a fat-soluble vitamin obtained from the diet, is required for gamma-glutamyl carboxylation of proteins involved in hemostasis, bone mineralization and vascular health. Fat malabsorption, hemorrhage in newborns, and poor diet can be associated with low vitamin K concentrations. Peripheral vitamin K concentration is correlated with dietary intake and can be important for anticoagulant dosing. However, low circulating concentrations make its measurement difficult. In our laboratory vitamin K_1 is measured by high-performance liquid chromatography (HPLC) using a single point internal standard calibration. In a recent multi-laboratory study, the vitamin K external quality assurance scheme (KEQAS) reported poor assay specificity for samples containing low amounts of vitamin K_1, providing an impetus for assay improvement. The purpose of this study was to develop and characterize the performance of a multi-point calibration curve for HPLC-based vitamin K_1 quantification.

Methods: Serum or plasma vitamin K_1 is routinely analyzed in our laboratory using a protocol that involves liquid-liquid and solid-phase extraction, reverse-phase HPLC, on-column reduction and fluorescence detection. Vitamin K_1 concentrations are determined by single-point calibration, using vitamin K_2 as an internal standard. The

new method utilizes a five-point calibration curve. Assay linearity, imprecision and carry-over were assessed using plasma samples containing known amounts of vitamin K_1. Method comparison was achieved by analyzing raw data using the established protocol as well as the multi-point calibration curve.

Results: A five-point calibration curve, spanning a 250-fold range (0.38-95 nmol/L) was used. Data were expressed as a ratio of vitamin K_1 to internal standard (vitamin K_2). There was an excellent linear relationship between vitamin K_1 and both peak area and peak height. Linear regression results for 10 replicates were: $y = 0.085x + 0.024$, $R^2 = 0.995$ (concentration vs. peak area ratio) and $y = 0.065x + 0.011$, $R^2 = 0.998$ (concentration vs. peak height ratio). Variability of the ratio was lower at each concentration when using peak height (CV: 3.7-11.8%) as opposed to peak area (CV: 5.9-13.4%); therefore, the use of peak height ratios was selected for implementation. Furthermore, to facilitate quantification of low concentration samples, the calibration curve was constructed using a y-intercept of zero. Imprecision was determined using spiked plasma at three concentrations. Total imprecision (CV), calculated from duplicate runs over 7 days, was 9.7%, 4.9% and 7.0% at 1.0 nmol/L, 7.1 nmol/L, and 13.9 nmol/L, respectively. Carry-over from high concentration samples (~300 nmol/L) was < 0.1%. To compare methods, spiked serum samples were diluted with saline to give 52 samples spanning a > 5000-fold range, then extracted and quantified using both methods. Comparison of the two quantification procedures yielded a Deming regression equation of $y = 1.381x - 1.77$, $R = 0.999$, $S_{y/x} = 7.1$.

Conclusion: The characteristics of a five-point calibration curve for vitamin K_1 measurement indicate it is suitable for clinical implementation, with excellent linearity, precision and agreement. Validation in a reference population is warranted.

B-131

The investigation of vitamin D and vitamin B12 deficiencies in post-partum women

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Background: Vitamin D and vitamin B12 deficiencies have been associated with a number of diseases such as the development of osteoporosis, increased risk of fractures in the elderly, decreased immune function, bone pain, and depression for vitamin D deficiency and the anemia and neurological deficits for vitamin B12 deficiency.

Objective: The primary objective of this study was to investigate the prevalence of vitamin D and vitamin B12 deficiencies in post-partum women in Amarillo, Texas.

Methods: 27 subjects were enrolled at the Department of OBGYN, Texas Tech University Health Sciences Center, Amarillo, Texas. Inclusion criteria included ages 18-55 year-old who were 4-8 weeks post-partum. The exclusion criterion included any antidepressant medication use in the past six months. Patients were asked to provide two blood samples on the day of their first post-partum visit for total vitamin D and vitamin B12 measurements, which were treated as routine clinical specimens. Subjects were asked to describe their prenatal and postnatal vitamin intake during interview. Vitamin D levels less than 20 ng/mL and 20- 29.9 ng/mL were considered vitamin D deficient and insufficient, respectively. Vitamin D levels of 30 ng/mL or above is considered sufficient. Vitamin B12 levels less than 200 pg/mL were considered deficient.

Results: 27 subjects were recruited. The results for 22 subjects were available (the results for 5 subjects are pending). Vitamin B12 levels of the 22 subjects have ranged from 296 to 1104 pg/mL (average: 546±182 pg/mL). None of the subjects were vitamin B12 deficient. Vitamin D levels of the 22 subjects have ranged from 11-45ng/mL. Of the 22 results available, 10 subjects (46%) had vitamin D deficiency levels (30ng/mL; average: 41±3 ng/mL). All the subjects but 2 reported taking prenatal vitamin supplements regularly on a daily basis during pregnancy. Six subjects reported no postnatal vitamin intake.

Conclusion: Despite taking prenatal vitamin supplements regularly on a daily basis, 87% of the subjects had vitamin D levels corresponding to vitamin D deficiency or insufficiency. All the subjects had vitamin B12 levels above minimum reference range. Pregnant women may need additional vitamin D supplements to attain a vitamin D level above 30 ng/mL. The lower level of vitamin D levels could be due to insufficient intake of vitamin D or an alteration in catabolism of vitamin D during pregnancy. Our findings are important because the investigation of vitamin D deficiency is not the routine part of post-partum visit. The vitamin D deficiency can impact both mother and fetus/baby during pregnancy and breast feeding. We will continue to recruit additional subjects and monitor the prevalence of vitamin D and vitamin B12 deficiencies in post-partum women.

B-132

Cocoa consumption effects on blood pressure, oxidative stress, metabolic profile and biomarkers of inflammation and endothelial function in individuals with stage 1 hypertension

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Background: Recent evidence suggests that the consumption of chocolate 70% cocoa decreases blood pressure and oxidative stress, while improves endothelial function and metabolic profile.

Objective: To evaluate in stage 1 hypertensive subjects, the effects of chocolate 70% cocoa intake on casual blood pressure, glucose metabolism, lipid profile, oxidative stress and biomarkers of inflammation and endothelial function.

Methods: Intervention clinical trial. Twenty stage 1 hypertensive subjects without previous antihypertensive treatment, of both sexes, aged 18-60 years were included in the present study. All patients received 50g of chocolate 70% cocoa/day (containing 2135mg polyphenols) for 4 weeks. To avoid weight gain during the study period, patients were instructed to reduce their habitual energy intake in 280Kcal/day.

Results: Comparison of pre versus post intervention data revealed significant reduction in casual blood pressure. Systolic blood pressure decreased from 146.5 ± 1.3 to 136.9 ± 2.6mmHg, $p < 0.001$; while diastolic blood pressure was considerably reduced from 93.2 ± 0.7 to 87.4 ± 1.8mmHg, $p< 0.03$. We observed a expressive reduction, however not statistically significant on plasma biomarkers of endothelial function: vascular cell adhesion molecule-1 (1037 ±44 vs.1019 ± 42ng/ml), intracellular adhesion molecule-1 (160 ± 12 vs.149 ± 10ng/ml), E-selectin (68 ± 7 vs. 64 ± 6ng/ml) and biomarkers of inflammation: high sensitivity C-reactive protein (9.3 ± 2.7 vs. 6.1 ± 1.2mg/l) and interleucin-6 (88 ± 21 vs. 69 ± 15pg/ml). HOMA-IR and serum levels of tumor necrosis factor-α, oxidized LDL, glucose, insulin, total cholesterol, LDL cholesterol, HDL cholesterol and tryglicerides remained almost unchanged during the study.

Conclusion: the results of this study suggest that chocolate 70% cocoa has a beneficial effect on blood pressure.

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Assessment of Iodine and Zinc status in school age children of Eastern Nepal

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Background: Iodine and zinc deficiencies present a significant health problem in Nepal. Co-existing micronutrient deficiencies during childhood contributes in impairment of growth, immune competence, and mental and physical development. The present study was designed to assess the iodine and zinc status, and investigate the association of iodine and zinc deficiencies in the school age children of Eastern Nepal.

Methods: We enrolled 386 school age children of 6-12 years of age from Sunsari and Dhankuta districts of Eastern Nepal from August 2009 to July 2010. Written consent was obtained from the guardians and school teachers of the school age children. Spot urine samples (n=386) were collected in plastic vials, blood samples (n=174) were collected in EDTA vials and transported to the laboratory maintaining a cold chain. Plasma and urine samples were stored at -20 ºC until analysis. Household salt samples (n=318) were collected in air-tight plastic pouch, salt types were categorized and salt iodine content was estimated by iodometric titration. Urinary iodine excretion (UIE) in spot urine samples was estimated by ammonium persulphate digestion microplate (APDM) method. Zinc was estimated in plasma by flame atomic absorption spectroscopy. At low (20 µg/L), medium (199 µg/L) and high (>300 µg/L) concentration of urinary iodine, intra assay coefficient of variation (CV) were 6.3%, 1.8% and 1.9% and inter assay CV were 11.9%, 4.9% and 6.2% respectively. Intra and inter assay CV from pooled plasma samples obtained from healthy volunteers for plasma zinc were 7.9% and 11.5% respectively. Iodometric titration showed intra and inter assay CV of 2.8% and 6.9% respectively.

Results: Our results showed median UIE 293.3 µg/L and 240.8 µg/L and mean±SD plasma zinc levels 7.8±2.5 µmol/L and 7.5±2.5 µmol/L in the school age children from Sunsari and Dhankuta districts respectively. We found 27(14.13%) and 46(23.58%)

school age children were iodine deficient (UIE<100 µg/L), 42(21.5%) and 34(17.1%) school age children consumed inadequately iodized salt (<15 ppm), and 65(76.4%) and 66(73.3%) school age children had low plasma zinc levels (<9.9 µmol/L) in Sunsari and Dhankuta districts respectively. Significant difference in median UIE was observed between gender (p=0.032), iodine status (p<0.0001), salt types (p<0.0001) and salt iodine content (p<0.0001) respectively. There was no significant difference between the median UIE of the two districts (p=0.186). We found significant difference in plasma zinc levels (p=0.043) between the age groups (8-10) and (10-12) years. No significant difference was observed in the mean plasma zinc levels between the two districts (p=0.399) and gender (p=0.579) respectively. There was no significant association (p=0.564) between school age children who were iodine deficient (UIE<100 µg/L) and had low plasma zinc levels (<9.9 µmol/L). However, 34(19.5%) school age children having low plasma zinc levels had co-existing iodine deficiency.

Conclusion: Our study showed remarkable iodine and zinc deficiencies in these regions. There was no significant association between the deficiencies of iodine and zinc. Universal salt iodization, zinc supplementation and awareness programs regarding adverse effects of these micronutrient deficiencies should be continued to minimize the risk in the vulnerable age groups.

B-134

Rapid determination of riboflavin in plasma using HPLC with fluorescence detection

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Background: Riboflavin is the central component of the cofactors FAD and FMN, and is therefore required by all flavoproteins. As such, riboflavin is required for a wide variety of cellular processes. It plays a key role in energy metabolism, and is required for the metabolism of fats, ketone bodies, carbohydrates, and proteins. The clinical manifestations of deficiency are non-specific. Clinical manifestations include mucocutaneous lesions of the mouth and skin, corneal vascularization, anemia, and personality changes. Riboflavin has been used in several clinical and therapeutic situations. For over 30 years, riboflavin supplements have been used as part of the phototherapy treatment of neonatal jaundice. More recently there has been growing evidence that supplemental riboflavin may be a useful additive along with beta-blockers in the prevention of migraine headaches.

Determination of free riboflavin in plasma is considered appropriate for general clinical analysis of riboflavin status and supplementation monitoring. Previously, measurement of plasma riboflavin ordered by the physicians here at the Cincinnati Children's Hospital Medical Center was performed at the reference laboratory. The results turnaround time were not always satisfied and service of monitoring was lagging. Therefore, rapid and reliable HPLC procedures based on direct HPLC injection after sample deproteinization or even without sample pretreatment are greatly desired.

The need for a quick measurement of riboflavin in plasma samples in a simplified manner and the need for a cost-effective procedure prompted the development of a rapid HPLC method. Here, a rapid and reliable HPLC method is described for determination of riboflavin concentrations in a small volume (100µL) of plasma that is suitable in pediatric practice.

Methods: Sample (100µL) was vortex-mixed with methanol, ascorbic acid solution, and internal standard lumiflavin for 1 minute and centrifuged at 10,350 g for 10 minutes at room temperature. The supernatant (ca. 300µL) was kept at dark for 30 min and transferred to an autosampler vial, 20µL of supernatant was injected directly onto the HPLC system. Separations of riboflavin and lumiflavin were achieved by using a 5-µm Microsorb-MV reversed-phase C18 column (150 x 4.6 mm) and a mobile phase consisting water, methanol and acetonitrile. The flow rate of HPLC run was at 0.8 mL/min and column temperature at 30°C. Peaks of riboflavin and lumiflavin were monitored at excitation 456 nm and emission 512 nm.

Results: The method achieved a linear concentration range of 2.66-132.85 nmol/L, which covered the reference range of 6.2-39 nmol/L. The limit of detection was 0.8 nmol/L. Both within-run and between-run precision for three fortified controls (19.9, 39.9, and 79.7 nmol/L) in plasma were lower than 6%. Analytical recoveries were greater than 96%. No interference was observed. The method was compared to a reference laboratory HPLC assay using 30 samples ranging from 5 to 100 nmol/L. The correlation showed a slope of 1.06, an intercept of 1.2 nmol/L and an *r* of 0.95.

Conclusion: This HPLC run is rapid (7 min) with excellent reproducibility. It requires no solid-phase extraction and one step deproteinization prior to chromatography. It is suitable for routine analysis of riboflavin in plasma.

B-135

Effect of temperature and light on the stability of serum carotene

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Background: Fat-soluble vitamins, such as carotene, are generally considered highly unstable at ambient temperature and when exposed to light and oxygen. Recent studies indicated that carotene concentrations in whole blood change insignificantly for several days; however stability data for serum carotene remain scarce. The aim of the study was to examine the effect of temperature and light on the stability of serum carotene.

Methods: Five serum pools were made using healthy volunteers and 7 day old left over laboratory samples. Carotene levels ranged from 2.50 - 4.27 µmol/L. Aliquots were stored under the following conditions: room temperature-dark, room temperature-light, refrigerated-dark, refrigerated-light, frozen (-30°C)-dark, and frozen (-70°C)-dark for the duration of 1, 3, 7, 10, or 14 days until analysis. Serum carotene concentrations were determined by spectrophotometer at a wavelength of 440 nm.

Results: Samples stored at -70°C for 14 days (the end of study controls) showed a minimal average change of only 3.04% compared with those measured freshly at day 0. Compared with the end of study controls, samples stored at room temperature-light for 10 and 14 days demonstrated a significant decrease (p<0.05) in carotene levels. Except for room temperature, all other storage conditions did not induce clinically significant changes of serum carotene.

Conclusion: Serum carotene levels are stable when stored at 4°C (in dark or light) or in the freezer for up to 14 days. Carotene stability is also acceptable at room temperature-dark for 7 days or room temperature-light for 3 days.

Table 1: Percentage change of serum carotene % (SE) at various conditions

	Room Temperature		Refrigerated (4°C)		Frozen (-30°C)	Frozen (-70°C)
	dark	light	dark	light	dark	dark
Day 1	2.05 (3.25)	-3.77 (2.31)	-0.60 (4.51)	-6.23 (3.74)	-2.54 (5.07)	
Day 3	-1.54 (2.85)	-5.95 (2.77)	-2.97 (4.08)	-2.15 (4.71)	-0.72 (5.08)	
Day 7	-6.90 (3.94)	-14.52 (2.77)	-2.40 (4.49)	-8.35 (4.31)	0.66 (3.79)	
Day 10	-11.91 (3.34)	-24.45 (4.18)*	-3.24 (3.66)	-9.87 (4.18)	-6.72 (2.56)	
Day 14	-14.60 (4.75)	-28.25 (3.88)*	-3.62 (3.56)	-9.64 (4.07)	-4.93 (4.21)	3.04 (8.77)

* $p < 0.05$ compared with percentage changes of frozen at -70°C for 14 days.

B-136

Determination of Cadmium in Whole Blood: Comparison of Proficiency Test Results with Isotope Dilution Inductively Coupled Plasma Mass Spectrometry

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Background: Recently, NIST issued SRM 955c Toxic Elements in Caprine Blood, a four-level frozen blood CRM intended for use in evaluating the accuracy of measurements of toxic elements and mercury species in whole blood. Certified concentration values, values of highest metrological order, are reported for Cd in Levels 1 and 3 based on ID ICP-MS measurements performed at NIST. Proficiency test (PT) data for SRM 955c were collected by NYSDOH and used to assign reference values for Levels 2 and 4. Now, additional ID ICP-MS measurements have been completed, enabling a comparison (Levels 2, 3, and 4) between the PT data and the ID ICP-MS data.

Methods: ID ICP-MS - Blood (1.0-g to 1.8-g) from six to eight vials of SRM 955c Levels 2, 3, and 4 was sampled, spiked with enriched ^{111}Cd, and digested with high purity HNO_3 in a high pressure microwave oven. Following digestion, a small portion of the sample was retained for analysis as the unseparated fraction and the remaining portion was separated off line using anion exchange chromatography. Samples were

introduced into the ICP-MS *via* a membrane desolvator to minimize oxide formation. Results were calculated using the $^{111}Cd/^{112}Cd$, $^{111}Cd/^{113}Cd$ and $^{111}Cd/^{114}Cd$ intensity ratios after correction for residual spectral inference from Mo and Sn. SRM 966, Toxic Elements in Bovine Blood was used for method validation.

Clinical Procedures - Single, blinded vials of each level of SRM 955c were distributed for analysis "as special" PT samples. Samples were analyzed in the same manner as routine patient specimens. A sub-set of the reported data, composed of results from a group of 22 experienced reference laboratories using ICP-MS (18), graphite furnace atomic absorption spectrometry (3) and atomic absorption spectrometry (1), were used for comparison to ID ICP-MS data.

Results: PT data are within -0.9 %, -3.3%, and -4.0 % of ID ICP-MS data for Cd in SRM 955c Level 2 (2.16 µg/L), Level 3 (5.20 µg/L), and Level 4 (10.26 µg/L), respectively. The approximate 95% confidence intervals for the difference between the means show no evidence of disagreement for Level 2, however small but statistically significant differences are revealed for Levels 3 and 4. Comparison of ID ICP-MS results for unseparated and separated sample fractions illustrate the adverse effect of blood matrix on the precision and accuracy of Cd measurements. CV's were 1.0 % for unseparated and 0.3 % for separated Cd Level 4 sample fractions (n=8) and means differed by +2.5 %. Matrix-induced signal suppression resulted in a 50 % decrease in signal intensity for unseparated samples.

Conclusion: It is possible that the small differences observed in the PT data and ID ICP-MS data are due to differing sample treatments. Matrix separation, used in the ID ICP-MS analyses was shown to improve accuracy and precision by reducing the effects of spectral and non-spectral interference. Reference values for Cd in Level 2 and Level 4 currently reported on the Certificate of Analysis for SRM 955c will be updated to reflect the additional ID ICP-MS data.

B-137

Evaluation of trace elements and biochemical parameters in blood samples from a healthy elderly population in a university hospital in Brazil

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Background: There are few data about reference values to be used in laboratory tests for elderly. This fact encouraged us to evaluate some biochemical parameters and trace elements concentrations present in blood samples from ambulatory elderly people.

Methods: This study was submitted and approved by our Internal Review Board (IRB). An elderly population, without clinical evidence of serious chronic diseases, from Clinical Hospital of São Paulo University Medical School was evaluated. The selection of these individuals was based on the SENIEUR protocol (*SENIor EURopean Protocol*). The blood samples of 125 elderly people (36 men and 89 women), aging 72 ± 8 years, were analyzed. The blood, after 12 hours fast, was collected by venipuncture using sterile standard metallic needles. It was collected in two types of evacuated tubes (Vacutainer Systems - Becton Dickinson, EUA): SST II *Advance* gel and clot activator tube and a specific tube for trace elements analysis, without heparin. An aliquot of serum (3.0 mL) was transferred to a flask (Nalgene) and freeze-dried for trace element determinations. Neutron activation analysis (NAA) was applied for trace elements determination. About 150 mg of freeze-dried serum were irradiated at the IEA-R1 research nuclear reactor together with elemental standards. Short and long irradiations were carried out under a thermal neutron flux of about 4 x 10^{12} n cm^{-2} $s^{-1}$for Br, Ca, Cl, Fe, Na, Rb, Se and Zn determinations. After adequate decay times, the irradiated samples and standards were measured using a Hyperpure Ge detector Model GX2020 coupled a gamma-ray spectrometer. The radioisotopes measured were identified according to their half-lives and gamma-ray energies and the element concentrations were calculated by comparative method. The certified reference material, NIST 1566b Oyster Tissue was analyzed to evaluate the accuracy and precision of the results.

Results: The mean concentration values obtained by NAA were: Br: 3.45± 0.84 mg/L, Ca: 9.58 ± 0.94 mg/dL, Cl: 89.19 ± 8.67 Meq/L, Fe: 132.4 ± 109.5 µg/L, Na: 133.3±12.5 Meq/L, Rb: 321.0± 57.8 µg/L, Se: 76.7 ± 25.0 µg/L and Zn 96.2 ± 14.5 µg/L. Biochemical analyses were carried out on Roche/Hitachi MODULAR *ANALYTICS* PP (Roche Diagnostics GmbH, Mannheim, Germany), using specific kits from Roche Diagnostics, too. The biochemical mean values obtained were: uric acid: 5.0±1.4 mg/dL, total bilirrubin:0.72±0.29 mg/dL, Na:141±3 mEq/L, K:4.5±0.4 mEq/L, Ca:9.4±1.1 mg/dL, ionized Ca:5.1±0.5 mg/dL, P: 3.5±0.5 mg/dL, Mg:2.09±0.28 mg/dL, glucose:93±10 mg/dL, urea:37±12 mg/dL, creatinine:0.84±0.19 mg/dL, Fe:105±32 µg/dL, total iron-binding capacity:304±41 µg/dL, ferritin:178±154 ηg/mL, total protein:7.4±0.5 g/dL, albumin:4.4±0.3 g/dL, total cholesterol:210±36 mg/dL, HDL-cholesterol 59±14 mg/dL, LDL-cholesterol:128±32 mg/dL, triglycerides:121±60 mg/dL, AST:22±7 U/L, ALT:20±11 U/L, alkaline phosphatase:79± 25U/L and GGT:25±29 U/L.

Conclusion: It can be concluded that the blood sera from this healthy elderly group do not present deficiency or excess of trace elements. The results of biochemical parameters suggest the need to establish specific reference values for elderly.

B-138

Hepcidin and ferritin in hemodialyzed patients

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Background: Hepcidin is a key systemic regulator of iron metabolism. Hepcidin binds to the iron cell exporter ferroportin so iron is kept in the cells unavailable for erythropoiesis. Hepcidin synthesis is up-regulated by high iron stores and inflammation. Dialyzed patients have very often impaired iron management - they suffer from anemia, which is caused by many factors including the state of microinflammation and hepcidin retention due to decreased glomerular filtration rate. Our aim was to describe the relationship of hepcidin and other parameters of iron metabolism, erythropoiesis and inflammation in these patients.

Methods: Complete blood cell count, iron, ferritin, trasferrin, CRP, albumin, creatinine, hepcidin (ELISA kit from DRG Diagnostics), soluble transferrin receptors (sTfR) and IL-6 were measured in samples from 164 patients treated in chronic hemodialysis program at the Ist Department of Internal Medicine, Faculty Hospital in Pilsen (age 66±13, 25-92 years), 63 women and 101 men and 37 control healthy volunteers (age 55±20, 21-92 years), 21 women and 16 men.

Results: Iron, transferrin and hemoglobin were significantly lower in the patients group ($p<0.0001$) while ferritin ($p<0.0001$), sTfR ($p<0.05$), hepcidin ($p=0.0003$), CRP and IL-6 ($p<0.0001$) were significantly higher in the patients group. Hepcidin weakly ($p<0,05$) positively correlated with CRP and ferritin and weakly negatively correlated with transferrin ($p<0,05$) in hemodialyzed patients. No correlation of hepcidin with other biochemical parameters in controls was shown.

Conclusion: Parameters of iron metabolism, erythropoiesis and inflammation were significantly different between hemodialyzed patients and a control group. Only weak correlation between hepcidin and ferritin and CRP and no correlation with IL-6 in hemodialyzed patients can indicate that the influence of inflammation as a factor causing increased hepcidin levels in hemodialyzed patients is not crucial and other factors including its retention in end-stage renal disease can concur.

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B-139

Modified Method for the Simultaneous Measurement of Retinol, α-Tocopherol, and β-Carotene in Human Serum

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Background: Vitamin A (retinol), Vitamin E (tocopherol), and carotene play an important role in human health. Vitamin A is necessary for normal vision, cellular differentiation, growth and reproduction. Vitamin E is known for its antioxidant properties and promoting immunity. β-Carotene possesses provitamin A activity and may have a protective effect in prevention of diseases such as cancer and cardiovascular disease. Existing methods employ a lengthy protocol for sample preparation and separation by high-performance liquid chromatography (HPLC). We developed and validated a simple, sensitive, and fast isocratic HPLC method for simultaneous quantification of these vitamins in human serum.

Methods: The developed method uses (1) liquid-liquid extraction followed by freezing with the removal of organic phase by decantation and (2) isocratic gradient with a short, small particle size C-18 column coupled with ultraviolet-visible detection for quantification.

Results: Sample preparation used in earlier published methods was enhanced by including a freezing step after liquid-liquid extraction. This enabled organic layer separation by decantation instead of pipetting. The freezing method shortened the time of sample handling, with complete recovery of the heptane layer and improved reproducibility of results. Isocratic separation of retinol, α-tocopherol, β-carotene, and internal standard on a C-18 column solves various issues in the determination of vitamins in serum, including: simultaneous selectivity, sensitivity, reliability, and analysis time. Analyte separation on the new column is as efficient as using a traditional C-18 column. The shorter length and smaller particle size results in a 60% reduction of the analysis time. Therefore, the new column is an ideal choice for high-throughput isocratic analysis of vitamins A, E, and β-carotene in serum. Evaluation of the method performance included precision, recovery, linear range and carry-over studies. Recoveries for all analytes were between 90% to 100%. Within day CVs ranged from 2.2% to 3.9%, and between-day CVs ranged from 5.7% to 14%. The assay was linear over the range 5.0-500 mcg/dL for retinol, 0.4-40 mg/L for α-tocopherol, and 2-200 mcg/dL for β-carotene. Carry-over was not detected. Retinol, α-tocopherol, and β-carotene in human serum of 200 patient samples were measured and compared with the previous column. The correlation between columns was good: for retinol (r=0.96), α-tocopherol (r=0.93), and β-carotene (r=0.98).

Conclusions: The overall findings suggest that the modified method is a simple, fast and reliable isocratic reversed-phase high-performance liquid chromatography (HPLC) method for the simultaneous determination of retinol, α-tocopherol, and β-carotene.

B-140

Stability of 24 Plasma Fatty Acids Stored at -20°C

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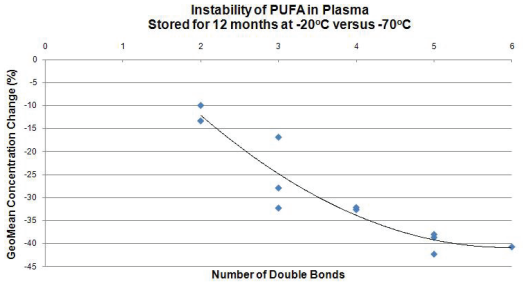
Background - Studies have shown a decrease or no change in the polyunsaturated fatty acid content of plasma/serum stored at -20°C for ≤10 years. This study reevaluates the stability of a panel of fatty acids in plasma stored at -20°C for ≤12 months. The panel includes six saturated (SFA), seven monounsaturated (MUFA), and 11 polyunsaturated (PUFA) fatty acids.

Methods - The analytical method used to quantitate fatty acid concentrations was a modification of Lagerstedt (Molecular Genetics and Metabolism 2001;73:38-45). Total fatty acids were hydrolyzed, hexane-extracted, derivatized to pentafluorobenzyl bromide esters, and detected using electron capture negative-ion gas chromatography-mass spectrometry. The analytical imprecision (CV_A) ranged from 2.3-5.6% (PUFA) to 2.6-11.1% (SFA and MUFA). Triplicate vials from three plasma QC pools were stored at -20°C for up to 12 months; at specified times vials were moved from -20°C to -70°C until batch analysis at the end of the study. Concentrations of fatty acids in the pools stored at -20°C were compared with those stored continuously at -70°C; all pools were stored in air atmosphere. A mixed effects model was used with a Satterthwaite approximation for the denominator degrees of freedom for analysis of pool-by-time effects.

Results - Ignoring pool-by-time interactions, PUFA showed significantly decreased geometric mean concentrations (10-45%) when stored at -20°C for 12 months ($p<0.05$); no significant decreases were seen in SFA or MUFA concentrations. In contrast, storage at -70°C for 12 months did not decrease PUFA concentrations (routine QC pool assessment; n = 132 assays). An association between decreased concentration and number of double bonds was observed suggesting autoxidation.

Conclusion - Plasma PUFA are not stable when stored in air atmosphere at -20°C for 12 months whereas SFA and MUFA are stable. Consideration should be given to storage temperature and addition of an inert gas/antioxidant when specimens are frozen for later analysis.



B-141

Dietary calcium intake and its relationship with intracellular calcium, adiposity, metabolic profile, blood pressure and endothelial function

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Background: Epidemiological studies consistently show an inverse association between calcium intake and adiposity and blood pressure. The main findings suggest that changes in intracellular calcium may mediate this phenomenon, but so far there are few studies evaluating the differences in intracellular calcium concentration in subjects with different levels of calcium intake.

This study aimed to evaluate the association of dietary calcium consumption with intracellular calcium, adiposity, metabolic profile, blood pressure and endothelial function.

Methods: Cross-sectional. 30 women, with mean age 30.91 ± 2.77 years, were submitted to evaluation of dietary intake, intracellular calcium, anthropometric parameters, % body fat, metabolic profile and blood pressures levels. Dietary intake was evaluated using a validated food frequency questionnaire and % body fat by bioimpedance. *Intra-erythrocyte calcium* was determined by *atomic* absorption spectroscopy. Endothelial function was evaluated by peripheral artery tonometry using Endo-PAT 2000® (Itamar Medical).

Results: Participants were classified into 2 groups, according to their mean dietary calcium intake (Group A < 870mg/day and Group B ≥870mg/day). There were no significant differences between groups of calcium intake with respect to age, race, alcohol intake, weight, height, body mass index, waist circumference, waist-to-hip ratio, body fat, metabolic variables, CRP- US and blood pressure and endothelial function. The lack of significant difference between the groups remained even after adjusting for confounding factors. The average calcium intake was 740.60 ± 53.12 mg / day in group A and 973.17 ± 40.55 in group B.

The assessment of endothelial function through the index of reactive hyperemia showed no difference between groups A and B (1.67±0.1 vs. 1.99±0.17). There were no significant differences in the concentrations of total serum calcium and serum ionized calcium between the two groups. Intracellular calcium was lower in group A compared to group B, however without reaching statistical significance (20.56 ± 2.97 vs 16.48 ± 2.55 mEq/L/Cel). There was no significant association between daily intake of calcium and intracellular calcium.

Conclusion: The findings of the present study suggest that the consumption of calcium is not associated with intracellular calcium, adiposity, metabolic profile, blood pressure and endothelial function.

Wednesday AM, July 27

Poster Session: 10:00 am - 12:30 pm
Cardiac Markers

C-01

Utility of Serum Concentration of High-Sensitivity Cardiac Troponin T as a Screening Marker of Cardiovascular Risk in Outpatients with Type 2 Diabetes

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A high-sensitive assay for troponin T (hsTnT) makes it possible to measure concentration >5-fold lower than the limit of the traditional assay. We evaluated the utility of hsTnT as a risk marker of cardiovascular disease in 385 outpatients with type 2 diabetes. **Results:** Serum hsTnT was detected (> lower detection limit of 2 pg/ml) in 79.5% of the patients, and was higher than 14 pg/ml (upper reference limit) in 19.3%. A history of cardiovascular disease was present in 94 (24.4%). During a median follow-up period of 12 months, there were 17 cardiovascular events. Higher hsTnT levels were associated with older age, higher levels of BNP, high-sensitivity CRP, cystatin C and urine albumin/creatinine ratio, a higher prevalence of cardiovascular disease, and a higher cardiovascular event rate (Table). On a stepwise Cox regression analysis including 12 clinical and biochemical variables, quartiles of hsTnT were independently (P=0.0003) associated with cardiovascular event. **Conclusion:** Serum hsTnT concentration may be useful as a screening marker of cardiovascular risk in outpatients with type 2 diabetes.

HsTnT quartile (pg/ml)	1st <3 (n=101)	2nd 3-7 (n=113)	3rd 7-11 (n=78)	4th >11 (n=93)	P value
Age(years)	60	66	68	73	<.0001
Male (%)	44.6	60.2	65.4	62.4	.008
Hemoglobin A1c (%)	6.9	6.6	7.0	6.7	.06
BNP (pg/ml)	16.1	17.8	32.0	69.5	<.0001
High-sensitivity CRP (mg/l)	0.36	0.50	0.50	0.72	.01
Cystatin C (mg/l)	0.63	0.69	0.79	1.06	<.0001
Urine albumin/creatinine ratio (mg/g Cr)	10.0	28.7	29.2	184.0	<.0001
Prevalence of cardio vascular disease (%)	11.9	20.4	24.4	43.0	<.0001
Cardio vascular event rate (%)	2.0	3.5	2.6	10.8	.009
Data are expressed as median or percent					

C-02

Myeloperoxidase and cardiac troponin I in patients with chest pain and suspicion of acute coronary syndrome

J. Racek[1], P. Brož[2], J. Lhotská[1], R. Rokyta[1], L. Trefil[2]. *[1]Charles University, Faculty of Medicine, Pilsen, Czech Republic, [2]Faculty Hospital, Pilsen, Czech Republic,*

Background: Myeloperoxidase (MPO), produced by activated neutrophils and macrophages in vulnerable atherosclerotic plaque, can be detected in plasma and serves as a prognostic marker in people with atherosclerosis. The aim of our study was to assess behavior of MPO and cardiac troponin I (cTnI) in patients admitted with chest pain and suspicion of acute coronary syndrome.

Methods: Concentrations of MPO and cTnI (both immunochemical determination on Architect 2000, Abbott) were measured in 57 patients (mean age 66.7 years, range 27-91 years) with chest pain, admitted at the Department of Internal Medicine, Faculty Hospital in Pilsen; three samples were obtained: on admission (sample A), then 6-8 h later (sample B) and finally 12-16 h after admission (sample C). The patients were divided into two groups: Group I (33 patients where diagnosis of MI was confirmed according to "universal definition of AMI") and Group II (24 patients) where MI diagnosis was excluded; the course of MPO and cTnI levels was compared in these two groups.

Results: Patients with MI presented with high MPO levels already on admission (sample A, median 1104.0 pmol/l) and its concentrations gradually decreased during next 12-16 hours (median of sample B 376.3 pmol/l, sample C 212.9 pmol/l). cTnI levels had an inverse trend: they rose from the lowest values on admission to the highest in the last sample (medians of samples A, B, C: 0,70; 4,77; 12.36 µg/l). 20% of patients with MI had cTnI levels below the cut-off for MI (0.04 µg/l) in the first sample and 33% had cTnI levels <0.1 µg/l; from these patients only one had normal MPO level (<250 pmol/l). Patients with chest pain and without MI had generally low levels of MPO (medians in samples A, B, C: 179.8; 227.0; 223.8 pmol/l) and their cTnI concentrations increased only slightly (medians of samples A, B, C: 0,04; 0.07; 0.08 µg/l). Total of 15 patients (62.5%) had normal MPO levels on admission and the rest of 9 patients (37.5%) had MPO>250 pmol/l; nevertheless, the levels of MPO in these patients were only slightly increased and only in one case exceeded 400 pmol/l. MPO values on admission were significantly lower on comparison with those in patients with MI (p<0.001).

Conclusion: We can conclude that high MPO levels on admission in patients with chest pain, together with cTnI, can indicate the patients with MI with a high probability. High MPO levels can serve as an early, although not specific, marker of ACS.

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C-03

An investigational-use high sensitivity cardiac troponin I assay measurement at presentation to predict short-term serious cardiac events in patients with potential acute coronary syndrome

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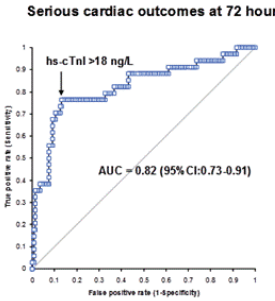
Background: There is uncertainty regarding which cutoffs should be used for high sensitivity cardiac troponin (hs-cTn) testing in the emergency setting. A common approach for identifying individuals at risk is to use the concentration cutoff derived from a healthy population (i.e., 99th percentile); however, a potentially more clinically relevant approach is to determine the concentration that optimizes both the sensitivity and specificity for an important event in the short-term (i.e., over 3 days). We sought to determine and compare hs-cTnI concentrations derived from receiver-operator characteristic (ROC) curve analyses versus the reported 99th percentiles (JACC 2009;54:1165-72) in a emergency department (ED) population with potential acute coronary syndrome (ACS).

Methods: An investigational-use hs-cTnI assay (Beckman Coulter) was measured in stored serum specimens (below -80C) in 179 subjects who presented to the ED with potential ACS. Patients were enrolled in the study if their symptoms occurred within 6 hours from pain onset and clinically had a cTn test ordered. Study subjects were followed for 72 hours for the following outcomes: death, myocardial infarction, congestive heart failure, serious arrhythmia or refractory ischemic cardiac pain that required hospitalization. ROC curve analyses were performed to identify the optimal concentration for predicting serious outcomes.

Results: The average age of the population was 62 years (SD=15). Using the reported 99th percentile cutoff for those ≤60 years (i.e., ≥10 ng/L) yielded the following sensitivity/specificity for the composite outcome: 76%(95%CI:59-89)/67%(95%CI:59-75); whereas employing the reported 99th percentile cutoff for those >60 years (i.e., ≥19 ng/L) yielded the following sensitivity/specificity estimates: 74%(95%CI:56-87)/87%(95%CI:80-92). The ROC derived cutoff for a serious cardiac event was >18 ng/L (Figure).

Conclusion: In patients presenting early with potential ACS the ROC-derived cutoff for a serious short-term outcome was the same as the 99th percentile in those >60 years. Future studies are needed to validate this cutoff.



C-04

Short and Long-term Biological Variability of Troponin-I Determined Using the ARCHITECT STAT High Sensitive Troponin-I Assay

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Current guidelines recommend serial measurements of cardiac troponin for the diagnosis of acute myocardial infarction (AMI). The ability to differentiate clinically significant changes over time is dependent upon multiple factors including biological variability (BV). High sensitive cardiac troponin assays are able to detect circulating troponin to differentiate normal variability from pathological processes.

This study evaluated the short-term (4 - 6 hours) and long-term BV (9 days) of troponin using the Abbott ARCHITECT *STAT* high sensitive troponin-I assay (hsTnI). The reagents, calibrators and controls used to perform the analysis were in final launch formulation.

Specimens were collected from apparently healthy volunteers using an IRB approved protocol. Serum and EDTA plasma samples were obtained from each subject (n=25) between 0800 and 1000 hours and between 1300 and 1500 hours on days 1, 5 and 9 and frozen until analysis. Analysis was performed on three analytical runs using a single reagent and calibrator lot.

Data was analyzed by ANOVA to determine the within subject (CVi), between subject (CVg) and analytical (CVa) components of variation. Data summarized below includes Relative Change Values (RCV) and Index of Individuality (II) using the following formulae[1]:

$RCV = 2^{0.5} * z * (CVa^2 + CVi^2)^{0.5}$, $II = (CVi^2 + CVa^2)^{0.5} / CVg$

Table 1. Short-and long-term biological variation in cTnI

		Serum		EDTA Plasma	
Variable		Short Term (4-6 hours)	Long Term (9 days)	Short Term (4-6 hours)	Long Term (9 days)
# of Subjects[a]		24	24	24	24
Mean hs cTnI	pg/mL	1.88		1.39	
Analytical Variation[b]	CVa%	16.8		16.9	
Biological Variation	CVi%	24.4	80.4	37.1	117
	CVg%	124		179.2	
Index of Individuality	II	0.24	0.66	0.23	0.66
RCV%	z=1.96	82	228	113	328

a. One subject removed as outlier b. Based on duplicate **Results: Conclusion:** A short-term change greater than the RCV (82% and 113% for serum and plasma, respectively) in patients with low concentrations of troponin may signify AMI in the appropriate clinical setting. The long-term RCVs were expectedly higher at 228% and 328%, as changes to the heart in healthy individuals are more likely to occur over days versus hours. The ARCHITECT *STAT* high sensitive Troponin-I assay enables the detection of small serial changes that may be clinically relevant.

1. Fraser CG, Biological Variation: From Principles to Practice, AACC Press 2001

C-05

Short-term Biological Variation of Cardiac Troponin I Measured with Three High-Sensitivity Assays

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The objective of this study was to determine the short term biological variation (BV) and associated reference change value (RCV) of three research high sensitivity cardiac troponin I (hs-cTnI) assays.

Blood (serum) was collected from 12 healthy volunteers (6 males and 6 females, ages 23 to 54 years) hourly at baseline and over 4 hours. High sensitivity cTnI was measured on three systems: Beckman Coulter Access II, Siemens Dimension Vista; Abbott ARCHITECT. Analytical, intraindividual, and interindividual CVs (CV_A, CV_I, CV_G; respectively) were calculated from the measured troponin concentrations (n=60 specimens). The table shows the findings.

Biological variations were similar for the three research high sensitivity cTnI assays, ranging between 44.5 to 50.1%. The within subject mean cTnI concentrations ranged from 3.5 to 5.5 pg/mL.

In conclusion, these findings provide preliminary evidence for what laboratorians and clinicians need to expect regarding interpretation of patient cardiac troponin I concentrations produced by serial testing using high sensitivity cTnI assays for detection of acute and chronic myocardial injury compared to findings within their respective normal individuals.

Analytical & Biological Variation by hs-cTnI Assay

Analytical Variation	Abbott	Beckman	Siemens
CV-A, %	13.8	14.5	13.0
Biological Variation			
CV-I, %	15.2	6.1	12.9
CV-G, %	70.5	34.8	12.3
Index of Individuality	0.22	0.46	0.11
RCV, %	50.1	44.5	47
RCV increase %	69.3	63.8	57.5
RCV decrease %	-40.9	-38.9	-36.5
Overall within Subject cTnI Mean, pg/mL	3.5	4.9	5.5

C-06

The impact of renal function on the efficiency of N-terminal pro B type natriuretic peptide measurement in Primary Care

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Objective: To determine the effect of renal function on the diagnostic utility of measurement of N-terminal pro B type natriuretic peptide (NTproBNP) measurement for detection of impaired LV ejection fraction in patients referred from primary-care with suspected cardiac failure.

Methods: Data from a series of studies of patients in primary care who had NTproBNP measurements performed plus echocardiographic estimation of ejection fraction were analysed. All samples were analysed for NTproBNP using an Elecsys 2010 (Roche Diagnostics, Lewes). The interassay %CV was 5.0 at 380 ng/L, 4.4 at 8700 ng/L, 5.0 at 13000 ng/L, with detection limit 20 ng/L and upper measuring limit 25000 ng/L. Estimated glomerular filtration rate (eGFR) was then calculated using creatinine measurements from the local laboratories (Jaffe reaction, %CV <5%) using the diet modification of renal disease equation. The data was then pooled and dichotomised using a eGFR of >60ml/min/1.73m^2 and then stratified by age as <60, 60-<75 and ≥ 75 years. Individual receiver operator characteristic curves (ROC) were calculated using ejection fraction as the dichotomous variable for all the data split by GFR and then for each age group split by eGFR. An ejection fraction of less than or equal to 40% was considered to be abnormal. The area under the curve (AUC) was then calculated.

Results: Data was obtained from 5224 patients (2399 males, 2825 females) from 18 centres in the UK, New Zealand, Europe and the US. The median age was 62 years (range 20.0 to 100.0 years, interquartile range 53. to 71 years) with a prevalence of systolic dysfunction of 23.2%.There were 1127 patients with an eGFR ≤60(median 51, range 5-60, interquartile range 42-56.3).Overall the AUC was (95% confidence intervals in parentheses) for eGFR ≤60 0.822 (0.797-0.847) vs. for eGFR >60 0.901 (0.887-0.916). Dividing by age the results were for eGFR ≤60 as follows Age <60 0.878 (0.814-0.941) 60-75 0.841 (0.804-0.877) ≥75 0.787 (0.745-0.829) and for eGFR >60 as follows age <60 0.937 (0.921-0.954) 60-75 0.876 (0.850-0.903) >75 0.829 (0.772-0.887). In all cases the AUC's were lower for an eGFR ≤60 but the confidence intervals overlap. The largest differences were seen in the younger age groups. There was no influence of gender. Using previously defined decision thresholds, the comparative sensitivity and specificities were as follows (eGFR <60 vs. eGFR >60) for each age group as follows. For age <60 (50 ng/L discriminant) sensitivity was 0.956 and specificity 0.352 (n = 167) vs. sensitivity 0.976 and specificity 0.524 (n = 2096). For age 60-75 (100 ng/L discriminant) sensitivity was 0.954 and specificity 0.416 (n = 469) vs. sensitivity 0.919 and specificity 0.563 (n =1561). For age ≥75 (250 ng/L discriminant) sensitivity was 0.873 and specificity 0.472 (n = 491) vs. sensitivity 0.827 and specificity 0.647 (n = 440).

Conclusion: This large multicentre study showed that impaired renal function reduced the diagnostic performance of NTproBNP measurement with a reduction in test specificity. However, the AUC's indicated test performance remained acceptable.

C-07

N-terminal and C-terminal fragments of IGFBP-4 as new markers for short-term risk assessment of major adverse cardiac events (MACE) in patients presenting with ischemia

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Background: Expression of the dimeric form of the Pregnancy Associated Plasma Protein A (dPAPP-A) is increased in vulnerable atherosclerotic plaques. Thus plasma PAPP-A was suggested to be used as a marker for MACE prediction. However dPAPP-A measurements in patient's blood are complicated by low analyte concentration and heparin interference. dPAPP-A is known as a protease specifically cleaving IGF-binding protein 4 (IGFBP-4) between amino acid residues Met135 and Lys136 to form its N-terminal and C-terminal fragments (NT- and CT-IGFBP-4) with molecular mass of 18 and 14 kDa, respectively. We supposed that IGFBP-4 fragments that are released from vulnerable atherosclerotic plaques as products of IGFBP-4 cleavage by dPAPP-A could be used for MACE risk assessment in patients presenting to the emergency department with the symptoms of acute ischemia.

Methods: Monoclonal antibodies (MAbs) specific to NT-IGFBP-4 (MAb IBP3) and to CT-IGFBP-4 (MAb IBP163) recognizing only novel epitopes raised by enzymatic cleavage of IGFBP-4 by dPAPP-A and having no cross-reaction with intact (full-length) IGFBP-4 molecule were obtained. Using one of these MAbs in pair with another MAb recognizing corresponding fragment as well as intact IGFBP-4, we developed two sandwich immunoassays for NT-IGFBP-4 and CT-IGFBP-4 measurements. Both assays were suitable for fragments quantification in human plasma regardless of the presence of full-length IGFBP-4 in the sample. Human recombinant NT-IGFBP-4 expressed in E. coli and CT-IGFBP-4 expressed in HEK293 cell line were used as calibrators. NT-IGFBP-4 and CT-IGFBP-4 were measured in EDTA-plasma of 166 patients (mean age 65.6 years, 80 men and 86 women) admitted to emergency department with symptoms of ischemia but w/o ST-segment elevation on electrocardiogram. The incidence of MACE (nonfatal myocardial infarction, cardiac death, percutaneous coronary intervention) was measured during 6 months (median) follow-up period after IGFBP-4 fragments baseline evaluation.

Results: The median plasma NT-IGFBP-4 concentration was 114 ng/ml (interquartile range 60-196 ng/ml). The median plasma CT-IGFBP-4 concentration was 86 ng/ml (interquartile range 58-124 ng/ml). During the follow-up, 17 (10.2%) patients met the endpoint (11 nonfatal myocardial infarctions, 5 cardiac deaths, and 1 percutaneous coronary intervention). Patients with plasma NT-IGFBP-4 concentrations >180 ng/ml (27.1% of population) had increased risk of developing future MACE [hazard ratio (HR) 12.5, 95% CI 3.8-41.6, P < 0.0001], and NT-IGFBP-4 as a single variable predictor of MACE showed an area under the ROC curve of 0.86. NT-IGFBP-4 concentrations above the indicated cutoff were 82% sensitive and 79% specific for MACE prediction. Patients with plasma CT-IGFBP-4 concentrations >100 ng/ml (36.8% of population) had increased risk of developing future MACE (HR 5.6, 95% CI 1.9-16.4, P = 0.0017), and CT-IGFBP-4 as a single variable predictor of MACE showed an area under the ROC curve of 0.80. CT-IGFBP-4 concentrations above the indicated cutoff were 77% sensitive and 68% specific for MACE prediction.

Conclusion: We report that IGFBP-4 fragments could be utilized as markers for MACE prediction in patients with suspected acute coronary syndrome.

C-08

Prognostic Utility of Combination of Cystatin C with BNP and Cardiac troponin T for Cardiac Mortality in Acute Heart Failure Patients without Advanced Renal Impairment

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Recently, we have reported that cystatin C may improve the early risk stratification compared with estimated glomerular filtration rate (GFR) in acute heart failure patients without advanced renal impairment. Thus, we investigated the prognostic value of combination of cystatin C with BNP and cardiac troponin T on admission in 362 consecutive patients hospitalized for worsening chronic heart failure with estimated GFR > or = 30 ml/min/1.73 m^2. **Results:** During a mean follow-up period of 694 days after admission, there were 59 (16%) cardiac deaths. Patients with cardiac death were older, had higher values of cystatin C, BNP, and cardiac troponin T, and had lower values of estimated GFR and hemoglobin compared to those without cardiac death (Table). On a stepwise Cox regression analysis including 10 clinical and biochemical variables, elevation (>median value) in cystatin C (> 1.09 mg/l; relative risk [RR] 4.7, P < 0.0001), BNP (> 790 pg/ml; RR 2.1, P = 0.02) and troponin T (> 0.029 ng/ml; RR 2.3, P=0.008) were independently associated with cardiac deaths. Each independent biomarker was assigned a number of points proportional to its RR as follows: cystatin C (2 points), BNP (1 point) and troponin T (1 point). When patients were categorized on the basis of the sum of their point numbers, cardiac mortality rate was 1.5% in patients with zero point, 8.1% in those with one, 7.2% in those with two, 23.5% in those with three, and 42.3% in those with four. **Conclusion:** The combination of cystatin C with BNP and troponin T on admission improves the early risk stratification for cardiac mortality in acute heart failure patients without advanced renal impairment.

	Cardiac death(-) (n=303)	Cardiac death(+) (n=59)	P value
Age(years)	73	77	.0009
Ischemic etiology (%)	37	46	NS
NYHA functional class IV (%)	61	71	NS
Left ventricular ejection fraction (%)	36	39	NS
Cystain C (mg/l)	1.05	1.41	<.0001
BNP (pg/ml)	732	1150	.0007
Troponin T (ng/ml)	0.020	0.050	<.0001
Estimated GFR (ml/min/1.73m2)	60.5	45.1	<.0001
Hemoglobin (g/dl)	12.7	11.3	.006
High-sensitivity CRP (mg/l)	5.0	11.0	.08
Data are expressed as median or percent; NS = not significant			

C-09

Daily measurement of C-Reactive protein in healthy volunteers

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Background: High sensitivity C-reactive protein (hs-CRP) is increasingly being suggested as a useful marker for cardiovascular disease risk. Most data collected is based on a single measurement of CRP. We wondered what the daily variation in hs-CRP might be and how much this daily variation might contribute to the accuracy of data interpretation.

Methods: Daily finger stick samples from 11 volunteers were collected over a 28 day period. Plasma hs-CRP levels were measured on a standard commercial laboratory analyzer.

Results: There were three episodes of URTIs, one episode of mild food poisoning and two episodes of soft tissue injury which gave dramatic changes in CRP values. The laboratory analyzer reported consistent results with CVs averaging <2% for samples over 0.75mg/L. In patients without any obvious inflammation hs-CRP levels varied over a 1.7-3.6 fold range. Some of this background variation could be explained by exercise events. A summary of the data is presented in the table below where the values represent the percentage of readings that each individual had in the risk ranges of the CDC/AHA guidelines. Measurement frequencies greater than 80% are highlighted to indicate the most likely risk category for a particular donor.

Conclusions: Hs-CRP values in this selection of normal volunteers varied considerably over the course of this four week period. Even allowing for adverse events, 8/11 candidates spent some time in more than one of the risk ranges of the CDC/AHA guidelines. This suggests that serial measurements of hs-CRP are much more likely to give a more accurate measurement of the underlying inflammation status of the donor than a single measurement. Serial measurement would reduce the likelihood of inaccurate categorization of individual patients and perhaps lead to a more powerful assessment of hs-CRP as a risk factor for cardiac disease.

Donor	F026	F032	F485	F516	M054	M134	M162	M233	M610	M698	M972
N (all data)	28	28	42	27	28	25	28	27	28	28	26
% < 1 mg/L	0	7	64	85	61	88	54	0	0	93	100
% between 1-3 mg/L	86	82	17	11	29	12	46	56	57	7	0
% > 3 mg/L	14	11	19	4	11	0	0	44	43	0	0
N (excl adverse events)	25	27	24	21	20	25	28	23	17	28	26
% < 1 mg/L	0	7	100	100	85	88	54	0	0	93	100
% between 1-3 mg/L	96	85	0	0	15	12	46	65	88	7	0
% > 3 mg/L	4	7	0	0	0	0	0	35	12	0	0

C-11

Performance Validation of the Presage ST2 Assay on the Triturus EIA System

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Background: Novel immunoassays which are only available in microtiter plate format are often prohibitively difficult for the typical clinical laboratory due to the staff burden imposed by assays in this format. Performance of such assays on an automated platform, such as the Grifols Triturus EIA System, can reduce the staff burden associated with performance of these assays. The Presage ST2 Assay is a novel, high sensitivity ELISA providing quantitative measurement of the serum protein soluble ST2. Published reports have shown that elevations of ST2 is powerfully prognostic in patients symptomatic for cardiac diseases and with diagnosed cardiac diseases, such as heart failure or acute coronary syndrome (ACS).

Methods: In the present analysis assay precision and detection limits were measured on the Triturus instrument and compared to results of the assay performed manually. To determine assay precision test samples at three (3) different analyte concentrations, spanning the clinically significant concentration range, were measured in groups of fifteen (15) replicates in two (2) replicate plates per run over three (3) consecutive days. Within run and between run variation was determined. To determine detection limits the Limit of Blank (LoB) was determined using fetal bovine serum (FBS) as a source of matrix known to not contain detectable ST2. Blank samples were measured in sixteen (16) replicates over four (4) consecutive runs. The Limit of Detection (LoD) and Limit of Quantitation (LoQ) were both determined per CLSI protocol EP5-A. Four (4) different plasma samples with low, normal ST2 concentrations measured in sets of sixteen (16) replicates over four (4) consecutive runs were used to determine assay variation and bias.

Results: Within run coefficient of variation (CV) on the Triturus was 7.5%, 4.4% and 5.1% at the respective ST2 concentrations from low to high. The ST2 concentrations in the samples as tested are 10.4, 42.1 and 74.0 ng/mL respectively. Between run CV on the Triturus was 10.0%, 5.0% and 7.0% respectively at the three (3) ST2 concentrations tested. The assay as performed on the Triturus does not exhibit any precision bias as a function of ST2 concentration. These results compare well with previously established values from the assay performed manually of 6.5%, 3.4% and 3.8% for intra-assay CV and 9.1%, 5.5% and 4.8% for inter-assay CV at low, medium and high ST2 concentrations respectively. LoB and LoD values were identical between manual and automated results at 0.47 ng/mL and 1.8 ng/mL respectively. The LoQ value obtained on the Triturus was slightly lower than the 2.4 ng/mL obtained manually but this difference is not statistically significant.

Conclusion: Performing an ELISA in microtiter plate format is a time consuming, effort intensive process. The Presage ST2 Assay as tested here requires a total elapsed time of approximately 4 hours and when performed as a manual test requires operator attention for at least 2 hours of this run time. The results reported here show that the Triturus EIA System provides comparable or slightly better performance than manual operation and with a fraction of the required operator time.

C-12

Immediate effects of cardiac resynchronization therapy

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Background: Heart failure (HF) is considered to be a complex syndrome associated with the neurohormonal and cytokine activation, that contribute to its progression. Cardiac resynchronization therapy (CRT) is an adjunctive treatment, indicated for HF patients, who remain symptomatic, despite optimal drug therapy. Left ventricle (LV) remodeling reflects the structural and functional deterioration that occurs in HF. The reverse remodeling is an accepted goal in the treatment of HF. In patients with HF, CRT leads to reverse remodeling of the LV. We aimed to investigate the immediate effects of CRT, on the serum levels of N-terminal pro-brain natriuretic peptide (NT-proBNP), proinflammatory cytokines (interleukins IL-1β, IL-6, IL-8, tumor necrosis factor TNF-α), matrix metalloproteinase-2 (MMP-2) and tissue inhibitor of metalloproteinase-2 (TIMP-2) in patients with HF.

Methods: 40 patients (21M/19F, 17 ischemic) with HF (III-IV NYHA functional class), wide QRS, normal PR interval, significant LV dyssynchrony (time difference in peak septal wall to postero-lateral wall strain >130 msec by speckle-tracking radial strain) and concordant LV lead position were investigated before, immediately and 1 week after CRT. The serum levels of NT-proBNP, IL-1β, IL-6, IL-8, TNF-α, MMP-2 and TIMP-2 were measured at the same time by ELISA. Cardiac function was assessed echocardiographically.

Results: Immediately after CRT, there was an unexpected mild reduction in serum levels of all analyzed biochemical parameters. CRT led to an early decrease in NT-proBNP potentially. Thus, after the initiation of CRT, the serum concentration of NT-proBNP decreased with 11.07%. Also, immediately after CRT there was a positive correlation between changes in NT-proBNP and IL-1β ($r=0.59$, $p<0.05$), NT-proBNP and TNF-α ($r=0.38$, $p<0.05$). The most convincing data, which demonstrate that CRT modifies the remodeling process are left ventricular telediastolic volume (LVTDV), left ventricular telesystolic volume (LVTSV), septal-to-posterior wall motion delay (SPWMDSax) and left ventricular ejection fraction (LVEF). LVEF had increased from 20.83±5.39% before CRT, to 29.31±6.04% at 1 week after CRT. One week after CRT, there was a good correlation between changes in LVEF and NT-proBNP ($r=0.45$, $p<0.05$), LVEF and IL-1β ($r=0.29$, $p<0.05$), LVEF and TNF-α ($r=0.24$, $p<0.05$), between changes in LVTDV and IL-1β ($r=0.42$, $p<0.05$), LVTDV and TNF-α ($r=0.26$, $p<0.05$) and between changes in SPWMDSax and MMP-2 ($r=0.40$, $p<0.05$), SPWMDSax and TIMP-2 ($r=0.31$, $p<0.05$)

Conclusions: After the initiation of CRT, neurohormonal and proinflammatory activity were reduced and the decrease in serum concentration of NT-proBNP, IL-1β and TNF-α predicts a clinical improvement during follow-up. The changes in proinflammatory cytokines activity were related to the changes in the NT-proBNP serum levels. Thus, the decrease in NT-proBNP potentially immediately post CRT, seem to be, at least in part, determined by the reduction of the peripheral markers of immune activation in patients responding to CRT. The precise molecular and genetic pathways responsible for reverse LV remodeling are not fully understand, but our results suggest that CRT could immediately improve the systolic and diastolic synchrony of the LV and ventricular function.

C-13

Highly sensitive cardiac troponin levels in pregnant women

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Background: Both gestational hypertension and chronic hypertension during pregnancy could damage the maternal heart. However, the implications of cardiac troponin (cTns) levels in pregnant women remain controversial. Furthermore, cardiac troponin I (cTnI) or troponin T (cTnT) levels in pregnant women show dissociated responses. Therefore, to elucidate their utility, we measured the highly sensitive cTnI and cTnT levels during pregnancy.

Methods: In this study, 200 pregnant women were prospectively enrolled; serum samples of 106 of the 200 women were obtained at enrolment and just before labor/delivery. The cTnI level was measured using the TnI-ultra, whereas the cTnT level was measured using the hs-cTnT. In addition, N-terminal natriuretic peptide (NT-proBNP) level was also measured. The limits of detection (LOD) were 6 ng/L for cTnI and 3 ng/L for cTnT, respectively. The control population comprised 212 age-matched non pregnant women in whom both cTnI and cTnT were measured: the 99 percentile value for both cTnI and cTnT was 4 ng/L; the cTnI level was over LOD in 1 (0.5%) and the cTnT level was over in 15 (7%) women. The eGFR was estimated by a formula specific for women by using both serum creatinine level and age.

Results: Among the 106 subjects, 86 women (Norm group: mean (SD) age, 34.1 (4.6) years) had no cardiovascular complication during the gestational period and just before labour/delivery, whereas 20 women (Comp group: 34.7 (5.9) years) had complications such as preeclampsia or chronic hypertension at both the time points. In the Norm group, the cTnT levels were over LOD in only 5 (6%) women at enrolment and in 23 (27%) women just before labour/delivery. In the Comp group, the corresponding numbers were 6 (30%) and 13 (65%). In the Norm group, the cTnI level was over LOD in 14 (19%) women at enrolment and in 34 women (43%) just before labour/delivery; the corresponding numbers in the Comp group were 4 (25%) and 10 (50%). Although the NT-proBNP level increased with advancing pregnancy in both the groups; however, it was elevated even at the enrolment stage in the Comp group. Similarly, the eGFR deceased with advancing pregnancy in both groups, but it was significantly lower in the Comp group. Furthermore, multiple regression analysis showed that the NT-proBNP level was an independent variable responsible for the levels of cTns.

Conclusion: The cTnI and cTnT levels were increased in some pregnant women with cardiovascular complications as well as in those with no cardiovascular complication; these elevations may be due to cardiac overload during pregnancy, which could be monitored by measuring NT-proBNP levels.

C-14

Evidence for processing of human pro-B-type natriuretic peptide in the circulation

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Background: B-type Natriuretic Peptide (BNP) is a cardiac hormone produced by the myocardium in response to volume overload and increased filling pressure. BNP consists of 32 amino acid residues (AAR). Active BNP hormone is produced along with the N-terminal fragment, NT-proBNP (76 AAR), from a precursor molecule, proBNP (108 AAR), by the proteolytic cleavage (processing). Both BNP and NT-proBNP are known to be powerful biomarkers of heart failure (HF) and risk assessment of cardiovascular complications. To date, the location where proBNP processing takes place is not precisely identified. Recent data show that unprocessed proBNP represents a substantial or even major part of BNP immunoreactivity in the plasma of HF patients and healthy donors. We have suggested that the circulation can be a place where proBNP is processed, releasing active BNP hormone. The goal of the present study was to examine this hypothesis.

Methods: As follows from recent *in vitro* and cell-based experiments, the efficiency of proBNP processing is suppressed by O-glycosylation of the threonine 71 residue located close to the known proBNP cleavage site. Considering this finding, two forms of recombinant proBNP, nonglycosylated (expressed in *E. coli*) and glycosylated (expressed in HEK 293 cells), were used in the study. Nonglycosylated or glycosylated proBNP were injected intravenously in rats (n=4 for each peptide). The rate and the products of proBNP cleavage were analyzed in the plasma samples collected at specific time points after injections. Immunoassays, gel filtration, and mass spectrometry (MS) techniques were applied to characterize proBNP processing.

Results: Nonglycosylated proBNP was efficiently processed in the circulation. BNP 1-32 as well as various N- and C-terminal truncated BNP forms (BNP 3-32, BNP 4-32, BNP 5-32, and BNP 5-31) were generated, as confirmed by MS. Analysis of the generated NT-proBNP forms revealed that cleavage of proBNP occurred exclusively at the site -R_{76}↓S_{77}-, suggesting that the observed multiple truncated BNP forms were the products of the subsequent proteolytic degradation of BNP 1-32. Glycosylation of the proBNP region located close to the cleavage-site suppressed proBNP processing in the circulation similar to that previously shown for furin- and corin-mediated proBNP processing.

Conclusion: The present study shows that proBNP is processed in the circulation with formation of mature BNP 1-32. This observation suggests that the circulation can be considered as a place where proBNP processing occurs. Further studies are needed to demonstrate the relevance of these animal-based experiments to humans.

C-15

Validation of BD Rapid Serum Tubes for STAT Troponin-T Testing on the Roche Cobas e411

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Background: Plasma separator tubes (PST) are often used for cardiac Troponin-T (cTnT) testing because they allow for a faster turn-around time compared to serum separator tubes (SST), which require up to 30 minutes to clot We have identified a small percentage of erroneous results (false positives) with PST samples when using the STAT Troponin-T reagent on the Roche Cobas e411 (Roche Diagnostics, Indianapolis, IN), presumably due to activated platelets, fibrin strands, residual platelets, and/or WBCs in PST samples. Use of the rapid serum tube (RST) from BD (Becton-Dickinson and Company, Franklin Lakes, NJ) potentially allows for a comparable turn-around-time to PST samples while using a specimen type (serum) historically associated with fewer false positive results. We compared cTnT results obtained from PST and RST samples on the Roche Cobas e411.

Methods: In order to find samples with measureable cTnT values, we implemented use of the RST tube for electrolyte panel testing on Emergency Department patients for one month. In our practice, ED patients with suspected myocardial damage receive a baseline cTnT followed by serial cTnTs at 3 and 6 hours. When a baseline cTnT (PST) and an electrolyte panel (RST) were collected in tandem the excess RST specimen was tested for cTnT and compared to the PST cTnT concentration. We determined the mean bias between the RST and PST cTnT concentrations, as well as concordance between RST and PST results. We defined concordant results as both sample types either detectable (≥0.01 ng/mL) or undetectable (< 0.01 ng/mL, 99th percentile), and if detectable the difference between the specimen types was <0.02 ng/mL.

Results: 432 of 443 (97.5%) cTnT RST results were concordant with PST concentrations. 108 (24.4%) samples had detectable cTnT levels (≥0.01 ng/mL) with both sample types. The mean difference between PST and RST cTnT for the 108 detectable cTnT comparisons was 0.000 (range -0.049 to 0.140) ng/mL. Four RST samples exhibited a bias ≥0.02 ng/mL compared to PST; however all had substantially elevated cTnT values (>0.250 ng/mL) with both sample types, thus not influencing diagnostic performance of the assay. Seven samples had detectable PST cTnT results with undetectable RST results. Based on the 3 and 6 hour serial cTnT results (PST samples only), two samples were deemed to have false negative RST cTnT results (cTnT increased over 3-6 hours), and five false positive PST cTnT results (cTnT value undetectable or patient discharged at 3 and/or 6 hours).

Conclusion: RST samples yield analytically and clinically concordant cTnT results with PST samples when tested on the Roche Cobas e411. Among rare discordant samples, erroneous cTnT results were more common with PST samples. Our data suggest that RST samples can be used for stat cTnT analysis, and may even reduce the frequency of erroneous cTnT results.

C-16

Vitamin D Deficiency In Heart failure With Normal Ejection Fraction

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Background: Low levels of vitamin D have high prevalence and is a factor of poor prognosis in heart failure (HF) with reduced ejection fraction. HF with normal ejection fraction(HFNEF) is now the most common form of HF particularly affecting the elderly with comorbidities and the relationship between low vitamin D and HFNEF is not established.

Aim of the study was assess the prevalence of vitamin D deficiency in outpatients with HFNEF and its correlation with diastolic dysfunction(DD).

Methods:A prospective study with 79 outpatients (age 70.7 ± 11.4years, female 72%) (BR latitude -22° South) with EF>50%. 30 pts were classified as HFNEF and 49 in the control group. All pts submitted measurement of 25(OH)D, ECG, B-type natriuretic peptide(BNP) and tissue Doppler imaging. HFNEF diagnosis was established by the criteria of the European Society of Cardiology.

Results: Pts with HFNEF had higher values of BNP and indices of DD (table). 25 (OH) D was lower in the group HFNEF, with statistical significance (34.3 ± 13.0 vs 45.6 ± 18.3 mcg / L, p = 0.004) and 47% of patients with HFNEF had vitamin D deficiency (25 (OH) D <30 mcg /L) against 24% in the group control. Moderate correlation was observed between vitamin D and E/E` (r -0.278 p=0.013), but not between age, BNP,LVEF, LAV-I and E` (r = -0.133 p = 0.244; r = -0.154 p = 0.176; r = 0.051 p= 0.656; r = -0.114 p = 0.319; r = 0.134 p = 0.238

Conclusion: Patients with HFNEF had, on average, lower levels of serum 25 (OH) D than the control group. Correlation was observed between 25 (OH)D and E/E` a noninvasive marker of LV filling pressure.

	HFNEF	Control	p
age(years)	76.2±10.9	67.4±10.4	0.001
LVEF(%)	70.1±8.8	70.1±8.8	0.051
E/E'	14.9±6.7	7.6±2.1	<0.0001
LAV-I ml/m²	42.8±12.2	29.1±7.1	<0.0001
E' cm/s	7.4±2.6	9.6±2.5	<0.0001
BNP pg/ml	131.6±93.3	29.7±41.4	<0.0001
25(OH)D mcg/L	34.3±13.0	45.6±18.3	0.004

C-17

Evaluation of Proadrenomedullin (MR-proADM) as a predictor of early death in AL amyloidosis

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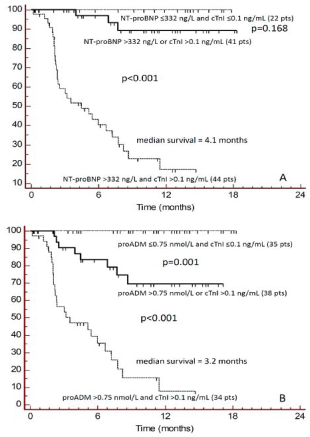
Background. In AL amyloidosis prediction of survival is contingent upon assessment of heart dysfunction, currently based on the cardiac biomarkers N-terminal proBNP (NT-proBNP) and troponins (cTn). The ability to accurately identify patients at risk of early death is crucial in the design of treatment strategy. Midregional proadrenomedullin (MR-proADM) is emerging as a powerful prognostic marker in cardiac diseases. Methods. We prospectively assessed the prognostic role of MR-proADM in 107 consecutive newly diagnosed patients enrolled between August 2009 and October 2010. Midregional proADM was measured using a commercial assay (BRAHMS, Germany). The 95 centile in normal subjects was 0.52 nmol/L. The withinrun %CV (n = 20) was < 4 at 1.2 nmol/L and the between-run %CV (n = 10) was < 9 at the same concentration. Results. Seventy-four patients (69%) had heart involvement by standard echocardiographic criteria. Overall median (range) MR-proADM was 0.81 nmol/L (0.12-4.35 nmol/L), NT-proBNP 2578 ng/L (31-77701 ng/L), cTnI 0.08 ng/mL (0-3.8 ng/mL) and serum creatinine 0.94 mg/dL (0.54-6.47 mg/dL). With a 10 month median follow-up of living patients, median survival was not reached and projected 6 month survival was 74%. The MR-proADM cutoff best predicting survival was 0.75 nmol/L (median 7.5 months vs. not reached, p<0.001, HR 6.4). The standard staging system based on NT-proBNP and cTnI was unable to detect an early survival difference between stage I and stage II patients (Fig. 1a). Whereas, substituting NT-proBNP with MR-proADM identified 3 groups with significantly different survivals (Fig. 1b). Conclusion. Midregional proADM improves the short-term prognostic discrimination of patients with AL amyloidosis and can provide guidance in the choice of therapy.



C-18

Establishing a reference interval for the high sensitive Troponin T assay in treatment-naive cancer patients

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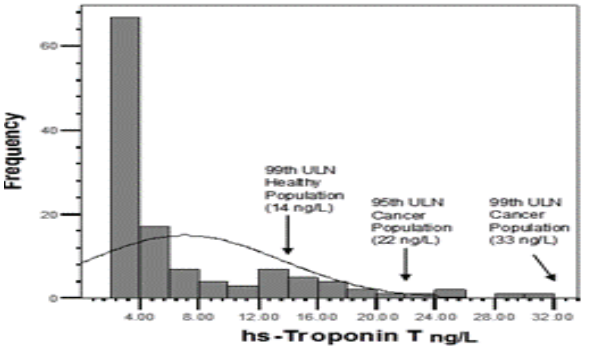
Background: Cardiac toxicity may be a complication of chemotherapy in cancer patients. However, defining the level/cutoff at what cardiac troponin (cTn) concentration is indicative of myocardial injury is not universally accepted in this setting. Moreover, with the advent of the high sensitivity cTn (hs-cTn) assays this issue will likely be more complex as the majority of individuals will have detectable concentrations prior to treatment. Our objective in this study was to measure hs-cTnT in cancer patients prior to treatment to establish a reference interval.

Methods: The study population consisted of 124 cancer patients (breast cancer (n=60), ovarian cancer (n=32), colorectal cancer (n=27) and melanoma (n=5)). Specimens (EDTA plasma) were collected prior to treatment and stored via the Ontario Tumour Bank (below -80C). The hs-cTnT assay (TnT hs, Roche Diagnostics) was measured in these specimens and a reference interval was established as per the recommendations of the Clinical and Laboratory Standards Institute (CLSI28-A3 guidelines).

Results: The median hs-cTnI concentration was 4 ng/L in the population (n= 101 female, n= 22 male; age range: 35-90 years; median age = 62 years) The data were non-Gaussian (Kolmogorov-Smirnov test: p<0.001), with only one concentration identified as an outlier by the Reed/Dixon method (outlier concentration from a breast cancer patient). The 95th and 99th percentile upper limits of normal (ULN) of hs-cTnT were 22 ng/L (90%CI:17-31 ng/L) and 33 ng/L, respectively. Twenty one (17%) of the patients had hs-cTnT concentrations that exceeded the published 99th percentile from a healthy population (≥ 14 ng/L) (Figure). In this population, hs-cTnT concentrations were not affected by gender and cancer types, however, they were affected by patients' age (multiple regression, p<0.05). **Conclusions:** A preliminary reference interval of the hs-cTnT assay for cancer patients has been established. Additional studies are needed correlating hs-cTnT elevations and heart function in this setting.



C-19

Distribution of GDF-15 in apparently healthy individuals assessed with a pre-commercial assay based on the ECLIA principle

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Background: Growth-differentiation factor 15 (GDF-15) is a member of the transforming growth factor ß (TGF-ß) super family. Cardiomyocytes and other cell types over express GDF-15 in response to various stressors, including pro-inflammatory cytokines and reactive oxygen species. Recent studies have demonstrated that GDF-15 is an independent risk predictor in patients with cardiovascular disease. Initial studies with a pre-commercial GDF-15 assay included evaluation of the technical performance and the assessment of the marker in healthy individuals.

Methods: Measurements were performed with an early development lot of a GDF-15 assay based on the ECLIA principle. The current assay on the Elecsys® platforms has a sandwich assay format with two monoclonal antibodies used for capture and detection of serum or plasma GDF-15. Pooled serum samples in the precision study had GDF-15 levels between 1100 and 16000 pg/mL. Preliminary reference values were evaluated in 973 apparently healthy individuals with exclusion of patients with chronic diseases identified with a standardized health questionnaire. The reference population was composed of 517 females and 456 males with ages ranging from 18 to 77 years. The diurnal variation of GDF-15 was studied in 10 smokers and 10 non-smokers.

Results: The between-day imprecision in human serum pools and control pools varied between 0.7 and 2.2 % CV, determined over 10 days in singlet determinations. Method comparison in serum samples to the initial research immunoradiometric assay (IRMA) for GDF-15 showed a slope and intercept of 1.06 and -145, respectively (PB regression, tau: 0.889, Deming's regression, slope 1.18, intercept -531, r: 0.963). The distribution of GDF-15 in apparently healthy females and males was not significantly different (97.5th percentiles for females and males were 1729 and 1889, respectively; Wilcoxon test: p = 0.44). A separate comparison of decades of age in male and female individuals showed significant differences between the lowest cohort of age and the other decades (Wilcoxon test : p < 0.05, trend: CHI² 269.7, p < 0.00001 (Kruskall-Wallis)). Smoking had an impact on the values of GDF-15. The mean values for individuals that never smoked vs. past and current smokers were 837 (SD: 376), 898 (SD: 413) and 1014 (SD: 1001) pg/mL, respectively. The three groups were significantly different (Wilcoxon P < 0.03; trend (CHI²) 29.2, p < 0.00001). GDF-15 showed a small diurnal trend in smokers, with GDF-15 values rising during the day. This trend was not observed in non-smokers. The median within-day variation of GDF-15 was 5% CV in non-smokers and 8% CV in smokers. The total intra-individual CV including test results collected over 4 weeks was rather low in both study groups (medians: 6.7% CV and 9.2% CV in non-smokers and smokers).

Conclusions: Initial studies with a pre-commercial assay for GDF-15 on Elecsys showed that the marker is influenced by age but not affected by gender. Smokers had slightly elevated GDF-15 values which might be related to increased cardiac risk in smokers. Future studies have to demonstrate whether the small but significant increase in GDF-15 has an influence on the prognosis of smokers and elderly, apparently healthy individuals.

C-20

Warning against Ongoing Myocardial Damage Diagnosed by High-Sensitivity Cardiac Troponin T Assay in Chronic Kidney Disease

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A new high-sensitive assay for troponin T (hsTnT) makes it possible to measure concentration >5-fold lower than the limit of the traditional assay. We prospectively investigated the clinical significance of hsTnT in 258 outpatients (median age, 68 yrs) with CKD (estimated GFR <60 ml/min/1.73 m^2) on not dialysis.

Results: Serum hsTnT was detected (> 2 pg/ml of lower detection limit) in 100% of the patients, and was higher than 14 pg/ml (upper reference limit) in 61%. Medical history of cardiac disease was present in 15%, and diabetes in 37%. During a median follow-up period of 16 months, there were 30 cardiac events defined as cardiac death or hospitalization for worsening heart failure or acute coronary syndrome. Higher hsTnT levels were associated with older age, higher levels of BNP, high-sensitivity CRP and cystatin C, a lower level of hemoglobin, higher prevalence f diabetes or cardiac disease, and a higher cardiac event rate (Table).On a multivariate Cox regression analysis, hsTnT (P=0.004) as well as BNP (P=0.0001) were independently associated with cardiac events. Prevalence of cardiac disease and cardiac event rate according to quartiles of hsTnT were shown in Table.

Conclusion: Ongoing myocardial damage diagnosed by hsTnT assay plays an important role in the progression and development of cardiac disease in CKD.

HsTnT quartile(pg/ml)	1st <10 (n=64)	2nd 10-19 (n=64)	3rd 19-32 (n=67)	4th >32 (n=63)	P value
Age (years)	61	66	73	72	<.0001
Diabetes	15.6%	29.7%	40.3%	61.9%	<.01
BNP (pg/ml)	17.8	28.5	58.6	121.0	<.0001
High-sensitivity CRP (mg/l)	0.45	0.77	0.81	0.94	.008
Cystatin C (mg/l)	1.37	1.91	2.38	3.08	<.0001
Hemoglobin (g/dl)	13.1	11.8	10.7	9.6	<.0001
Prevalence of medical history of cardiac disease	3.1%	9.4%	22.4%	25.4%	.0008
Cardiac event	0%	1.6%	6%	20.6%	<.0001
Data are expressed as median or percent					

C-22

Evaluation of the Elecsys Troponin T high sensitive STAT assay on Roche Cobas e601 Platform

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Introduction: Because of its high cardio-specificity and sensitivity for myocardial damage, Troponin T is currently one of the cardiac markers of choice for diagnosis of acute myocardial infarction (AMI). The Elecsys Troponin T high sensitive (TnT hs) STAT assay is reported to be highly sensitive and meets the precision requirements set by the ESC/ACCF/AHA/WHF guidelines for AMI which requires coefficient of variation (CV) of ≤10% at the 99th percentile of normal reference population. Our objective was to determine the analytical performance of this new assay, as well as to compare the STAT (9 minutes) reagent formulation to the standard Troponin T (18 minutes) reagent.

Materials and Methods: The analytical performance of the assay was evaluated for precision, linearity and analytical sensitivity. Precision was measured in accordance to CLSI EP5-A2 protocol by testing two quality control materials in three replicates over five days. Linearity was determined by the serial dilution of a high concentration of patient sample in triplicates and was demonstrated acceptable by the recovery of expected value within ±20% in all 5 serial dilutions. Method comparison was done against the standard Troponin T reagent using 98 serum samples spanning the analytical measuring range.

Results:

Within-Run Imprecision (CV)	2.3% at 22 ng/L, 0.5% at 191 ng/L
Total Imprecision (CV)	2.7% at 22 ng/L, 1.5% at 191 ng/L
Linearity	3 - 8600 ng/L
Dilution Recovery	97 - 100 %
Deming	y = 0.96x + 0.02 (n=98)
r^2	0.99

Conclusion: The analytical performance of the Elecsys TnT hs STAT assay was validated and found to be acceptable as per the manufacturer's claims. Comparison studies showed that the STAT reagent correlated well with the routine reagent formulation. In summary, with higher degree of precision and shorter assay duration, this new assay could aid the diagnosis of AMI with a faster turnaround time.

C-23

Distribution and clinical correlates of cardiac troponin I in the Framingham Heart Study using an ultrasensitive assay

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Background: The use of cardiac troponins to diagnose myocardial injury in the acute setting is well-established. With the advent of highly-sensitive troponin assays, it is now recognized that circulating troponins may be detectable in individuals who are not acutely ill. We sought to define the distribution and clinical correlates of cardiac troponins in rigorously-characterized, healthy individuals drawn from a large epidemiologic cohort, using a new "ultrasensitive" assay.

Methods: We measured high-sensitivity cardiac troponin I (Erenna cTnI, Singulex) in 3,450 Framingham Heart Study participants who attended a routine examination. The lower limit of detection for this assay is 0.78 pg/mL. A healthy reference sample was obtained by excluding individuals with prevalent coronary disease, heart failure, atrial fibrillation, diabetes, hypertension, obesity, valvular disease, left ventricular systolic dysfunction, pulmonary disease (abnormal forced expiratory volume in 1 second), and renal dysfunction (GFR<60 ml/min).

Results: A total of 1,159 subjects comprised the reference population (mean age 55, 60% women). cTnI was measurable in 73% of individuals. In multivariable linear regression, male sex (p<0.008) and increasing age (p=0.002) were associated with higher cTnI concentrations. On the other hand, cTnI concentrations were not associated with systolic blood pressure, body mass index, smoking status, total cholesterol, or HDL cholesterol. Reference limits were determined using a quantile regression approach (Table, values in pg/mL). The upper 99th percentile values ranged from 7.3 to 25.2 pg/mL in men (depending on age group), and 14.3 to 24.8 pg/mL in women. Sex pooled upper 99th percentile values ranged from 12.8 to 25.8 pg/mL.

Conclusions: Circulating cTnI is detectable in nearly three-quarters of healthy, middle-aged adults. "Normal" values of cTnI increase with age and are higher in men.

	Quantile regression reference limits for cardiac troponin I measured by an ultrasensitive assay					
	Males (M)			Females (F)		
Age,years	2.5th %ile	50th %ile	97.5th %ile	2.5th%ile	50th %ile	97.5th %ile
30-39	0.78	0.9	7.9	0.78	0.8	8.2
40-49	0.78	1.1	8.9	0.78	0. 9	8.4
50-59	0.78	1.2	9.9	0.78	1.0	8.7
60-69	0.78	1.4	11.1	0.78	1.1	8.9
70-79	0.78	1.6	12.4	0.78	1.2	9.1

C-24

Clinical Validation of the Diagnostic Accuracy of the Abbott ARCHITECT & i-STAT BNP Assays

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The demand for accurate measurement of B-type natriuretic peptide (BNP) is valuable in ruling in and assisting in the diagnosis of heart failure (HF). The objective of this study was to provide a clinical validation of the diagnostic accuracy of the Abbott ARCHITECT and Abbott i-STAT BNP assays in comparison to the Inverness Biosite Triage BNP assay. 994 patients presenting through the emergency room at Hennepin County Medical Center to rule out or rule in HF were enrolled. Waste plasma (EDTA) collected as part of the patient's emergency room evaluation was banked at -80C. BNP was measured along manufacturers' guidelines by all three assays. Electronic health records were reviewed for clinical diagnosis and demographic information. Patients with HF were classified along New York Heart Association (NYHA) classification stages I, II, III, and IV. The mean age of patients was 61 years, with HF age greater than no HF age patients (64y v. 58y). 52% of subjects were male. 40% of patients had a diagnosis of HF. Diagnostic accuracy for HF detection showed the following ROC areas under the curves and sensitivities and specificities: ARCHITECT 0.79

(95% CI 0.76, 0.81), sensitivity 77%; specificity 66%; i-STAT 0.78 (95% CI 0.75, 0.81), sensitivity 72%; specificity 71%; Triage 0.80 (95% CI 0.77, 0.82), sensitivity 78%, specificity 69%. The median BNP concentration for each assay was significantly associated with the severity of clinical symptoms as shown in the table.

In conclusion both the Abbott ARCHITECT and Abbott i-STAT BNP assays were clinically comparable to the Inverness Biosite Triage BNP assay in ruling out and for the diagnosis of heart failure in patients presenting to an inter-city emergency room.

BNP Results by NYHA Classification					
BNP, ng/L	No HF	NYHA I	NYHA II	NYHA III	NYHA IV
ARCHITECT	56	114	364	760	829
i-STAT	36	81	341	677	660
Triage	44	108	326	635	716

C-25

Development of an Immunoassay for BNP on the Dimension® EXL™ integrated chemistry system with LOCI® Module

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Background: Brain natriuretic peptide (BNP) is an established marker that aids in the diagnosis of congestive heart failure. It is a 32 amino acid polypeptide, with a 17 amino acid ring structure, produced by cleavage of the parent proBNP hormone following its release from cardiac ventricles. We describe the development and initial analytical performance of a monoclonal antibody-based immunoassay for measurement of BNP in patient samples on the Dimension® EXL™ integrated chemistry system with LOCI® Module.

Methods: This method is a one-step sandwich immunoassay based on LOCI technology. The LOCI assay model has developed as a homogenous, chemiluminescent technology that includes two synthetic bead reagents and a biotinylated monoclonal antibody fragment which recognizes an epitope located at the C-terminus of the BNP peptide. The first bead reagent (Sensibeads) is coated with streptavidin and contains photosensitive dye. The second bead reagent (Chemibeads) is coated with a second antibody specific for a second independent epitope in the ring of the BNP peptide and contains chemiluminescent dye. Sample is incubated with Chemibeads and biotinylated antibody to form a particle/BNP/biotinylated antibody sandwich. Sensibeads then are added and bind to the biotin to form a bead-aggregated immunocomplex. Illumination of the complex by light at 680 nm generates singlet oxygen from Sensibeads, which diffuses to the Chemibeads and triggers a chemiluminescent reaction. The resulting chemiluminescent signal is measured at 612 nm and is directly proportional to the concentration of BNP in the sample.

Results: The Dimension EXL BNP* method is a 10 minute method with an assay range of 5 - 5,000 pg/mL. The functional sensitivity (analyte concentration corresponding to 20% CV total precision) is less than 10 pg/mL. Repeatability (within-run) and within-lab (total) precision estimates were evaluated by following CLSI EP5-A2 protocol over a 20 day testing interval using patient pools and quality control materials. Repeatability and within-lab precision were less than 4.0 and 6.0% respectively across the range.

No significant interference (<10 % bias) was seen from lipemia (3000 mg/dL), hemoglobin (500 mg/dL), or icterus (20 mg/dL). Comparative results from patient samples representative of normal and abnormal levels of analyte were evaluated by Passing-Bablok regression and showed good agreement between this new Dimension EXL method and the Dimension Vista® BNP method. The regression statistics are as follows: Dimension EXL Method (Y-axis) versus Dimension Vista BNP Method (X-axis): Slope = 1.01, Y-Int = -6.4 pg/mL, r = 0.99, N = 109, range 0-4931 pg/mL.

Conclusion: The BNP method on the Dimension EXL integrated chemistry system with LOCI Module method has demonstrate excellent precision, sensitivity, and agreement with its comparative method.

* Product under development_Not available for sale

C-26

Measurement of Nt-proBNP with LOCI assay in heart failure patients

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Background: Natriuretic peptides are important biomarkers for the diagnosis, monitoring and prognosis of heart failure (HF). The circulating levels of Nt-proBNP (N-terminal pro-brain natriuretic peptide), one fragment derived from proBNP, can be measured by a broad spectrum of immunoassays. Recently, luminescent oxygen channelling assay (LOCI) has been developed for Nt-proBNP testing. LOCI assay is a homogeneous chemiluminescent immunoassay offering a higher sensitivity and a reduced turn around time of analysis in comparison to many other immunoassays. The aim of this study was to assess the reliability of LOCI Nt-proBNP assay in HF patients.

Methods: Nt-proBNP concentrations were measured at baseline in 71 patients with severe HF (females n=17; males n=54; NYHA II-IV; mean age: 68 ± 12 years; mean ejection fraction: 22 ± 7%; etiology: ischemic n=52, non ischemic n=19) with LOCI immunoassay performed on Dimension Vista analyzer (Siemens). Circulating levels of Nt-proBNP and BNP were also determined using conventional immunoassays.

Results: Within-run and between run imprecision for the LOCI Nt-proBNP were 5.4% and 6.8%, respectively. The LOCI Nt-proBNP was significantly correlated with the conventional Nt-proBNP ($r = 0.99$, $p < 0.0001$) and conventional BNP ($r = 0.94$, $p < 0.0001$) assays. Regression analysis using conventional Nt-proBNP as reference showed a slope of 0.95 and an intercept of 58.1 with LOCI Nt-proBNP assay. The kappa coefficient between the two Nt-proBNP assays was 0.94, indicating an excellent agreement between the results. The time to obtain the results with LOCI assay was reduced of 8 minutes in comparison to conventional immunoassay.

Conclusions: Our study shows that LOCI Nt-proBNP is closely related to conventional immunoassay and appears as a reliable assay. Moreover, LOCI technology offers the possibility to perform Nt-proBNP testing with a significantly reduced turn around time of analysis, allowing a faster delivery of results to the physicians.

C-27

High sensitive troponin I for identifying myocardial damage in ICU patients

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Background: Elevated cardiac troponin (Tn) concentrations are often detected in intensive care unit (ICU) patients even in absence of coronary ischemia or structural cardiac disease. Elevated Tn concentrations are associated with poorer evolution and outcome. However, given the suboptimal detectability of current Tn assays some ICU patients with myocardial damage and poor prognosis may remain undetected. The new, high-sensitive methods allow detection of Tn (hs-Tn) concentrations 5-10-fold lower than those detected with the current assays. We hypothesized that hs-Tn would be detectable in more ICU-patients by means of the high-sensitive methods than by using current methods and this may help to stratify the risk in such patients.

Patients, Methods: The study included 108 patients consecutively admitted to a general ICU; patients with clinical, electrocardiographic or biological evidence of coronary or structural cardiac disease were excluded. Blood samples were collected at ICU admission in lithium-heparinized tubes and TnT (4th generation assay, Elecsys 2010, Roche Diagnostics) and hs-TnI (STAT high-sensitivity Troponin, ARCHITECT i2000 SR, Abbott Diagnostics) were measured. To define myocardial injury we used, according to our routine clinical practice, the TnT value measured with a 10% imprecision (0.035 ug/L), whereas for hs-TnI we used the 99th reference percentile recommended by the manufacturer (22 ng/L) that can be measured with an imprecision <5%. According to the clinical picture, patients were classified as having systemic inflammatory response syndrome (SIRS), severe sepsis or septic shock.

Results: Mean age of the patients (38% female) was 58.9±17.5 years and the length of ICU stay was 10.3±9.8 days; 62% patients developed SIRS, 17.6% severe sepsis and 20.4% septic shock. ICU- and 28 day-mortalities were 9 and 13.4% in SIRS, 31.6 and 36.8% in severe sepsis and 40.9 and 50% in septic shock, respectively. hs-TnI (median (IQR), ng/L) was of 13.6 (6.9-68.3) in SIRS, 37.1 (9.3-543) in severe sepsis and 95.1 (17.6-630) in septic shock (p=0.01), whereas TnT values (ug/L) were 0.010 (0.010-0.026) in SIRS, 0.016 (0.010-0.182) in severe sepsis and 0.033 (0.010-0.227) in septic shock (not significant). TnT and hs-TnI concentrations were higher than the thresholds in 29 and 53% of patients, respectively. Neither TnT nor hs-TnI concentrations were statistically associated with ICU- or 28-day mortalities, as previously described. Nevertheless, elevated hs-TnI values were more frequent than elevated TnT values in patients who died: 53 and 61% of patients dying in ICU or after the 28-day follow-up, respectively, showed elevated hs-TnI values, whereas only 39 and 40% showed elevated TnT.

Conclusion: In non-coronary ICU patients, hs-TnI, but not TnT concentrations increased according to the severity of the disease which, in turn, was associated with higher mortality. Elevated hs-TnI values were more frequently observed in patients who died than elevated TnT values. Although confirmation in larger populations is required, these results suggest the high sensitive TnI assay is superior for identifying ICU patients with a very high mortality risk. The superiority is based on the capability of the high-sensitive assay to detect very low TnI concentrations with an appropriate analytical imprecision.

C-30

Evaluation of an investigation use high-sensitivity troponin I assay for risk stratification in the Heart Outcomes Prevention Evaluation (HOPE) study

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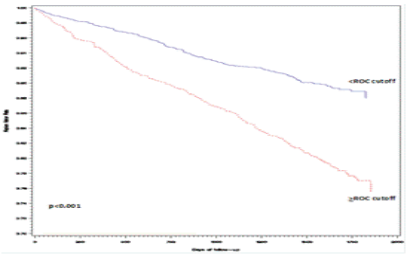
Background: High sensitivity cardiac troponin (hs-cTn) testing may be useful in identifying patients in the stable setting with known cardiovascular disease (CVD) at subsequent risk for an ischemic event. The majority of studies to date have assessed the ability of the hs-cTnT and not hs-cTnI assays for prognostication outside of the acute coronary syndrome setting. This study's aim was to assess the ability of an investigational-use hs-cTnI assay to predict future ischemic cardiovascular events in people with stable CVD.

Methods: Baseline serum specimens were measured with an investigational-use hs-cTnI assay (hs-cTnI IUO; Beckman Coulter; preliminary 99th percentile=10 ng/L) in 2572 participants from the Heart Outcomes Prevention Evaluation (HOPE) study. Briefly men and women ≥55 years of age with a history of coronary artery disease, stroke, diabetes, or peripheral vascular disease and at least one additional risk factor (e.g., smoking, hypertension, microalbuminuria, etc.) and without heart failure were enrolled in the study. Receiver operator characteristic (ROC) curve analysis was performed for hs-cTnI based on the primary outcome of the HOPE study (a composite of myocardial infarction, stroke, or cardiovascular death). Kaplan-Meier and Cox proportional hazard analyses were performed (mean follow-up 4.5 years) based on the ROC curve concentration. The coefficient of variation for the hs-cTnI assay over the course of the study was 14.7 % at a mean concentration of 14 ng/L (serum pool; n=86).

Results: The optimized concentration from ROC analysis was determined to be 6 ng/L. Subjects with concentrations ≥6 ng/L had a higher probability for primary outcome (Figure). After adjusting for important variables, the hazard ratio was 1.9 (95%CI:1.5-2.3) for those ≥6 ng/L as compared to the <6 ng/L group.

Conclusion: High sensitivity cardiac troponin I concentrations below the 99th percentile in patients with stable cardiovascular disease are of prognostic importance.



C-31

Pearl-Top Tubes are suitable for specimen collection for B-type natriuretic peptide measurements on Centaur CP and Triage meter instruments

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Background: Whole blood or plasma specimens, collected in EDTA tubes (purple-top), without serum separator are used to measure B-type natriuretic peptide (BNP). BNP is a marker for ventricular wall function and is used to identify patients with congestive heart failure. Plasma collected in tubes with no serum separator is more prone to contamination with intracellular organelles after long storage. However, use of EDTA tubes with serum separator gels such as pearl-top tubes (PTT) with EDTA, can provide a clean plasma sample, almost free of intracellular contamination. It has been shown that serum separator gels can effect the concentration of some analytes such as drugs in serum specimens. There has been no study to evaluate the use of PTT for BNP measurement. Thus in this study, we evaluated the use of PTT to measure BNP by Triage meter by Alere and Siemens Centaur CP instruments.

Methods: A total of 50mls of blood was collected in 5 EDTA tubes and 5 PTT (5ml/tube) from healthy volunteers (n=5, 2 males, 3 females). The study was approved by our institution's internal review board. BNP was measured in whole blood, plasma and serum, at 0, 4, 8, 12, and 24 hrs stored at RT or 4°C, on Alere Triage meter and Siemens Centaur CP instruments.

Results: The preliminary results show that there was no significant difference between BNP values generated from specimens collected in PTT or EDTA tubes that were stored in RT or 4°C, and analyzed by both methods. However, there was some decrement in stability of BNP in both tubes. Specifically, BNP concentrations from EDTA samples stored at RT were reduced after 24 hour storage when measured on Triage meter ($p<0.05$; conc. at 0hr vs. 24hr). The decrement in BNP stability in both PTT and EDTA specimens stored at RT and run on Centaur was significant ($p<0.004$ and $p<0.005$; EDTA vs. PTT, respectively).

Conclusion: Both PTT and EDTA tubes are suitable for collecting specimen to measure BNP on automated analyzers or Triage meter. Also, the stability of BNP in specimens collected in PTT and/or EDTA tubes and stored for 24 hours at RT is significantly reduced.

C-32

Natriuretic Peptides Are Associated With Social Deprivation And Subclinical Atherosclerosis

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Background: The natriuretic peptides have been shown to be independent predictors of cardiovascular risk in asymptomatic individuals, even with measured concentrations well below current cut-offs used in the diagnosis of heart failure, and when adjusted for classical cardiovascular risk factors. The association between social deprivation and cardiovascular risk is now well recognised, and is not adequately explained by classical cardiovascular risk factors. The contribution of natriuretic peptides to the socioeconomic gradient in cardiovascular risk has not been previously studied. The aim of this work was to determine if the natriuretic peptides are associated with social deprivation and subclinical atherosclerosis, and if they help to explain socioeconomic differences in cardiovascular risk.

Methods: B-type natriuretic peptide (BNP) and N-terminal proBNP (NTpro-BNP) were measured on plasma samples (n=490) from the Psychological, Social and Biological Determinants of Ill Health (pSoBid) study. This was a cross-sectional, population based study in which participants were selected on the basis of area-level deprivation. The study population consisted of approximately equal numbers of males and females, equal numbers from the two extremes of social deprivation and equal numbers in each age tertile from 35-64 years. Fasting blood samples were separated and frozen at -80°C within one hour of venepuncture. BNP was measured on Siemens ADVIA Centaur and NTpro-BNP on Siemens Immulite 2500. Ultrasound assessment of the carotid arteries for plaque presence and carotid artery intima-media thickness (cIMT) was performed on a Siemens Acuson Sequoia 512 scanner.

Results: BNP and NTpro-BNP were both higher in the most deprived (MD) compared to the least deprived (LD) participants: BNP (median (IQR)) was 15.5 (8.3, 30.3) pg/mL in MD and 11.1 (7.1, 21.2) pg/mL in LD ($p=0.0008$); NTpro-BNP was 51 (23, 96) pg/mL in MD and 26 (10, 54) pg/mL in LD ($p<0.0001$). On univariate analysis, both markers were associated with ultrasound indicators of subclinical atherosclerosis (common carotid intima-media thickness (cIMT) and plaque presence) ($p<0.04$ in all cases). On multivariate analysis, adjusting for age, sex and BNP or NTpro-BNP removed the significance of area level deprivation as a predictor of cIMT. However, deprivation remained a significant predictor of plaque presence after adjusting for age, sex and BNP or NTpro-BNP.

Conclusion: The natriuretic peptides are associated with subclinical atherosclerosis and social deprivation. The fact that BNP and NTpro-BNP remove the significance of deprivation as a predictor of cIMT suggests potential utility for these biomarkers in unravelling the factors underlying the socioeconomic gradient in cardiovascular risk.

C-33

Autophagy activity in macrophage invasion and cytokine secretion

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Background: Macrophages play a central role in the development of atherosclerosis. They facilitate the formation of atherosclerotic plaque by invading through the extracellular matrix (ECM) and secreting pro-inflammatory cytokines. The regulation of autophagy in macrophages was recently found to have significant impact on the disease progress. Autophagy is a lysosome dependent degradation pathway that can be activated by insufficient nutrient supply through the mammalian target of rapamycin (mTOR). The induction of autophagy through mTOR is mediated by an autophagy protein complex with two core components: Unc-51 like kinase-1 (ULK-1) and Atg13. The molecular linkage between autophagy and macrophage function in atherosclerosis is poorly understood. The objective of this study is to investigate the role of ULK1 complex in the invading capacity and cytokine secretion of macrophages.

Methods: Lentiviral sh-RNA mediated silencing of ULK1, Atg13, Atg5 (another key factor in autophagy), and green fluorescent protein (GFP, as control) was used to generate stable RAW264.7 macrophage cell lines named as RAW^{ULK1}, RAW^{Atg13}, RAW^{Atg5}, RAW^{GFP}, respectively. BD BioCoat™ Matrigel™ Invasion assay kit (BD Biosciences) was used to measure cell invasion. An equal number of the four stable cells were plated in the chamber inserts coated with Matrigel™ that closely mimics the basement membrane of ECM. Medium beneath the insert contains 10% FBS as chemoattractant. After 24 hours of starvation to induce autophagy, cells invaded through the membrane to the well bottom was washed and stained by 1% Toluidine blue. The staining was extracted and colormetric assay was used to determine the cell number. Macrophage cytokine secretion was measured using the cytokine panel kit (R&D system). An equal number of plated stable RAW264.7 cells were stimulated by lipopolysacharride (LPS) at 100 nM for 2 h. Cell culture medium was collected and was applied to the test panel membrane pre-coated with antibody against an array of cytokines in duplicates. The signal from the horseradish peroxisome conjugated with secondary antibody was captured by film and quantified.

Results: RAW^{ULK1} exhibited a significant reduction in the invasion ability compared to RAW^{GFP}. About 40% of reduction occurred with ULK1 knockdown in a significant range ($p < 0.05$). On the other hand, knockdown of either Atg13 or Atg5 reduced the invading activity to a lesser extent. Compared with GFP knocked cells without LPS stimulation, LPS stimulation induced the production of many cytokines including monocyte chemoattrantant protein-1 (MCP-1), macrophage inflammatory protein (MIP; MIP1α, MIP1β and MIP2) from the control cells. On the other hand, knockdown of either ULK1 or Atg5 suppressed secretion of these cytokines, among which three kinds of MIP (MIP1α, MIP1β and MIP2) were most significantly suppressed in secretion in comparison to those from the control cells.

Conclusion: ULK1 complex plays a critical role in macrophage function including invading capacity and cytokine secretion. The level of MIPs may serve as an indicator of altered autophagy activity in macrophages.

C-34

High-sensitivity (level 4) cardiac troponin T assay versus two guideline acceptable (level 1) troponin I assays and a clinically usable (level 1) troponin T assay in detecting early AMI presenters at Emergency Room

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Background: Cardiac troponins (cTn) are currently the markers of choice for diagnosis of acute myocardial infarction (AMI). However, a major limitation of standard troponin assays is the lack of sensitivity at presentation caused by a delayed increase of circulating levels. With the introduction of the new high-sensitivity cardiac cTnT (hs-cTnT) assay, may be possible to measure cTnT even in early AMI presenters. The aim of our study is to compare hs-cTnT versus two SIEMENS (Centaur Ultra and LOCI-VISTA) troponin I assays, and versus cTn T STAT 4º generation ROCHE assay.

Methods: Seventy six consecutive patients with suspicion of acute coronary syndrome (ACS) were included (sex: 27 females [37%]; age: median, 77 y; range: 26-93 y). Inclusion criteria were: 1. A negative (<0.10 ng/mL) Dimension RxL cTnI (routine method) value at presentation followed by a rising and/or falling pattern of cTn I as indicative of an evolving AMI, 2. time from onset of symptoms less than 4 hours, and 3. discharge diagnosis of ACS. Leftover plasma samples at presentation were frozen at - 30 C until measuring on ADVIA-Centaur and Vista (SIEMENS) platforms, and eCobas 411 (ROCHE) platform for cTnI and cTnT, respectively. 99th percentils were chosen as cutoff (ng/mL) for the 4 **Methods:** Roche hs-cTnT, 0.013; Roche cTnT STAT, 0.010; Siemens Centaur, 0.04; Siemens VISTA, 0.045, as reported in previous studies.

Results: The sensitivities (S) of the different methods in detecting a positive value of troponin at presentation were the following: Siemens VISTA, 17 out of 76, S = 22.4 %; Siemens Centaur Ultra, 22 out of 76, S = 29.3 %; Roche cTn T STAT, 16 out of 76, S = 21.1 %; Roche hs-cTnT, 57 out of 76, S = 75 %). hs-cTnT showed better sensitivity than the rest of methods ($p<0.0001$). The differences between the sensitivities of cTnT STAT assay and the cTnI assays were not statistically significant.

Conclusion: As expected, hs cTnT assay identifies more (nearly an additional 50% of negative Dimension cTnI patients are detected) early AMI presenters at presentation than guideline acceptable cTnI assays. However, since high sensitive assays also detect non ischemic elevations of troponin further studies are needed to elucidate the impact of using one of these methods at Emergency Room.

C-35

AQT90 Flex Troponin T compared with Modular Troponin T 4th and 5th generation

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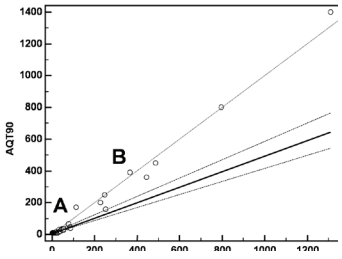
Background: The measurement of troponins (TnTs) is currently recommended for the biochemical diagnosis of myocardial cell injury. The new architecture of many Health Management Organizations requires the availability of Point-of Care devices that allow a measurement of TnTs not only rapid and analytically reliable but also consistent with the analyzers located in the main laboratory. The aim of the study was to compare the analytical performance of the new POCT AQT90 Flex analyzer for cardiac troponin T with Modular Troponin T 4th (TnT4) and 5th generation assays (TnT5).

Methods: We measured troponin T in 80 consecutive plasma samples received by the LRR of Ravenna in the January 2011 using AQT90 Flex analyzer (Radiometer Medical, Aps, Denmark); TnT4 and TnT5 (Modular E170 analyzer, Roche, Mannheim, Germany). We analyzed the results according CLSI EP9A3 standard using the softwares Statis Pro (CLSI, Wayne, USA), Medcalc (Mariekerke, Belgium) and Validator (Marquis, Metz, France).

Results: AQT90, TnT4 and TnT5 yielded respectively the following **Results:** lowest value: 10 ng/L, 10 ng/L and 3 ng/L; highest value 1400 ng/L, 1250 ng/L and 1310 ng/L; mean: 74.55 ng/L , 71.57 and 83.85 ng/L; Standard deviation: 195.47 ng/L, 181.98 and 188.90 ng/L; median: 10.5 ng/L, 10 ng/L and 23 ng/L. Comparison data yielded the following **Results:** Passing-Bablok regression analysis:AQT90= 1.2500 + 1.1250 TnT4; correlation coefficient= r : 0.959 (95% confidence interval 0.9244-0.9786). AQT90= 5.1026 + 0.4872 TnT5; AQT vs TnT4 correlation coefficient= r : 0.994 (95% confidence interval 0.9244-0.9786); AQT vs TnT5 correlation coefficient= r : 0.959 (95% confidence interval 0.990-0.996). Figure shows that the regression analysis of AQT90 TnT vs TnT5 in values < 100 ng/L (A) is different from that in 200-400 ng/L interval (B).

Conclusion: The TnT results yielded by AQT90 are consistent not only with those of TnT4 but also with those of TnT5.



C-36

Label-Free Detection of Cardiac Markers in Human Serum Analyzed by Guided-Mode Resonance Biosensor

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1. Background Cardiac makers such as cardiac troponin I (cTnI), creatine kinase MB (CK-MB), and myoglobin (MYO) are very useful tools for diagnosing acute myocardial infarction (AMI).[1] Presently, hospital laboratories use immunoassays

with various methods for labeling to detect cardiac makers such as cTnI, CK-MB, and MYO, but this type of analysis needs more time than label-free detection of the direct interaction between antigen and antibody without the detection antibody. A guided-mode resonance (GMR) biosensor has been accepted as a powerful technique to monitor reflection processes at the interfaces in different chemical and biological research areas.[2] We report the detection of cardiac markers in human serum samples by using a guided-mode resonance biosensor.

2. Methods We have fabricated the guided-mode resonance filter (GMRF) using plastic based nano-imprinting method, and immobilized the antibody on the GMRF for detecting cardiac markers. After imprinting, high refractive index material was deposited on the nanometer-scale grating structure using PECVD technique in order to create the guided mode. The deposited Si_3N_4 layer was treated by oxygen plasma to form the hydroxyl group (OH) for chemical conjugation between antibody and Si_3N_4 surface. The antibody immobilization process on the GMRF surface was performed by self-assembled monolayers (SAMs) formation, surface aldehyde formation, sequentially. Finally, a GMR biosensor chip was fabricated by immobilization of the antibodies of cardiac markers on the GMRF surface. The specific binding between the antibody immobilized on the GMR biosensor chip and the antigen in the human serum took place as the human serum was inserted.

3. Results We measured the relative shift of peak wavelength between the initial peak wavelength and peak wavelength after 30 min for the various concentrations of antigens. Dose-response curves ranging from 0.05 to 10 ng/mL for cTnI, from 0.1 to 10 ng/mL for CK-MB, and from 0.03 to 1.7 μg/ mL for MYO were obtained. The shift of peak wavelength was increased as the concentrations of cardiac markers were increased. The limits of detection (LOD) of cTnI, CK-MB, and MYO were estimated to be 0.05, 0.1, and 35 ng/mL, respectively. These results can apply to AMI screening as the boundary concentration between a healthy people and a patient for cTnI, CK-MB, and MYO are 0.05, 3.6, and 92 ng/mL, respectively.[3] The coefficient of variation (CV) from mean and standard deviation (SD) was below 20%.

4. Conclusion GMR biosensor chips were evaluated for the analysis of cTnI, CK-MB, and MYO concentrations in human serum to diagnose cardiac disease. We are able to distinguish between normal and elevated levels of these antigens in human serum samples because the LOD are less than the boundary concentrations between a healthy person and a patient. GMR sensor technology will be useful in developing low-cost and portable biosensors that can screen for cardiac diseases.

References (1) Bhayana, V. *Lancet* 1993, *342*, 1554. (2) Hong, J.; Kim, K.-H.; Huh, C.; Sung, G. Y. *OPTICS EXPRESS* 2007, *15*, 8972-8978. (3) Burtis, C. A. *Tietz Textbook of Clinical Chemistry and Molecular Diagnostics* 4th ed.; Elsevier Saunders, 2006.

C-37

High Sensitive Cardiac troponin T measurements for predicting short-term serious cardiac outcomes in emergency department patients with suspected acute coronary syndrome

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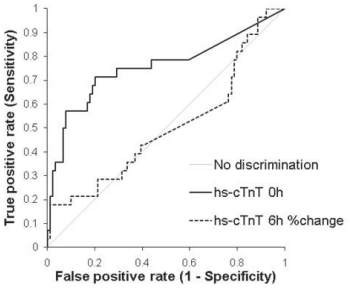
Background: Recent data using the high sensitive cardiac troponin T assay (hs-cTnT) suggest improved specificity for the diagnosis of non-ST-segment myocardial infarction when change criteria are employed (*Clin Chem* 2010;56:642-650). Our objective was to determine hs-cTnT capability to predict short-term serious cardiac outcomes in patients presenting with suspected acute coronary syndrome (ACS).

Methods: We used the Roche Elecsys hs-cTnT assay on stored serum specimens (below -80°C) from 117 emergency department patients with suspected ACS obtained at presentation (0h) and 6 hours later (6h). The clinical endpoint was a composite of death, myocardial infarction, congestive heart failure, serious arrhythmia and refractory ischemic cardiac pain that required hospitalization, at 72 hours. We used receiver operating characteristic (ROC) curve analyses to evaluate the hs-cTnT performance at 0h, 6h, and for the relative (%) change between 0h and 6h (6h %change).

Results: The hs-cTnT concentrations were higher in those with the composite outcome versus without (median [25th-75th percentile] hs-cTnT: 39 (8-75) ng/L vs. 4 (<3-11) ng/L at 0h, and 42 (8-140) ng/L vs. 5 (<3-13) ng/L at 6h; both p<0.001). There were no significant differences in the hs-cTnT concentrations between 0h and 6h time-points in those with (p=0.75) or without outcomes (p=0.87). The ROC curve comparison between 0h and 6h time-points showed the same area under the curve (AUC=0.76 [95%CI:0.64-0.88]). When the % change was applied, the AUC was 0.50 (95%CI:0.37-0.63), and the test performance was inferior compared to the 0h (p=0.004) (Figure).

Conclusion: Our preliminary data suggest that hs-cTnT performs similarly at presentation and 6 hours later in those patients presenting with suspected ACS in the emergency setting to predict a serious short-term cardiac outcome. Change in serial measurements may be less informative for diagnostic endpoints other than myocardial infarction.



Wednesday AM, July 27

Poster Session: 10:00 am - 12:30 pm
Automation/Computer Applications

C-39

Workflow and Turnaround Time Improvements in a High Volume Hematology Department

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Background: The challenges facing laboratory managers over the past twenty years include staff shortages, the rising cost of providing testing services, and the significant number of error opportunities. Responding to these challenges, Saint Francis Health System implemented a series of improvements to include faster, more sensitive analyzers, high volume lab automation, and new management approaches through a continuous improvement philosophy. The most recent addition was the Abbott High-Efficiency Hematology (HEH) system implemented in November of 2010.

The Abbott HEH system integrates the Abbott CELL-DYN™ Sapphire, Aim Lab Pathfinder™, and the IMSolutions™ middleware products. Together this Abbott HEH system was designed to ease the manual labor requirements on the medical technologists while improving the overall workflow and laboratory turnaround times.

Methods: In order to understand the effectiveness of HEH, a detailed study protocol was developed that would use Lean methodologies, time studies and existing metrics to objectively measure performance. Prior to HEH implementation, this benchmarking exercise was performed to document the pre-installation turnaround times and manual time requirements. Implementation and integration of the HEH system occurred in November 2010, and after the new process was familiar to the medical technologists in the lab, a "post-implementation" study was performed so that the impact of Abbott's HEH system could be objectively evaluated.

Results: The benchmarking phase revealed that the hematology department processed approximately 1,200 CBCs per day with a differential rate of 17 percent. The urine volume was approximately 350 tubes per day and was handled outside of the lab automation line. Depending on the time of day, the hematology area was staffed by 3 to 6 medical technologists and the CBC turnaround time goals were generally being met.

The improvements from Abbott's HEH system included a decrease in pre-analytical turnaround times from 57 to 21 minutes during the busy time periods. Overall, the turnaround time metrics also generally showed an improvement with routine CBC turnaround times decreasing 28 percent and other metrics improving by 2 to 21 percent. Specifically, the routine CBC turnaround time went from 27.9 minutes on average (with a 0.3 CV) to 20.2 minutes (0.23 CV). The routine TSH turnaround time went from 53.2 minutes on average (with a 0.14 CV) to 41.8 minutes (0.23 CV).

The HEH system also impacted the manual time required by the technologists by allowing the registering of urine tubes that could not previously by handled by automation. This resulted in a savings of almost 70 minutes per day. Another "hands on" benefit was that the system allowed normal sample results to be auto-resulted in a more efficient manner. This saved 140 minutes per day in manual labor time and decreased the opportunity for errors.

Conclusion: Responding to various macroeconomic challenges, Saint Francis was able to continuously improve their laboratory operation through various means, the most recent improvement being the Abbott HEH system. The system has important benefits such as automated sample handling, auto-release functionality and, if integrated into the lab correctly, it can help improve lab turnaround times and reduce manual labor.

C-40

Integration of a Multisite Enterprise Quality Control Program on a Wide Area Network and Use of Sigma Statistics to Standardize Westgard QC Rules

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The objective was to create an efficient infrastructure to electronically integrate quality control (QC) across a multisite enterprise so that QC data for 15 common chemistry analytes could be compared at nine different sites (2 "H"ospital; 7 "R"apid "R"esponse labs) utilizing four discrete chemistry analyzers (Roche Diagnostics, Indianapolis, IN - P modular (H1); c501 (H2); I400 (RR1-4); and c311 (RR5-7). Sigma statistics from the 9 sites could then determine if method performance was similar enough to apply shared test-specific run acceptance and rejection rules.

Harmonized reagent systems and shared lots of calibrator and QC material (BioRad Labs, Hercules, CA) were employed. QC results interfaced to a Sunquest LIS with standardized test codes and a common Westgard rule set (1_{2s}, 1_{3s}, 2_{2s}, 2_{3s}, 4_{1s}). Monthly data passed from the LIS to BioRad Unity and external peer QC net client servers. Peer comparison statistics (e.g. bias from peer means) were used to calculate site sigmas. Total allowable error was taken from CLIA or CAP proficiency testing limits. Representative sigma statistics for 4 tests with two QC levels tabulated across the enterprise were:

Test	H1	H2	RR1	RR2	RR3	RR4	RR5	RR6	RR7
Alk Phos	8.9	12.8	9.4	11.5	10.3	6.4	11.9	7.4	7.3
	12.9	15.1	21.5	26.5	15.5	9.0	20.0	17.6	11.1
Glucose	3.7	6.7	6.3	9.1	7.6	5.1	8.9	6.0	4.5
	4.7	7.9	7.9	11.1	5.8	6.4	9.2	8.1	5.2
Potassium	8	7.4	13.2	12.2	15.2	6.4	10.9	9.1	8.0
	7	7.4	8.0	9.2	8.7	5.3	8.5	7.2	5.3
Sodium	3.8	2.8	1.8	2.8	3.5	2.4	3.7	3.4	1.9
	3.8	3.1	2.7	1.5	3.6	2.3	3.4	3.0	1.7

Monthly sigmas showed that tests such as ALKP consistently exceeded 4 sigma; tests such as glucose commonly exceeded 4 sigma with occasional outliers <4; and tests such as sodium consistently yielded a sigma <4. Using Westgard OPspecs charts with >4 sigma values, a less stringent Westgard 1_{3s} rule set was applied across the enterprise to several tests. Tests with sigma <4 were maintained with a full Westgard rule set.

We conclude that an enterprise method of QC standardization may be extended with sigma analysis to differentiate those analytes that perform consistently >4 sigma from those <4. Further, individual outlier sites can have more stringent rules applied as needed. Analytical QC rules may thus be selectively assigned to analytes that require different levels of error detection.

C-41

An Evaluation of the Automated Analysis of Urinary Casts, Crystals and Organisms on the Sysmex UF1000i

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Background: Urine microscopy is a routinely ordered test to screen for urinary tract infections and renal abnormalities. A study was conducted in Khoo Teck Puat Hospital, a 550-bed acute care hospital, to evaluate the Sysmex UF1000i [Sysmex Corporation, Kobe, Japan] fluorescence flow cytometer in automating urine analysis. While microscopy remains the gold standard in urine formed elements analysis, the methodology is labour-intensive, time-consuming and fraught with variability in technique due to operator differences. By automating this process, we aim to increase analytical productivity and ensure consistency in our reporting.

Methods: We collected 282 random urine collections from both healthy and diseased patients and analyzed them concurrently, using the conventional manual microscopy method and automated flow cytometry. The fresh mid-stream urine samples were collected and promptly sent to the laboratory for examination. The UF1000i uses the principle of fluorescence flow cytometry. The manual method is performed by concentrating 10 ml of each urine sample to 1 ml after centrifugation at 1500rpm for 5 minutes, charged to a Kova slide and analyzed by a technician under 40x magnifications on the microscope. Results were analyzed using frequency analysis, quantitative and semi-quantitative comparisons. Observations were made on the type of casts and crystals found and its influence on quantitation.

Results: We found good correlation between the automated quantification of bacteria and semi-quantitative microscopic observations on the UF1000i. We noted that operators had a tendency to misclassify yeasts as bacteria and vice versa. After excluding these outliers (n=20), a log-linear relationship was evident. Our findings indicated that the cut-off values of 2 casts per microlitre and 4 crystals per microlitre were appropriate. At the cut-off value of 2 casts per microlitre, the sensitivity and specificity were 78.3% and 56.9% respectively, with a negative predictive value of 77.8%. At 4 crystals per microlitre, the sensitivity and specificity were 79.7% and 91.0% respectively and yielded a negative predictive value of 94.4%. For the analysis of bacteria, sensitivity and specificity was 82.5% and 50.0% respectively at a cut-off of 20 bacteria per microlitre, with a negative predictive value of 67.4%. For yeast-

like cells, a cut-off of 10 yeast-like cells per microlitre yielded a sensitivity of 71.3% and specificity of 72.8%, with a negative predictive value of 70.7%. An imprecision study performed using quality control [QC] material yielded Coefficients of Variation for Casts and Bacteria of 16.7% and 7.4% at the low QC level and 19.69% and 5.82% at the high QC level, respectively. These values fall within the manufacturer's recommendations of less than 40% and 10%.

Conclusion: Microscopic examination of urinary formed elements remains the gold standard analysis method. However, its use is compromised by operator variability. Our study shows that automated urine analysis on the UF1000i is a good surrogate for the quantification of bacteria, yeasts, casts and crystals. However, microscopy is still required for the further elucidation of cast and crystals identification and can be an adjunct in confirming findings for these two elements.

C-42

Disease Management Panel Program: Cost Effective Patient Screening in Asymptomatic Adult Individuals

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Background: The association between elevated blood cholesterol and cardiovascular disease as well as the long term consequences of diabetes mellitus is well known to both the medical community and the general population, yet many patients are not adequately screened for these serious public health issues. Because many patients would otherwise not seek out screening for these conditions, admission to the hospital for unrelated conditions presents an excellent opportunity for at risk population screening and education. To address the need for increased patient screening, a Disease Management Profile (DMP) was implemented at Dartmouth-Hitchcock Medical Center in 2005. Included in the DMP are a fasting glucose, hemoglobin A1c (HbA1c) and a lipid panel (Total Cholesterol, High Density Lipoprotein Cholesterol, triglycerides and a calculated low density lipoprotein cholesterol). In an effort to constrain cost and prevent unnecessary laboratory testing two strategies were employed. The first was the incorporation of a questionnaire as part of the DMP screening process, that limits testing to only those patients with any of the following: a known family history of diabetes mellitus, a preexisting diagnosis of cardiovascular disease, age greater than or equal to 45 or obesity. The second strategy involved the creation and implementation of a Cerner Command Language (CCL) program (LIS: Cerner Corporation, Kansas City, Mo.) to query the patient record for DMP orders or for the individual DMP components within the previous 12 months. Previous results for the entire DMP or either the HbA1c or lipid panel results in DMP or individual DMP component cancellation.

Objective: The objective of this study was to review the performance of the CCL program and its utility in guiding appropriate test utilization for screening an at risk patient population.

Methods: In order to measure the effectiveness of the CCL program we took a retrospective look at the number of DMPs ordered from July of 2008 until January of 2011 through the use of a secondary CCL program. Data analysis included total number of DMPs ordered as well as the number of HbA1c tests automatically canceled by the CCL program.

Results: For the eighteen month period investigated a total of 6,713 DMPs were ordered by our providers. Of the 6,713 DMPs ordered 31.1% or 2,091 had the HbA1c automatically canceled by the program due to a previous HbA1c value in the patient record within the previous 12 month period.

Conclusions: The development and use of a unique CCL program has permitted the laboratory to continue its support of the DMP process while reducing superfluous testing and constraining cost to the hospital and patient.

C-43

The Use of Mehtod Evaluation Protocols

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Background: Evaluation Protocols are necessary for all types of laboratory test systems used in the clinical laboratory and manufacturer settings. The CLSI documents are used throughout the world on a daily basis to address the areas of evaluation protocols, which include linearities, precision, trueness, carryover, drift, comparability, reference interval, and limit of detection. Software has been developed and is available from CLSI that incorporates the 7 protocols and can address the difficult and time-consuming task that laboratorians face when performing these evaluations. The use of CLSI developed software ensures automatic calculations and alignment with CLSI guidelines. Demonstration of compliance with accreditation requirements for verification and validation of clinical laboratory assays is simplified with the use of this software.

Methods: Two of the 7 CLSI evaluation protocols incorporated into this software are used for calibration verification procedures. These documents are (1) *User Verification of Performance for Precision and Trueness; Approved Guideline_Second Edition* (EP15-A2) for verifying the accuracy of the calibration using 3 levels of reference materials, and (2) *Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline* (EP06-A) for proof of linearity across the reference range.

Results: In the example shown, the StatisPro software is used to perform the linearity portion of the calibration verification of Busulfan using LC-MS/MS methodology at Johns Hopkins University Clinical Reference Laboratory in Baltimore, Maryland. The study design and data input fields were simple and easy to use enabling report generation and sign-off efficient and organized as demonstrated by this table.

Conclusions: The StatisPro software developed to faithfully implement 7 CLSI method evaluation guidelines is easy to use and satisfies the laboratory's need for various protocols that meet regulatory and accreditation requirements.

Linearity (CLSI EP06-A) Busulfan (ug/mL) At Johns Hopkins Laboratory								
Level	Replicates	Expected Value	Mean	Mean SE	Linear Fit	Allowable Nonlinearity	SD	CV
1	2	0.039	0.0440	0.00100	0.0417	0.002	0.0014	3.20%
2	2	0.078	0.0900	0.00200	0.0832	0.0039	0.0028	3.10%
3	2	0.156	0.1680	0.00600	0.1662	0.0078	0.0085	5.10%
4	2	0.3125	0.3370	0.17000	0.3326	0.0156	0.0240	7.10%
5	2	0.625	0.6340	0.01000	0.6649	0.0313	0.0141	2.20%
6	2	1.25	1.3640	0.06500	1.3296	0.0625	0.0919	6.70%
7	2	2.5	2.6320	0.10200	2.659	0.125	0.1442	5.50%
8	2	5	5.3260	0.11800	5.3177	0.25	0.1669	3.10%
Pooled	16						0.0851	4.80%

C-44

Automated analysis of blood cells in cerebrospinal fluid and other body fluids

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Background: Routinely, determination of cell counts in body fluid is performed by microscopic examination. Conventional methods for the cytological analysis of body fluids samples require manual chamber counting of red and white blood cells (WBC) and leukocytes differentiation using a cytocentrifuged and stained preparation. This is a time-consuming procedure, which is also subjective and prone to interoperator variability and low precision. The objective of this study was evaluate the performance of the automated global and differential cell counts in several body fluids compared to the microscopic analysis.

Methods: We studied, 216 samples (112 cerebrospinal fluids, 74 ascitic fluids, 30 pleural fluids). All samples were analyzed up to 2 hours after collection. The standard routine laboratory included manual erythrocytes (RBC) and WBC total and differential counts (cytocentrifuged air-dried hematological staining of May-Grunwaldt) and automated total and differential cell counts (Sysmex XE-5000). Paired Student's t-test and simple linear regression (least square method) were used to evaluate the correlation between both methods.

Results: The automated WBC and RBC counts were highly correlated with that of the microscopic reference method ($r \geq 0.90$ in all cases). Considering pleural and peritoneal fluids, a good agreement between both methods was also observed for mononuclear cells ($r=0.86$) and polimorphonuclear cells ($r=0.88$). Despite these good correlations, polimorphonuclear cells showed a significant reduction of the percentages obtained by the Sysmex XE-5000 compared to manual method. This reduction is probably due to changes of size and shape of these cells, which are frequently observed in these fluids. High fluorescence cells > 2.0 / 100 WBC suggest the presence of macrophages and/or mesothelial cells and/or neoplastic cells. On the other hand, for CSF samples, despite the high correlation for the entire range of WBC, at the low end

of the data spectrum (WBC≤5mm³) a weak correlation was observed. In this range, 20% of the samples were misclassified as abnormal by the automated analyzer and polimorphonuclear (PMN) and mononuclear cell (MN) counts is of limited value. Despite the good correlation for WBC>5/mm³ (r=0.91), PMN are overestimated and MN are underestimated by the automated analyzer (p<0.001).

Conclusion: Automated RBC, WBC and differential leukocytes counts lead us to two different situations depending on the origin of the samples. For pleural and peritoneal fluids, as the results show good correlation with the standard method, the use of this automated analyzer has the potential of reducing the time to report a preliminary result to the clinician. Based on the results obtained for CSF samples, most patients were correctly classified as normal or abnormal, however a careful review of the results is still mandatory. The results observed at the low end of the data suggest that larger studies may be necessary to determine the need of a new reference range for automated CSF WBC counts.

C-45

Laboratory Efficiencies when Using the Roche cobas p 701 Post Analytical Unit

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Introduction: The Automated Laboratory receives, processes, analyzes, and stores an average of 4750 Chemistry, Hematology, Coagulation, Immunology, Infectious Disease, A1C, and Electrophoresis blood samples per day. In our laboratory, it is essential to have a reliable system to store specimens in a controlled refrigerated environment. Our post-analytical specimen storage solution must also be able to perform the time consuming task of locating and retrieving 150+ daily requests for additional testing on previously drawn specimens. We evaluated the Roche Diagnostics cobas p 701 post analytical stand-alone unit for its specimen storage and retrieval functionality. In addition to providing a robust and reliable storage and retrieval system, the cobas p 701 post analytical unit provides our laboratory many more additional efficiencies.

Objectives: This study looks to evaluate the efficiencies gained when using the cobas p 701 post analytical unit in our laboratory setting. Our expectation was to see time saving efficiencies regarding specimen storage and add-on specimen retrieval, however, we discovered several other benefits when using the post-analytical unit.

Methods: We compared the amount of time required from add-on specimen order receipt to refrigerated storage over selected days prior to and after the use of the automated storage system. Second, we compared the time saving in eliminating manually restoppering specimens after analysis. Third, we compared the efficiencies of the automated specimen location and retrieval process. Fourth, we compared efficiencies in specimen disposal. Fifth, we compared the efficiencies in refrigerated space and finally we assessed the quality of temperature storage.

Results: The time frame spanning specimen receipt, analysis and specimen placement into refrigerated storage was averaged over four days prior to the use of the system and was determined to take 4 hours, and 31 minutes. After the system was in use, a three day average was reduced to 2 hours and 29 minutes. On a typical day, manually capping 1226 specimens required 2 hours of Technologist time verse no time spent manually capping with the use of the system. On a typical week day 5000 expired specimens are disposed averaging 45 minutes of Technologist time compared to less than 10 minutes with the use of the system. The refrigerated storage footprint has been reduced by 50% with the use of the automated storage and retrieval system. The system maintained 2 - 8 °C 99.98% of the time during a 12 week observation time.
Conclusion: The cobas p 701 post analytical unit is a robust, reliable, and efficient specimen storage solution. Additionally, the system reduces the time of specimen location and retrieval, manual capping of specimens, and manually disposing of expired specimens, minimizing the exposure to specimens in a greatly reduced footprint. The system also reduces the amount of time specimens are exposed to room temperature and better maintains the refrigerated temperature for specimens in our laboratory, thus providing improved specimen quality.

C-46

A Simple, Practical And Profitable Tool For Validation Of New Analysers In An Accredited Laboratory

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Background: The objective of this work is to validate two new biochemistry analyzers versus a previously accredited one with a procedure consisting of the application of both EP-9 and EP-10 CLIA guidelines according to the quality standard --- ISO 15189 : 2007

Methods: Plasma samples (Lithium heparin) from 50 patients were used to perform sequential measurements of glucose, Na, K, Cl, urea, creatinin, calcium, magnesium, albumin, total proteins, CK, CKMB,ALT, amylase, total bilirrubin, Troponin I and lactate, processed in the Dimension EXL and RXL analyzers in a 15 days time-frame, according to the EP-9 guideline. Outliers and bias paired results were searched for using linear regression analysis and Bland-Altman plots. The acceptance criterion was to get a bias lower than quality specifications defined in the accredited laboratory (ENAC 630/LE 1377). In addition, the EP-10 guideline was followed to check the imprecision, proportional and constant bias, linearity, carry-over, and drift of these parameters in the new EXL analyzers.

Results: All parameters studied fulfilled the EP-9 guide. Correlation coefficients were excellent (r>0,98) for all parameters, except for Na (0,970)and CKMB (0,972). These parameters showed a interval of results that made it difficult to obtain good correlation coefficients. In these cases more attention was paid to the Bland-Altman plots, which showed less than 4% of the data over the 3 standard deviations. Moreover, all parameters fulfilled the EP-10 guide. Results showed a statistically significant difference (p=0.01) for slope and non-statistically significant difference for linearity, carry over and drift. All results fulfilled the criteria of imprecision and systematic bias, which had been indirectly shown with the EP-9 guide.

Conclusion: This work shows a simple, practical and profitable way of validating new analyzers. Common statistical methods are used and it has to be recalled that when some parameters show a narrow interval of results, it makes it very difficult to obtain good correlation coefficients. In these situations (Na and CKMB, in our study) it seems appropriate to pay more attention to de Bland-Altman plots to check the agreement degree between the different analysers results. Furthermore, it is convenient to emphasize that a good correlation coefficient does not imply that the results between both methods are interchangeable. In summary, we recommend this procedure as a suitable way to validate new analyzers according to the accredited Laboratories requirements.

C-47

Assessment of Turn around Time (TAT) Improvement with Implementation of Roche Modular Pre-Analytic (MPA) Plus System

R. Ybabez, N. Baumann, C. Yoch, S. Cedotal, L. Jonsgaard. *Mayo Clinic, Rochester, MN,*

Objective: To assess whether implementation of Roche MPA and Modular automation improved TAT of routine chemistry and immunoassay tests in a high-volume clinical core laboratory.

Background: Clinical core laboratories can gain efficiencies in staffing and turn around time (TAT) by utilizing automation. Additionally, many of the pre-analytic steps in the clinical laboratory can be accomplished using automation. Prior to automation, our laboratory practice for handling most chemistry and immunoassay samples was manual processing (centrifugation, aliquoting, routing) and manual loading on to instruments for testing. The implementation of the Roche Modular Pre-Analytic (MPA) Plus System allowed for automated processing and aliquoting of samples. The expectation was an overall improvement in turn around time for routine chemistry and immunoassay testing.

Methods: Turn around time (TAT) data were collected for six months (Feb-Aug) pre- and post-implementation of the Roche MPA and Modular analytics. Data were collected from a random sampling of chemistry (Electrolyte Panel [ELPN], n=930) and immunoassay (Troponin T [TPNT] n=12425, and Thyrotropin Stimulating Hormone [TSH] n=966) tests performed during the specified time interval. Laboratory information system (LIS) reports were generated to obtain the TAT from sample collection to result reporting. ELPN and TPNT testing was performed on Roche Modular analyzers (D mod and E mod, respectively). TSH samples were centrifuged

and aliquoted by the Roche MPA prior to testing on a stand-alone immunoassay analyzer.

Results: TAT for ELPN was 83 +/-32 mins prior to implementing automation and 63 +/-26 mins after implementation of automation (23% improvement). TPNT TAT pre- and post-automation was 87 +/-7 mins and 78 +/-20 mins, respectively (10% improvement). TSH TAT was 149 +/-50 mins before implementation and 123 +/-35 mins after implementation of the Roche MPA (18% improvement).

Conclusion: In conclusion, there was improvement in mean turn around times for all chemistry and immunoassays tests monitored after implementing automation in the laboratory. Comparison of pre- and post-automation data showed a 10-25% improvement for the noted tests. The MPA automated system also improved TAT for tests that are performed on stand-alone platforms not connected to the automation (e.g. TSH) by reducing the pre-analytical processing time. Overall, the TAT improvement can be attributed to the practice change from manual processing and loading onto analyzers to the usage of Roche MPA automation system with Modular analytics.

C-48

Comparative Analysis of Physical and Digital Representations of Immunofixation Electrophoretic Gel Patterns

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Background: Examination of digital representations of physical specimens is an increasingly attractive option for the modern laboratory due to ease of data storage, remote evaluation and the potential for image enhancement. One such technique where electronic imaging may be of use is serum immunofixation electrophoresis (IFE), where electronic representations of physical gel could be potentially utilized for analysis and data storage. Sebia has recently released a new gel scanning system, GELSCAN. A validation of clinical utility of gel analysis using digital representation was undertaken.

Methods: In the present study, a comparison of the number of observed protein bands identified on physical IFE gels and corresponding digital gel representations was performed. Fifty three IFE gels were prepared using Sebia HYDRASYS HYDRAGEL kit from randomly selected myeloma patient serum samples. The physical gels and digital gel representations were reviewed independently by five experienced medical directors. The digital images were generated and visualized by three different **Methods:** A) scanned on an Epson Perfection V700 photo scanner and visualized using the Sebia Phoresis software (v.6.1.2) (designated Epson), B) scanned on a Sebia GELSCAN and visualized by Phoresis software (v 7.4.5) (designated Phoresis), and C) scanned on a Sebia GELSCAN with an image bitmap and visualized by Microsoft picture viewer (designated Bitmap). All observed distinct and indistinct bands (not limited to monoclonal protein bands) observed on the original gel and the three digital representations were recorded by each observer.

Results: The mean (±S.D) absolute number of bands/gel observed directly on the physical gels was 1.03(±0.19). There were significant inter-observer differences in absolute number of bands seen. In order to normalize differences in absolute number of bands seen between observers, mean intra-observer ratios of (observed bands on image representation)/(observed bands on physical gel) were generated. The mean intra-observer ratios were 0.71(±0.11) with the Epson scanner, 0.99(±0.16) with Phoresis, and 1.04(±0.14) with Bitmap. Significant difference ($p<0.05$), was established by ANOVA between the intra-observer rations of the physical gel and Epson imaging method, but not between the examination of the physical gel and other two imaging methods utilizing the Sebia GELSCAN.

Conclusions: Physical examination of gels yielded differences among observers, which is likely due to variation in visual acuity and/or interpretation of what constitutes a protein band. However, the relative number of bands seen for each observer using different imaging methods versus direct gel examination was remarkably consistent among the observers and is likely due to differences in the quality of the gel representation. While significantly fewer bands were observed using the Epson scanning system as compared to physical gels, the relative number of bands seen utilizing Sebia GELSCAN representations and the physical gel were similar for all observers.

This study indicates that band representation by digital images of IFE gels scanned by the Sebia GELSCAN are comparable to visual evaluation of the physical gel and therefore may be suitable for diagnostic purposes and data storage. Similar studies should be undertaken to evaluate any digital imaging system for suitability in clinical practice prior to implementation.

C-49

Effects of modest central selection bias on parametric reference range determination from a normal distribution: an example by simulation

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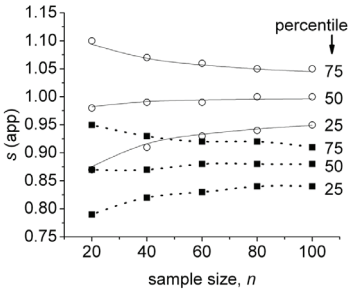
Background: Reference range determination may be adversely affected by inadvertent selection bias in the population used for study. For instance, selection of patients having no abnormal results on a comprehensive metabolic panel might inadvertently also similarly select for more "central" results for an additional analyte, even if all tests rendered are ostensibly independent. We examined this effect for a specific theoretical test case, that in which a parametric reference range determination is based on the apparent standard deviation of data that are sampled only from within the central 95% of a normal distribution.

Methods: Distributions of apparent standard deviations *s*(app) were obtained by 10,000 replicates of sample size *n* (*n*=20-100) obtained from the restricted central 95% of a standard normal distribution (mean=0, standard deviation=1*s*). Sampling was by computer simulation using a pseudo-random number generator according to the probabilities of the standard normal distribution.

Results: see Figure. As a control for the computation algorithm, distributions of *s*(app) obtained by sampling of size *n* from the unrestricted normal distribution (circles) were shown appropriately to correspond to those computed independently from the theoretical chi-squared distribution as a function of *n* (lines). In comparison, for sampling restricted to the central 95% of the normal distribution (squares, dashed lines), the distributions of *s*(app) as a function of *n* were shifted downward by more than 10%. For all *n*, the 50th percentile for *s*(app) for central 95% restricted sampling was less than the 25th percentile for *s*(app) for unrestricted sampling. Importantly, this modest extent of data restriction was not a recognizable aspect of data distributions as evaluated by Hoffman plot analyses.

Conclusions: Modest population selection bias that excludes exterior segments of normally distributed population data can dramatically reduce the apparent standard deviation of a sample distribution of such data. Such bias may go unrecognized for small sample size (*n*<100).



C-50

Performance Evaluation of a New Assay for β-$_2$-Microglobulin on the ADVIA® Chemistry Systems

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Introduction and Objective: β_2-Microglobulin(B2M) has a molecular weight of 11,800 Da and occurs on all nucleated cells as a component of the HLA complex. It is constantly released into the blood in small quantities. B2M is freely filtered in the kidneys, where it is reabsorbed and degraded in the renal tubules. The serum levels of B2M found in healthy individuals consistently remain low. A rise of serum concentrations occurs as a result of a higher release of B2M due to increased activity of the immune system, such as in infections, rheumatic diseases, and in cell death; or as a result of diminished elimination due to renal damage. The serum concentration of B2M could thus be a sensitive marker for the glomerular filtration capacity if other relevant disease mechanisms are ruled out. Elevated concentrations of B2M in serum are also found in patients with multiple myeloma and chronic lymphatic leukemia. In situations with increased cell proliferation, its specificity can be enhanced by determining the B2M-to-cystatin C ratio. A new assay* for B2M on automated clinical chemistry analyzers is under development. The objective of this study was to evaluate the performance of this new assay on the ADVIA Chemistry systems

(Siemens Healthcare Diagnostics, Tarrytown, NY, US).

Materials and Methods: In the ADVIA Chemistry B2M assay, sample is diluted and reacted with a buffer that contains latex particles coated with antibodies specific to B2M. The formation of the antibody-antigen complex during the reaction results in an increase in turbidity, the extent of which is measured at 545 nm. The B2M concentration in a sample is determined from a standard curve with a reagent blank and a single-level-calibrator. The performance evaluation in this study included precision, interference, linearity, and correlation. The data were collected on the ADVIA 1200, 1650, 1800, and 2400 systems. All ADVIA Chemistry systems use the same ADVIA Chemistry B2M reagent packs, calibrator, and commercial controls. Correlations were made between the B2M method and the N Latex β2-Microglobulin method performed on the Siemens BN™ II system.

Results: The imprecision(%CV) of the new B2M method with three-level commercial controls and three serum pools (ranging from ~0.6 to ~16 mg/L; n = 80) on all ADVIA Chemistry systems (1200/1650/1800/2400) was ≤3.5% (within-run) and ≤4.5% (total), respectively. The analytical range of the new methods was from 0.25 to 18 mg/L. The method correlated well with the N Latex β2-Microglobulin method on the Siemens BN II system: y = 1.00 (BNII) - 0.35 (r = 0.99; n = 63; sample range: 0.65-16.0 mg/L). The new method also showed no interference at a B2M level of ~1.2 mg/L with unconjugated or conjugated bilirubin (up to 60 mg/dL), hemoglobin (up to 1000 mg/dL), lipids (Intralipid, Fresenius Kabi AB; up to 1000 mg/dL), and rheumatoid factor (up to 2500 IU/mL). The method exhibited a maximum of 60 days of on-system stability and a minimum calibration frequency of 21 days.

Conclusion: The data demonstrate good performance of the B2M assay on the high-throughput ADVIA Chemistry systems.

* Under development. Not available for sale.

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A New, High-Throughput Assay for Ferritin, Evaluated on the ADVIA® Chemistry Systems

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Introduction and Objective: Ferritin is a high-molecular-weight (~240 kDa) protein that functions as the primary iron-storage compound in the body. It is composed of 24 subunits in a spherical assembly around an iron inner core. There are two forms of the subunits: an acidic H form and a basic L form. Different combinations of these subunits yield a family of isoferritins, with electrophoretic and immunogenic heterogeneity. Though synthesized by all human cells, the predominant isoferritins are composed primarily of L subunits and occur in liver, spleen, and blood. Measurements of ferritin may aid in the diagnosis of diseases affecting iron metabolism, such as hemochromatosis and iron deficiency anemia. Ferritin also has proven to be a useful marker for monitoring iron therapy. Historically, this assay was run on immunoanalyzers with low to moderate throughput. A new ferritin assay* for high-throughput automated clinical chemistry analyzers to meet the needs of the high-volume laboratory operation is under development. The objective of this study was to evaluate the performance of this new assay on the ADVIA Chemistry systems (Siemens Healthcare Diagnostics, Tarrytown, NY, US).

Materials and Methods: This new assay is a latex-enhanced immunoturbidimetric assay. Reagent R1 contains buffer, and reagent R2 contains latex microparticles linked to antibodies against ferritin. In the assay, the turbidity signal from agglutination of latex particles due to the presence of ferritin in the test sample is measured at 658 nm. The ferritin concentration in the sample is read off a six-level standard curve. The performance evaluation included precision, interference, linearity, and correlation studies. The data were collected on the ADVIA 1200, 1650, 1800, and 2400 systems. All ADVIA Chemistry systems use the same ADVIA Chemistry Ferritin reagent packs, calibrators, and commercial controls. Correlations were made between the ADVIA Chemistry Ferritin method and the N Latex Ferritin method performed on the Siemens BN™ II system.

Results: The new ferritin assay on the ADVIA Chemistry systems showed good precision performance. The within-run and total %CV results for four ADVIA Chemistry systems (1200/1650/1800/2400) were 1.4%-4.9% and 3.1%-6.1% (at a ferritin level of ~30 ng/mL), and 0.4%-0.8% and 0.8%-1.5% (at a ferritin level of ~300 ng/mL), respectively. The assay correlated well with the existing N Latex Ferritin method on the Siemens BN II system (y = 1.00x + 0.19; r = 0.99, n = 47, sample range: 6-431 ng/mL). The new method also showed <±10% interference at a ferritin level of ~78 ng/mL with bilirubin (conjugated or free, up to 60 mg/dL), hemoglobin (up to 500 mg/dL), lipids (Intralipid, Fresenius Kabi AB; up to1000 mg/dL), and rheumatoid factor (up to 2500 IU/mL). The analytical linearity range for this new method was 6-450 ng/mL, with a low limit of detection of 6 ng/mL.

Conclusion: The data demonstrate good performance of the ferritin assay on the high-throughput ADVIA Chemistry systems from Siemens Healthcare Diagnostics.

* Under development. Not available for sale.

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Automated, on-board preparation of whole blood samples for HbA1c

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Background: According to the International Diabetes Federation (IDF), the numbers of persons worldwide with diabetes mellitus is expected to explode from 285 million in 2010 to 438 million in 2030. As disturbing as these statistics may be, an even more distressing fact is that an estimated full third of these individuals today are unaware they have the disease. Given such an alarming prediction, the need for accurate and reliable diabetes diagnosis has never been more acute. To that end, the glycemic control marker HbA_{1c} has proven to be useful in monitoring a patient's historic overall blood glucose levels. A potential new indication for HbA_{1c} is in the diagnosis of diabetes mellitus, provided it meets the performance standards recommended by the National Glycohemoglobin Standardization Program (NGSP) and other professional bodies. HbA_{1c} measurements require lysis of red blood cells from either whole blood or packed cell samples. Results are typically reported as a ratio of HbA_{1c} and total hemoglobin in percent or in mmol/mol.

Methods: There are three major analytical methodologies adapted for automated clinical chemistry analyzers. The enzymatic methodology utilizes a protease to selectively cleave the N-terminal fructosyl dipeptide from the beta-chain of HbA_{1c}, the microparticle methodology measures the agglutination of monoclonal antibodies to HbA_{1c}, and the turbidometric inhibition assay measures unreacted HbA_{1c} antibodies as insoluble antibody-polyhapten complexes.

In order to process these whole blood samples, The ARCHITECT *c*8000 clinical chemistry analyzer has automated the RBC lysis procedure onboard to avoid inconvenient off-line pretreatment of samples. The sample probe descends to a sample depth of 70% from the top of the sample. This operation ensures adequate aspiration of red blood cells even if sedimentation has occurred. The increased dive depth and added exposure of the exterior sample probe to red blood cells, requires that the sample probe be washed externally over the entire exposed region to control carryover within specification.

Results: Limited early feasibility data with HbA_{1c} reagent using enzymatic methodology has demonstrated good precision and robustness to RBC settling with 0.23% CV for HbA_{1c} levels of 31.06 mmol/mol and 0.16% CV for HbA_{1c} levels of 87.53 mmol/mol for n=10 replicates (well-mixed). The same samples after a two hour settling time have %CVs of 0.42% and 0.16% respectively. Correlation of 50 samples to High Performance Liquid Chromotography (gold standard reference method), ranging from 22.12 mmol/mol to 88.37 mmol/mol, yielded a slope of 0.9916 and R^2 of 0.9961 for well-mixed samples and a 1.042 slope and R^2 of 0.9965 for the same samples after 2 hours settling time.

Conclusion: Feasibility results demonstrate excellent HbA_{1c} assay performance regardless of the RBC status (mixed or settled). The combination of the HbA_{1c} hardware modifications and the enzymatic methodology assay produces results well within the ±6% Total Allowable Error specification used by the CAP PT Survey. This is also consistent with the Carmen Ricos' biological variability goal for Hb and HbA_{1c}.

C-54

Developing an Educational Resource for Teaching Evidence-Based Laboratory Medicine

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Background: While educational resources exist for teaching evidence-based medicine principles, resources are limited for teaching the application of these principles for laboratory medicine quality improvement. Since 2006, the Centers for Disease Control and Prevention (CDC) has sponsored the Laboratory Medicine Best Practices (LMBP) initiative which has produced new systematic review methods to evaluate published and unpublished evidence of effectiveness for clinical laboratory quality improvement practices (pre- and post-analytic). Our objective was to develop a web-based resource that would increase the knowledge of evidence- based laboratory medicine (EBLM) principles while offering Continuing Education (CE) credit(s).

Methods: The LMBP 'A-6' systematic review methods have been applied by several multi-disciplinary teams, expert panels, and the LMBP Workgroup, described in a detailed technical report in 2010 (www.futurelabmedicine.org). An LMBP pilot project demonstrated that many laboratory quality improvement projects failed to meet minimum research standards for good study design making them unacceptable for inclusion in an evidence review. Specific requirements integral to the practice of evidence-based laboratory medicine are (1) ASK: formulating answerable questions, (2) AQUIRE: searching for published and unpublished evidence, (3) APPRAISE: critical appraisal of data, (4) ANALYZE: synthesis of eligible evidence, (5) APPLY: disseminating findings; and (6)ASSESS: measuring and monitoring targeted outcomes. An aim of the LMBP initiative is to facilitate the adoption of evidence-based practice methods. The LMBP Web-Based Tutorial was developed as an approach, to introduce professionals to the LMBP A-6 methods, to educate professionals about completing assessments that meet minimum systematic review inclusion and quality criteria, and to provide a resource for quality improvement curriculum material. Feedback from multiple laboratory professionals and health care educators' conferences was integral to the development of the curriculum.

Results: Initiated in August 2010, tutorial development had four phases: (1) Design, (2) Development, (3) Production and (4) Evaluation. Design: concept and content development, content analysis by experts in the field of evidence based methodology, Development: the projects' instructional designer created storyboards to visually outline the sequence of the content, images and related interactive features of the tutorial, Production: building the tutorial using authoring software, then pilot testing, and completing continuing education accreditation of the finalized product; Evaluation: Outcomes to be evaluated are knowledge enhancement and learner satisfaction through a survey In addition; the number of learners receiving CE credit will provide an indication of need. Course content for Module 1 covers the utility of evidence-based approaches, application of systematic reviews, and validated steps for reviewing and evaluating laboratory medicine quality improvement practices. Course content for Module 2 accents key elements for improving the rigor of quality improvement assessments

Conclusion: Teaching laboratory professionals how to improve the design rigor of quality improvement studies or assessments is essential to expanding the body of evidence of effective laboratory medicine quality improvement practices. The LMBP Web-Based tutorial is the first tutorial designed to accomplish this and teach the principles of EBLM. A live demonstration model at the AACC Annual Meeting will increase awareness of this resource and contribute to its evaluation.

C-55

Lower human intervention and higher automation in clinical chemistry and hematology processes in order to assure better quality and productivity

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Background: Nowadays, optimizing clinical chemistry to have cost-effective results is a great challenge. Converging related processes and sharing samples is a solution used in Fleury Group to make the workflow leaner and more efficient. However, developing more complex flows create more decision nodes. Consequently, human dependence and mistakes probability increase.

Hands-on time in routines like sorting tubes, staining, screening slides and verifying/releasing results is massive in high-volume laboratories. On the other hand, having well-trained, specialized staff is not an easy task. High quality level is essential in health care and, if the staff is focused in repetitive jobs, the training and quality control can be mistakenly put in the second place.

Thus, new solutions using robotics and informatics are essential to allow staff to dedicate the appropriate amount of time to quality.

Aim: Evaluate automation methods to make Fleury Group´s clinical chemistry and hematology process leaner, avoiding mistakes, increasing productivity, standardization and quality.

Methods: During one year, data have been extracted from an 88,000 tubes/month laboratory routine that shared samples: 45.6% Counting Blood Cells (CBC), 31.1% A1c Hemoglobin (A1c), 0.6% Erythrocyte Sedimentation Rate (ESR), 11.3% CBC + A1c, 7.3% CBC + ESR, 0.2% A1c + ESR, 3.9% CBC + A1c + ESR.

The process is composed by a PVT RSD *Pro* sorter, two Sysmex HST402 and a Cella Vision™ DM96 system for CBCs, four BIO-RAD Variant II for A1c, an Alifax Test1 for ESR and a refrigerated sample storage room. All the test results are analyzed by a decision support algorithm that combines technical and clinical knowledge, able to release automatically coherent results.

Results: Using the described system, the human intervention and decision were lower. The team productivity grew 80%, with a delayed result rate of 0.06%. Because 79.27% of the results were automatically verified and released, the average time between blood draw and result releasing was 3hours and 14minutes for all outpatient samples. The remaining results (20.73%) were not automatically released and were analyzed by specialized technicians and the physician staff for quality assurance. Data correction rates after results were released were 0.03%. The microscopy productivity increased 141% and, additionally, the images generated by slide scanning could also be used for training and educational purposes.

Conclusion: Robotics and informatics solutions are very adequate resources for high-volume clinical chemistry and hematology laboratories with high levels of quality. They allow fast, efficient processing of high number of samples and tests. Importantly, the quality of the entire testing process is also improved because the technical and medical staff can dedicate more time to quality control and assurance, as well as training and educational activities.

C-56

Validation of an Automated Wash process for Agencourt® FormaPure® RNA Extraction from FFPE samples using the Eppendorf Ep Motion® 5075

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Background: The Pathwork® Tissue of Origin (TOO) Test uses the Agencourt FormaPure kit for extracting RNA from formalin-fixed, paraffin-embedded (FFPE) tissue. This procedure involves several repetitive wash steps that are both time-consuming and tiring. Additionally, the manual procedure is more prone to variability in pipette mixing especially given the large number of samples processed by Pathwork Diagnostic laboratory (PWDL). We therefore sought an automated solution using the Eppendorf Ep Motion 5075 system. The Eppendorf Ep Motion system is designed to aspirate and dispense liquid from a source position to a destination, and can transport plates to destination positions on the instrument deck using a gripper arm. It is also customized with a thermal module for cooling and heating microplates to temperatures ranging from 0°C to 95°C. PWDL developed and validated a procedure for automating the RNA extraction wash steps.

Methodology: Using predefined commands in the Ep Motion's software, we developed a protocol to perform the washing steps during RNA extraction. We validated this procedure using samples that reflect the different types of FFPE samples typically received and processed by PWDL. Four different sample pools consisting of either 5-µm-thick paraffin curls or microdissected tissues were tested in triplicate using the Ep Motion method and were compared to extractions performed manually. Negative controls were also included. Both the manual and automated methods were run simultaneously by two operators. Extracted RNA concentrations and purity (A260/A280 ratios) were measured using the Nanodrop™ 2000 instrument.

Results: RNA obtained using the Ep Motion procedure had a median concentration of 22.8 ng/µl with a range of 9.3 ng/µl to 57.9 ng/µl. The CV among triplicates ranged from 5.5 to 6.9. In contrast, RNA concentrations obtained from these same samples using the manual method had median value of 15.1 ng/µl and a range of 8.3 ng/µl to 33.5 ng/µl. The CV among triplicates in the manual method was more variable, ranging from 0.9 to 35.7. All negative control samples had concentrations of -0.4 to 1.0 ng/µL. A260/A280 ratios for both methods ranged from 1.7 to 1.9, indicating good purity. Three representative RNA samples from the automated extraction were processed through the TOO Test and produced the expected TOO Test result (both tissue type and similarity score).

Conclusion: The automated Ep Motion procedure developed by PWDL produced equivalent or higher RNA yields relative to the manual method. RNA yields were also more consistent when compared to the manual method as demonstrated by lower and less variable CVs. A robust automated system saves time, reduces operator fatigue and allows for the opportunity to increase the sample batch size. In conclusion, this study validates that the Ep Motion can be used to perform wash steps during RNA extraction for patient testing in our clinical diagnostic laboratory.

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Process Improvement Through Automated Semen Analysis

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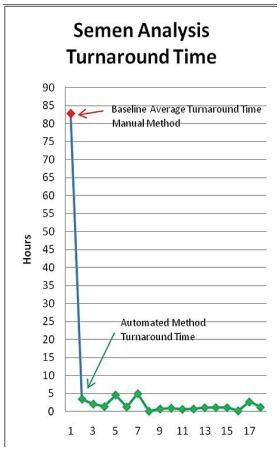
Semen analysis has significantly benefited from automation. With manual testing, the Laboratory was faced with the challenges of maintaining standards of efficiency, consistency and competency. Predicted staffing shortages also influenced our decision

to automate. To improve services, cost efficiency and physician satisfaction, and decrease turn-around-time, we decided to automate semen analysis with Medical Electronics SQA-V ™ Semen Analyzer. Use of LEAN principles identified items to streamline testing while providing clinicians with timely, accurate and consistent results. The SQA-V Semen analyzer is a bench top device that rapidly determines the total sperm count, motility and morphology of semen specimens. SQA-V technology is based on electro-optical signal processing in conjunction with algorithms built into the software. The analyzer can accommodate fresh, post-vasectomy, frozen and washed specimens.

37 patient samples including normal and abnormal specimens were used to validate the system. The manual testing was performed by those staff members trained in manual semen analysis using WHO 3rd classification. Training was completed in 5 days and after implementation, our goal of a 3 hour turn-around-time (figure 1) was consistently met. The manual semen analysis average turn-around-time was 3 days due to delays in manual processing, including transport, staining and microscopic examination. Our average turn-around-time per case was reduced from 82 hours to 3 hours. Annualized, this freed up 0.5 FTE. We performed 161 cases in 2010. Overall, labor costs for testing were reduced by $14,560.00.

Automating semen analysis has enabled our Laboratory to focus on delivering accurate test results in an efficient and timely manner. Testing is now available on demand and many of the delays involved in manual processing have been eliminated. Scarce resources are maximized and our Scientists are free to perform other tasks. Our physicians can now rely on having accurate test results consistently available in less than 3 hours.



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cobas® 8000 modular analyzer series Workflow Analysis

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Objective: To see how the laboratory would benefit from using a consolidated workstation (cobas 8000 modular analyzer series) in place of several stand alone instruments (2-Olympus AU2700™, Olympus AU640™, and Roche COBAS Integra® 800). In an effort to optimize workflow process, two cobas c 701 analytical modules in combination with a cobas c 502 module were used, utilizing a tailor made reagent concept, to see how the system would handle large sample workloads.

Study Design: Divided into four main sections: 1.) Analysis of current routine workflow to gain an understanding of the process to develop an appropriate site specific protocol and documentation logs, 2.) Documentation of routine operation with current instrumentation, 3.) Documentation of routine operation simulated on cobas 8000 modular analyzer series, and 4.) Assessment of Practicability for routine instrumentation and for cobas 8000 modular analyzer using a questionnaire covering all aspects of the system.

Results: The Turn-Around-Time (TAT) for the specimens studied are listed below in Table 1. Using this consolidated workstation resulted in a 5% reduction of samples due to unneeded aliquots and decreased TAT. Optimized TAT was accomplished by using a tailor made reagent concept. In using this consolidated workstation, Hands-On-Time was reduced by nearly 3 hours over a 24 hour period for maintenance, reagent handling, calibration and quality control, and reduced needed technical personnel. When compared to this institutions routine instrumentation, the cobas 8000 modular analyzer series practicability was rated 25% higher.

Conclusion: When compared to the routine instrumentation in this laboratory, the cobas 8000 modular analyzer series showed a reduction on sample TAT, Hands-On-Time of technical personnel and an increase of practicability.

Table 1.

Run #	Run Characteristics	cobas 8000 TAT	Routine TAT
1	Peak time, 2 hours, 927 samples, HbA1c evenly spaced	36m	53m
2	Peak time, 3 hours, 1467 samples	40m	1h:06m
3a	Peak time of day, 2 hours, 1064 samples, <c701/c701/c502>	37m	1h:03m
3b	Peak time of day, 2 hours, 1063 samples, <c701 /c502>	1h:18m	1h:03m
4	Peak time of day, 3 hours with 1246 consolidated aliquots (due to running samples from 4 separate routine analyzers)	44m	1h:00m

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Reducing turnaround time and streamlining a dedicated specimen processing workstation using high speed IRIS® StatSpin® Express 4 centrifuges

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Implementation of two StatSpin® Express 4 centrifuges (IRIS® International) can improve the turnaround time of plasma chemistry and immunoassay tests (TAT) performed in the central laboratory when compared to traditional 10min centrifugation times. Two StatSpin Express 4 centrifuges replaced three Eppendorf® 5702 (Hamburg, Germany) centrifuges in the rapid processing workstation as phase 2 of a process improvement project to decrease chemistry-immunology test turnaround time, phase 1 consisting of creation of a dedicated plasma specimen processing workstation to minimize processing delays related to specimen process flow. A StatSpin Express 4 centrifuge can centrifuge 8 tubes for 3min at 4000g (cycle time 3.7min). Each Eppendorf 5702 centrifuge, as configured, can centrifuge 16 tubes for 10min at 2500g (cycle time 10.8min). Specimens were collected in Becton-Dickinson Vacutainer®PST™Gel and Lithium Heparin Blood Collection Tubes by standard phlebotomy technique. Potassium and troponin I test TAT results were monitored for 60 days before and after implementing StatSpin Express 4 centrifuges. Two Siemens Dimension® Vista® 500 Intelligent Lab Systems were loaded manually by technologists, and results verified in a Meditech LIS system. The minimum measured TAT, defined as time received to time verified, was 15min for troponin I and 10min potassium using a StatSpin centrifuge; 7.1min longer if collection tube centrifuged using an Eppendorf centrifuge. The data had a right skewed distribution, so other parametric tests were not performed. The table summarizes the data:

	Potassium		Troponin I	
Turnaround Time	Before	After	Before	After
	StatSpin	StatSpin	StatSpin	StatSpin
Mean [SD] (min)	31 [9]	24 [8]	33 [8]	28 [8]
Median (min)	29	22	31	26
75th %tile (min)	35	27	36	30
90th %tile (min)	42	34	43	39
N	3499	3628	333	368
# of Centrifuges	3	2	3	2

Importantly, the 90th percentile TAT for potassium improved 8min to 34min (19%) and troponin I 4min to 39min (9%) after implementation of the StatSpin centrifuges. The TAT improvement of the tests are close that predicted by the StatSpin centrifuge's shorter centrifugation cycle time. Using StatSpin Express 4 centrifuges at a manual collection tube processing workstation can modestly improve turnaround time for plasma test results in the clinical laboratory setting.

C-60

Evaluation of the Analytical Performance of the Siemens Dimension® EXL 200™

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The new Dimension® EXL 200™ (EXL) integrated chemistry system from Siemens Healthcare Diagnostics utilizes photometry and turbidimetry for general chemistry

tests, integrated ion-selective multisensor technology (IMT) for electrolytes, chromium-based heterogeneous immunoassays (HM module) and high sensitivity homogenous chemiluminescent immunoassays (LOCI module). This integrated platform enables analyzer consolidation and improved workflow.

This study evaluated analytical performance for 28 tests prior to analyzer implementation. For each assay, evaluation included initial calibration / calibration verification followed by validation of claims for assay sensitivity, linearity/reportable range and within-run precision. The CLSI EP10 (Preliminary Evaluation of Quantitative Clinical Laboratory Measurement Procedures) protocol was run to assess bias, precision, linearity, carryover and drift. Two levels of Bio-Rad quality control materials were run over a minimum of 20 days and results (mean and SD) were compared to the peer group data for Dimension Series analyzers and the separate peer group data for the Siemens Dimension Vista® 1500 (Vista). Patient correlation studies (EXL versus Vista) included 20 - 40 specimens over the assay range. The table shows the results for two representative assays and technologies: glucose (photometry) and troponin I (LOCI module).

	Assay	Sensitivity		Linear	Within Run Precision			
	Range				Guidelines		Observed	
		Claim	Actual	Actual	Level	SD	Level	SD
Glucose	0.0 to	0.06	0.01	0.0 to	4.3	< 0.3	7.5	0.1
mmol/L	27.8			30	14.7	< 0.7	15.2	0.2
TnI	0.02 to	0.01	0.00	0.0 to	0.60	< .12	0.56	0.01
ug/L	40.0			40.3	6.00	< .56	6.14	0.09
	Between Run Precision							
	Allowed		Observed		Peer Dimension		Peer Vista	
	Mean	SD	Mean	SD	Mean	SD	Level	SD
Glucose	4.3	< 0.3	4.67	0.11	4.65	0.11	4.52	0.13
	14.7	< 0.7	15.8	0.2	15.7	0.3	15.3	0.5
TnI	0.60	< 0.12	0.40	0.03	N/A	N/A	0.46	0.09
	6.00	< 0.56	8.07	0.37	N/A	N/A	9.50	0.90
	EP10 - Bias				EP10 - Precision			
		Low	Mid	High		Low	Mid	High
Glucose	Target	4.65	10.18	15.70	WR SD	0.025	0.049	0.091
	Actual	4.617	10.13	15.65	TotalSD	0.049	0.129	0.164
	Bias	-0.033	-0.046	-0.049	TotalCV	1.1	1.3	1.0
	Allowed	0.186	0.407	0.628	Allowed	2.0	2.0	2.0
TnI	Target	0.45	4.01	7.57	WR SD	0.008	0.040	0.112
	Actual	0.476	4.005	7.583	TotalSD	0.015	0.080	0.176
	Bias	0.026	-0.005	0.013	TotalCV	3.2	2.0	2.3
	Allowed	0.059	0.521	0.984	Allowed	6.5	6.5	6.5
	EP10 - Linearity, Carryover and Drift					Patient Correlations		
	t Values - All Runs - Acceptable ≤ 4.6					EXL 200 vs Vista		
	y-Int	Slope	Carry	Linear	Drift	y-Int	Slope	r
Glucose	-1.9	-0.2	-0.5	0.1	-0.8	0.16	1.015	0.9992
TnI	1.2	-0.5	-0.5	0.7	1.2	-0.02	0.903	0.9999

Overall the results met the claims of the manufacturer for sensitivity, linearity and for both within run and between run precision. The Bio-Rad quality control results showed acceptable comparison to the peer group data for Siemens Dimension Series and Vista (Vista only for troponin I). The CLSI EP10 protocol confirmed results within the allowed limits for bias, precision, linearity, carryover and drift. The patient specimen correlation results showed acceptable agreement between EXL and Vista.

C-61

Evaluation of digital imaging technology for interpretation of serum protein immunofixation gels

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Background and Objectives: Digital imaging technology is being adopted in several areas of pathology. Advantages of digital images include linking results to charts for convenient access and affording remote interpretation. The purpose of this study was to compare traditional serum protein electrophoresis immunofixation (IFX) to digital scans using a new GelScan system (Sebia, USA).

Methods: Five experienced interpreters, who were blinded to the data, analyzed 200 consecutively-ordered IFX tests run on Sebia's HYDRASYS with IF2/4 agarose Hydragel. Interpreters reviewed the samples using 2 different digital images as well as the physical IFX gels. Digital images included a low-resolution (LR) image (816 x 600) and a high-resolution (HR) image (1300 x 1200). LR images were viewed using Sebia's phoresis software (version 6.5.0), whereas HR images were viewed using Microsoft Office Picture Manager, which allowed zooming and additional manipulation. The extent of image manipulation was left to the discretion of the reviewer. LR images could not be resized, but color inversion was possible. The physical gel interpretations were used as the gold standard.

Results: Based on the physical gel gold standard, the prevalence of abnormalities identified was 50.5%. The sensitivity for detection of abnormalities using the LR images was 82.0% ± 20.6 (mean ± 95% confidence interval), and the specificity was 70.1% (± 16.7). The sensitivity using HR images was 80.4% (± 7.4), with a specificity of 90.5% (± 6.8). There were 14 samples that the majority of the interpreters identified as normal using both the LR and HR images, but abnormal using the physical gels. Of these, 4 patients had a previous history of multiple myeloma. Based on review of recent charts (20 months after the sample used in the study), all but one is currently in complete remission. The patient not in complete remission is currently on chemotherapy and continues to have abnormal free light chain concentrations. Review of the remaining 10 patients reveals that 2 have been lost to follow up, while the remaining 8 have been seen subsequently, but no additional IFX has been performed.

Conclusions: The data show that interpreters were able to identify abnormalities more readily by physical gel than by digital images. However, the subsequent chart review showed that the abnormalities identified using the physical gel were of limited clinical significance and would not have affected patient management. Digital images have several added benefits over physical gels including the ability to link the gel to the patient file, remote interpretation, and ease of review of previous results. These data support implementation of digital images for interpretation into routine clinical practice.

Wednesday AM, July 27

Poster Session: 10:00 am - 12:30 pm
Clinical Studies/Outcomes

C-63

Early Second-Trimester Serum MiRNA Profiling Predict Gestational Diabetes Mellitus

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Background: Gestational diabetes mellitus (GDM) is one of the most common pregnancy complications and affects approximately 3-8% of all pregnancies. Detection of women at higher risk for GDM early in pregnancy is a desirable goal because interventions such as diet, medication, and exercise may be applied earlier and has a positive effect on maternal and fetal outcomes. The aim of this study is to systematically assess whether the serum microRNAs (miRNAs) profiling can predict GDM in advance of the measurement of blood glucose.

Methods: Serum samples collected at 16-19 gestational weeks, were obtained from 92 healthy pregnant women and 92 women who subsequently developed GDM. miRNA profiling was performed on total RNA extracted from serum obtained from 48 individuals (24 controls and 24 patients) by systematically TaqMan Low Density Array. Individual quantitative reverse transcription polymerase chain reaction assay was used to validate the profiling results in the discovery set and in a validation set of 92 controls and 92 GDM patients. Logistic regression was used to estimate the associations between miRNA expression levels and GDM risk.

Results: Two miRNAs (miR-29a and miR-222) were identified and consistently validated as efficient predictors. Taken together, women with high serum miRNA expression levels had significantly decreased risks of developing GDM for the two miRNAs (Odds ratio [OR] = 0.25, 95% confidence interval [CI] = 0.13-0.47 for miR-29a; and OR = 0.34, 95%CI = 0.18-0.64 for miR-222).

Conclusion: MiR-29a and miR-222 in serum may serve as noninvasive biomarkers for predicting GDM development.

C-64

Monoclonal IgA Proteins Migrating into the β Region of Serum Protein Electrophoresis Gels Can be Easily Identified and Quantified Using IgAκ and IgAλ Measurements

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Accurate quantification of IgA monoclonal protein (M-protein) by serum protein electrophoresis (SPE) can be difficult especially when they co-migrate with other proteins. Polyclonal antibodies have been developed which recognise junctional epitopes between immunoglobulin light chains and their heavy chain partners (heavy/light chain; HLC). Here we describe these assays and their utility for the detection and quantification of IgA monoclonal proteins. Serum IgAκ and IgAλ concentrations were retrospectively measured in 210 IgA (145 IgAκ and 65 IgAλ) multiple myeloma (MM) patients at presentation. Results were compared to previously published IgAκ/IgAλ reference ranges, historic SPE and immunofixation data. Experimental sensitivities were tested by spiking well characterised, pooled normal human sera with known concentrations of monoclonal proteins, and the results were compared with SPE. The assays nephelometric measuring ranges and (sensitivities) were: IgAκ 0.35- 11.20g/L (0.018g/L) and IgAλ 0.33- 10.40g/L (0.016g/L). Intra- and inter- assay CV's were measured using analytes at 3 different IgAk concentrations (9.144, 2.480, and 0.558g/L) the measured intra- assay CV's were 1.8%, 1.8%, and 3.2% and the measured inter- assay CVs were 0.5%, 2.3%, and 1.0%, respectively. Similarly, 3 different concentrations of IgAλ (8.445, 1.926, and 0.5g/L) were used to determine IgAλ inter- and intra- assay CV's; the inter- assay CV measurements were 2.1%, 2.6%, and 3.2% and the measured intra- assay CVs were 2.5%, 0.6%, and 1.1%. Abnormal IgA HLC ratios (HLCr) were recorded in 145/145 IgAκ patients (median 350; range 7.03- 7353) and 65/65 IgAλ patients (0.01; 0- 0.37). In 83/210 patients (40%) it was not possible to quantify the monoclonal protein accurately by SPE due to its co-migration with other serum proteins; however abnormal IgA HLC ratios were calculated for these patients. The sensitivity limits of the IgA HLCr for detecting 3 IgA M-proteins diluted in normal human sera were compared to SPE. SPE sensitivity was approximately 1g/L for 3 different monoclonal IgA proteins. In 1/3 cases HLCr was similar to SPE and in 2/3 cases HLCr was more sensitive (below 0.5g/L). In conclusion, the IgA HLC assays have a comparable or better sensitivity than SPE for measuring IgA M-proteins. In addition, the IgA HLC assays provide an accurate measurement, especially where IgA M-protein co-migrates with other serum proteins.

C-65

Evaluation of Paraoxonase and Arylesterase Activity With Oxidative Status in Children With Sickle Cell Anemia

O. H. Ozturk[1], N. Yilmaz[1], K. T. Ulutas[1], M. Tutanc[2], V. Arica[2], O. Y. Kurtgoz[1], A. B. Gurpınar[1], Z. Yonden[1], S. Sogut[3], G. Oktay[4], M. Arslan[5]. *[1]Mustafa Kemal University, Faculty of Medicine, Department of Biochemistry, Hatay, Turkey, [2]Mustafa Kemal University, Faculty of Medicine, Department of Pediatrics, Hatay, Turkey, [3]Bezmialem Vakif University, Faculty of Medicine, Department of Biochemistry, Istanbul, Turkey, [4]Antakya State Hospital, Diagnosis of Hereditary Blood disease Center, Hatay, Turkey, [5]AkdenizUniversity, Faculty of Medicine, Department of Biochemistry, Antalya, Turkey,*

Background: Hemoglobinopathies are the most common genetic diseases around Mediterranean area, south of Turkey. Current evidence revealed that oxidative stress has an important role in the pathophysiology of the vaso-occlusive crises observed in sickle cell anemia (SCA) patients. This study was performed in order to determine oxidative status including paraoxanase and Arylesterase in the patients with SCA and SCA crisis and healthy subjects.

Methods: All patients were assigned to two subgroups as following; SCA and SCA crisis. All participants were enrolled in the study after confirmation with hemoglobin S (HbS) ratio analysis. Oxidative status parameters which were shown in the table were studied from the serum.

Results: We observed that oxidative stress parameters (NO and MDA levels) increased in SCA compared to control, while there was no increase for serum levels of NO and MDA in SCA crisis. However, antioxidant parameters as total antioxidant status (TAS) and total thiol levels (TTL) were found decrased in SCA crisis. Serum paraoxonase activity was decreased in patients with both SCA and SCA crisis, whereas there were no differences for arylesterase (ARES) among the groups.

Parameters	SCA HbS/S (n=38)	SCA HbS/S Crisis (n=20)	Control (n=28)
TAS (Total antioxidant status) (µmol trolox equiv/L)	1,38 ± 0,34	1,26 ± 0,48 (a)	1,52 ± 0,13
PON (Paraoxanase)(U/L)	36,89 ± 26,3 (b)	29,91 ± 25,6 (b)	96,2 ± 53,8
TTL (Total thiol)(µmol/L)	934,8 ± 307,5	798,9 ± 365,1 (a)	1010 ± 126,8
ARES (Arylesterase)(U/L)	225,39 ± 17,2	227,1 ± 15,3	221,64 ± 0,12
NO (Nitrate/nitrite)(mmol/L)	80,7 ± 7,5 (a)	79,9 ± 8,3	75,9 ± 8,2
MDA (Malondialdehyde)(µmol/L)	0.87 ± 0,1 (a) (c)	0.61 ± 0,2 (b)	0.77 ± 0,07

Values are mean±SD; One-Way Anova, and posthoc analysis by Tukey's test. P value of less than 0.05 was accepted as significant. a: p value was less than 0.05 compared with control; b: p value was less than 0.001 compared with control; c: p value was less than 0.001 compared with SCA Crisis.

Conclusion: Significant reduction of paraoxonase activity seems to be related with both the degree of oxidative stress and sickle cell anemia. Therefore we suggested that oxidative stress could be very crucial for pathophysiology of SCA.

C-66

Analytical and Clinical Performance of the Enhanced Liver Fibrosis (ELF™) Test on the ADVIA Centaur® Immunoassay Systems from Siemens

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Background: The ELF test, a noninvasive marker of liver fibrosis calculated from three direct markers of fibrosis (HA, PIIINP, and TIMP-1), which is CE marked and available in Europe on the Immuno1™ (Siemens) immunoassay system but is unavailable in the US, performs well clinically (Rosenberg et al., Gastroenterology, 2004, 1704-1713). ADVIA Centaur ELF reagents are in development but the individual assay and ELF test parameters have been finalized. Cutoffs at 7.7 and 9.8 score were defined using biopsy and score data from the 921 mixed-etiology samples used to define the Immuno1 coefficients.

Objectives: Estimate and validate ELF score coefficients for the ADVIA Centaur systems. Assess their analytical and clinical performance.

Methods and Materials: ADVIA Centaur HA, PIIINP, and TIMP-1 reagents and ELF calibrators (two lots each), ELF controls, serum pools, and patient samples (184 routine remnants from iQur with Immuno1 results plus 164 remnants from two clinical trials [unrelated; no Immuno1 data]) assayed (singleton) with each reagent lot were used on two Centaur XP and two CP systems in Siemens' Tarrytown laboratory over 16 days with multiple replicates of each calibrator lot in each run. All system responses were reduced to doses with multiple offline calibrations. Median doses were used for each platform.

Using 98 samples, log-log Passing-Bablok correlations vs. Immuno1 for the three methods were used to calculate ELF coefficients for each ADVIA Centaur platform. ELF scores were calculated for all samples with these. The coefficients were validated using the remaining 86 routine remnants. ROC curves were estimated from 40 of these with biopsy data.

Reagent and calibrator lot-to-lot correlations were estimated with all 348 samples.

Precision was estimated (for each reagent lot and system) with a CLSI EP05-A2 protocol using controls, calibrators, and serum pools.

Results: ELF correlations:

98-sample initial (training) fits:

(XP-ELF)=0.995(Immuno1-ELF)+0.051,r=0.978

(CP-ELF)=0.992(Immuno1-ELF)+0.085,r=0.975

86-sample validation fits:

(XP-ELF)=1.048(Immuno1-ELF)-0.556,r=0.962

(CP-ELF)=1.038(Immuno1-ELF)-0.439,r=0.959

Both training and the CP validation correlations were statistically (95% confidence) indistinguishable from identity. The XP validation slope was indistinguishable from 1 (95% CI 0.991-1.095) but its intercept was slightly significantly negative (95% CI -1.007 to -0.035).

The areas under the ROC curves (AUROCs) to distinguish Ishak biopsy 0-2 vs. 3-6 (cutoff 7.7) are 0.84, 0.85 and 0.85 for XP, CP and Immuno1. All sensitivities are 100%, both Centaur specificities are 11.8%, and the Immuno1 specificity is 0%. To distinguish Ishak 0-4 vs. 5-6 (cutoff: 9.8), AUROCs were 0.91, 0.91, and 0.90 for XP, CP, and Immuno1. For each, sensitivity=78.6%, for both Centaurs specificity=92.3%, and for Immuno1 specificity=84.6%. The AUROCs were statistically indistinguishable across platforms.

Lot-to-lot correlations:

XP: (RgtLot2)=1.013(RgtLot1)-0.148,r=0.999

CP: (RgtLot2)=1.011(RgtLot1)-0.117,r=0.998

XP: (CalLot2)=0.947 (CalLot1)+0.305,r=1.000

CP: (CalLot2)=0.998(CalLot1)-0.015,r=1.000

CLSI EP05-A2 ELF precision estimates:

XP: Mean=4.9-13.5,WRSD=0.03-0.14,TSD=0.06-0.16

CP: Mean=5.0-13.7,WRSD=0.03-0.15,TSD=0.06-0.19

Conclusions: The ADVIA Centaur XP and CP ELF tests were defined and validated. Their ELF scores and clinical performances were nearly indistinguishable from those for Immuno1. ELF scores separated moderate from mild fibrosis at the lower cutoff with high sensitivity, and severe from moderate fibrosis at the higher cutoff with high specificity. The ADVIA Centaur ELF scores had acceptable lot-to-lot variabilities and precisions.

C-67

Urine NGAL predicts AKI, Clinical Outcomes and Survival in Patients with Traumatic Brain Injury

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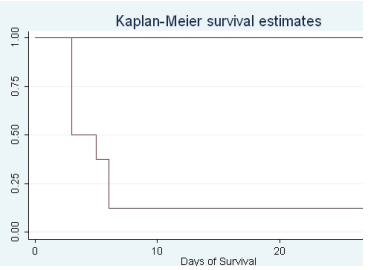
Background: AKIN criteria have been adopted clinically for prognosticating traumatic brain injuries (TBI). Urine NGAL as established acute kidney injury marker may substitute the AKIN criteria by single quantifiable urine marker. **Methods:** Both serum creatinine and urine NGAL were measured in a cohort of consecutively enrolled 32 patients with traumatic brain injury at single research hospital on admission, day 2 and 3. Urine NGAL concentration was determined on Architect platform (Abbott Diagnostics, USA) using manufacturer' recommendation. Serum creatinine and urine output were used to determine the incidence of AKI according to AKIN criteria published in 2008. **Results:** Age, admission Glasgow coma scale (GCS), presenting systolic and mean blood pressure are similar in both AKI and Non-AKI patients. Urine NGAL levels are significantly higher among patients with AKI ($p<0.01$). Peak urine NGAL concentration >80ug/L during the first 3 days after admission predicts all AKI cases at specificity of 87.5%, while Glasgow outcome score (GOS) is significantly lower for the same population. Patients with peak urine NGAL concentration less than 80 ug/L have significantly better survival ($p<0.05$) at 30 days. No cases survived at a month with greater than 120ug/L of peak urine NGAL level during the first 3 days. **Conclusion:** The urine NGAL peak concentration may predict not only the AKI occurrence but also the clinical outcome and survival benefits among TBI patients. Thus it may be a clinically promising single biomarker for both AKI and prognosis prediction for patients with severe brain trauma.



C-68

YKL-40 Levels in Rescently Diagnosed Essential Hypertention Patients and Its Relation with Carotid Intima Media Thickness and Insulin Resistance

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Background: Hypertension (HT) or high blood pressure is a chronic medical condition accompanied by cardiac and vascular changes, such as thickening of the media layer of the arterial wall, narrowing of the vessel lumen, and resulting increase in arterial resistance. It has been shown that YKL-40 was secreted from macrophages in atherosclerotic plaques and its levels increased in low-grade inflammatory responses. Aim of this study was to evaluate YKL-40 levels in recently diagnosed essential hypertension patients as an indicator of the inflammatory process related to hypertension and its connection with Carotid Intima Media Thickness (CIMT).

Materials and Methods: 50 recently diagnosed hypertension patients who applied Şişli Etfal Research and Training Hospital Hypertension Clinics and 30 healthy volunteer control subjects were recruited to this study. Patients with diabetes mellitus, secondary hypertension, osteoarthritis, romatoid arthritis, kidney and liver diseases were excluded from the hypertensive patient and control groups. Serum YKL-40 glucose, insulin, homocysteine, hs-CRP, total cholesterol, HDL cholesterol, LDL cholesterol, VLDL cholesterol and triglyceride levels were measured after 12 hours of fasting. Glucose and total cholesterol with an enzymatic colorimetric method, hs-CRP with immunoturbidimetric, insulin and homocysteine with chemiluminescence and finally YKL-40 were measured with enzyme immunoassay

CIMTs were measured by the same radiologist with use of same duplex doppler ultrasound instrument (Aplio XV, Toshiba, Tokyo, Japan) and 14 MHz probe.

Student T test, Mann Whitney U test and Pearson correlation analysis and Spearman's Rho correlation were used for data analysis. NCSS 2007 & PASS 2008 Statistical Software (Utah, USA) were used for all statistical analysis. Results were evaluated

95% confidence level and p values less than 0.05 were considered as statistically significant.

Results: YKL-40, insulin, hs-CRP, homocysteine levels and CIMT were found to be significantly higher in patients with hypertension in respect to the healthy control group ($p<0.01$). Our data also demonstrated statistically important correlation between YKL-40 and HOMA-IR, age, BMI, waist circumference, and triglyceride levels, but not between serum YKL 40 levels and serum total cholesterol, hs-CRP, homocysteine levels, CIMT, systolic and diastolic blood pressure, and gender ($p>0.05$) in the patient group.

Since it has been proven that the macrophages in atherosclerotic plaques secreted YKL-40 and CIMT was the early atherosclerotic indicator of HT, we investigated the correlation between YKL-40 and CIMT. We could not show any correlation between YKL-40 and CIMT levels ($p>0.05$). This might arise from the very approximate CIMT levels measured in the patients with hypertension.

Conclusion: YKL-40 levels were found to be increased in recently diagnosed HT patients. High YKL-40 levels may be a new risk factor reflecting inflammatory atherosclerotic processes acting in the pathogenesis of essential hypertension. Further studies demonstrating the effects of the antihypertensive treatment on YKL-40 levels and inflammatory process will help to clarify its role in the pathogenesis of HT.

C-69

Markedly Reduced Overall Survival of CYP2C19 *2/*2 Homozygotes After Myeloablative Hematopoietic Stem Cell Transplantation

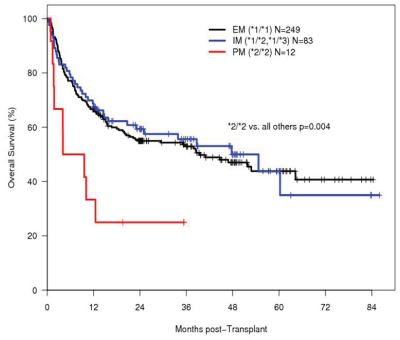
S. E. Melanson[1], K. Stevenson[2], H. Kim[2], J. Antin[2], V. Ho[2], J. Ritz[2], R. Soiffer[2], M. Court[3], F. Kuo[1], J. Longtine[1], P. Jarolim[1]. *[1]Brigham and Womens Hospital, Boston, MA, [2]Dana Farber Cancer Institute, Boston, MA, [3]Tufts University School of Medicine, Boston, MA,*

Background: Patients with allelic variations of cytochrome P450 enzymes that affect drug metabolism may have worse outcomes after hematopoietic stem cell transplantation (HSCT); in particular carriers of the cytochrome P450 2C19 (CYP2C19) loss-of-function allele, *2. Based on the number of loss of function alleles in CYP2C19 patients can be classified as extensive metabolizers (EM) (e.g., *1/*1), intermediate metabolizers (IM) (e.g., *1/*2, *1/*3) and poor metabolizers (PM) (e.g., 2/*2, *2/*3 and *3/*3). **Objective:** To determine if patients with allelic variations in CYP2C19 have worse outcomes after HSCT.

Methods: We analyzed DNA samples from 344 patients who had allogeneic HSCT between 2002 and 2008. Genotyping was performed to detect the *1,*2 and *3 allelic variants of CYP2C19. Progression-free survival (PFS) and overall survival (OS) were determined for EM, IM and PM. We recorded all medications in the PM group.

Results: The EM, IM and PM phenotype was found in 249 (72.4%), 83 (24.1%) and 12 (3.5%) patients, respectively. CYP2C19 PM had significantly worse overall survival (3-yr: 25%) than EM and IM (55%) (Figure, adjusted HR=2.78, p=0.004). PFS was also significantly worse in PM (3-yr: 25%) versus EM and IM (53%) (adjusted HR=2.38, p=0.008). A total of 192 different medications were administered to the twelve PM. Of the drugs metabolized by CYP2C19, seven of the nine patients who died received voriconazole and four received omeprazole. None of the three surviving patients received either drug.

Conclusions: The significantly worse overall survival in CYP2C19 *2 homozygotes may be due to significantly higher concentrations of CYP2C19 substrates such as voriconazole. This may lead to not only direct voriconazole toxicity but also to exacerbated drug-drug interactions and altered concentrations of immunosuppressants and antibiotics. We propose testing for the CYP2C19 *2 allele in patients undergoing HSCT and careful monitoring of drug levels in the homozygotes.



C-70

Urinary Cystatin-C As An Early Biomarker Of Acute Kidney Injury Following Abdominal Aortic Aneyrysm Repair

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Background: Acute kidney injury (AKI) is a common and often serious complication after surgical abdominal aortic aneurysm repair (AAAr). Recent findings demonstrate that elevated levels of urinary Cystatin-C (uCys-C) may reflect tubular dysfunction. Our aim was to evaluate the utility of uCys-C as a diagnostic marker of AKI in patients undergoing AAAr surgery.

Methods: In a prospective study we included 50 patients undergoing elective AAAr surgery. All patients had serum and spot urine collections before surgery (baseline) and at 6, 24 and 48 hours post-surgery thereafter. A final collection was made on day 5. Exclusion criteria included neoplasia, end-stage renal and liver disease, and recent surgery.

uCysC was quantified by latex particle-enhanced turbidimetric immunoassay using a commercial CysC kit (Sentinel, Milan, Italy) on Architect ci8000 analyzer (Abbott Laboratories, Il.) with intra- and inter-assay coefficient of variation <5.0%. Serum Creatinine (sCr) was determined with the Jaffe reaction using Abbott reagents on the same analyzer. Estimated glomerular filtration rate (eGFR) was calculated with the MDRD equation.

AKI was defined by using the AKIN (Acute Kidney Injury Network) criterion: an absolute increase in sCr above baseline of at least 0.3 mg/dL or a percentage increase of at least 50%. AKI status was determined at baseline and approximately 48 h later.

Results: The mean age (±SD) of the patients was 72.5 (±8.8) years. Ten patients (20%) developed AKI. The following table summarizes our results. Subsequent post-hoc comparisons indicated that as early as 6 hours after surgery those who developed AKI increased their uCysC levels significantly from baseline as well as from those that do not develop AKI. Same analysis for sCr shows similar significant increase at 48 hours.

Conclusions: Our results indicate that uCysC is superior to sCr in the early diagnosis of AKI following abdominal aortic aneurysm repair surgery.

		Baseline Median(IR)	6_hours Median(IR)	24_hours Median(IR)	48_hours Median(IR)	Day_5 Median(IR)	*Difference p(*)*
sCrea mg/dL	AKI	1.09 (0.28)	1.04 (0.33)	1.20 (0.68)	1.48 (0.62)	1.47 (0.52)	*p<0.05*
	noAKI	0.90 (0.31)	0.82 (0.30)	0.95 (0.44)	0.85 (0.26)	0.87 (0.38)	*ns*
	Difference p(*)	*ns*	*ns*	*ns*	*p<0.001*	*p<0.005*	
eGFR ml/ min	AKI	66.99 (23.62)	76.55 (31.31)	61.31 (35.98)	43.36 (33.17)	48.51 (29.86)	*p<0.05*
	noAKI	87.87 (29.00)	99.19 (36.43)	92.08 (45.42)	94.48 (32.77)	91.41 (44.09)	*ns*
	Difference p(*)	*ns*	*ns*	*p<0.05*	*p<0.0005*	*p<0.001*	
uCusC mg/L	AKI	0.08 (0.06)	0.35 (0.49)	0.81 (1.29)	1.10 (4.17)	0.39 (3.94)	*p<0.0001*
	noAKI	0.07 (0.07)	0.05 (0.05)	0.09 (0.10)	0.09 (0.12)	0.07 (0.05)	*ns*
	Difference p(*)	*ns*	*p<0.0001*	*p<0.001*	*p<0.0005*	*p<0.0005*	

(*)Kruskall-Wallis

C-71

Low Serum Albumin Levels At 48-Hours After Acute Ischemic Stroke Correlate With Outcome

K. Makris[1], K. Koniari[2], L. Spanou[1], H. Gialouri[2], O. Glezakou[2], I. Papassotiriou[3], I. Drakopoulos[1]. *[1]Department of Clinical Biochemistry, "KAT" General Hospital, Athens, Greece, [2]Department of Internal Medicine, "KAT" General Hospital, Athens, Greece, [3]Department of Clinical Biochemistry, "Aghia Sophia" Children's Hospital, Athens, Greece,*

Background: Animal studies show a neuroprotective effect of serum albumin in ischemic stroke. The neuroprotective effect of albumin in ischemic stroke in humans is not well studied. Our aim was to determine whether serum albumin levels are correlated with severity, outcome and mortality after ischemic stroke.

Methods: In a prospective study, we included 80 patients with ischemic stroke. Serum albumin was measured at the time of admission and at 24, 48 and 72 hours

thereafter. A final measurement was performed on day 7. Measurements performed on Architect-8200 biochemistry analyzer, (Abbott,Il) with intra- and inter-assay coefficients of variation of 0.8 and 0.9% respectively. Stroke severity was assessed at the time of admission with the Scandinavian Stroke Scale (SSS). Functional outcome was measured with the modified Rankin scale (mRS) on day 7. Patients categorized into 3 severity groups according to mRS-score: mild (mRS-score 0-2), moderate (mRS-score 3-4) and severe (mRS-score 5-6).

Results: The table summarizes the results of the study. The mean age (±SD) of the patients was 75.8 (±8.8) years. Twenty three patients (28.8%) died during a follow-up period of 1 year. At baseline median serum albumin levels were comparable among three patient groups as well as between survivors and non-survivors. At 48 hours survivors had significantly higher ($p<0.0001$) albumin levels (3.95mg/dL) compared to non-survivors (3.50mg/dL). In the severe group serum albumin decreased significantly to a minimum of 3.45mg/dL at 48 hours and remained low until day7 (3.50mg/dL), while in the mild and moderate groups similar significant decreases (3.95 and 3.65 mg/dL respectively) were followed by a slight recovery (4.10 and 3.95 mg/dL respectively).

Conclusions: Our results indicate that high serum albumin is associated with better outcome and lower mortality in ischemic stroke patients. High serum albumin may be neuroprotective in ischemic stroke in humans

	baseline Median(IR) mg/dL	24_hours Median(IR) mg/dL	48_hours Median(IR) mg/dL	72_hours Median(IR) mg/dL	Day_7 Median(IR) mg/dL	*Difference (p*)*
Survivors	4.20 (0.45)	4.00 (0.50)	3.95 (0.50)	4.05 (0.60)	4.10 (0.60)	*p<0.005*
Non-survivors	4.10 (0.55)	3.80 (0.55)	3.50 (0.38)	3.50 (0.50)	3.70 (0.73)	*p<0.0005*
Difference (p)*	*ns*	*ns*	*p<0.0001*	*p<0.0001*	*p<0.005*	
Mild	4.20 (0.50)	4.00 (0.55)	3.95 (0.40)	4.10 (0.55)	4.10 (0.60)	*p<0.005*
Moderate	4.30 (0.43)	4.00 (0.35)	3.65 (0.78)	3.85 (0.85)	3.95 (0.33)	*p<0.05*
Severe	4.10 (0.60)	3.80 (0.70)	3.45 (0.53)	3.50 (0.70)	3.50 (0.90)	*p<0.0005*
Difference (p)*	*ns*	*ns*	*p<0.0001*	*p<0.0005*	*p<0.,005*	

* *Kruskall-Wallis-test*

C-72

The Effect of Glitazones on Levels of Asymmetric Dimethylarginine in Type 2 Diabetes

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Background:Cardiovasculer events are the leading cause of death in type 2 diabetic. Therefore, it is very important to know cardiovascular effects of antidiabetic drugs. Thiazolidinediones (TZDs) are effective drugs in glysemic control of type 2 diabetes. The aim of this study was to assess the effects of TZDs on cardiovascular risk factors and oxidative stress in patients with type 2 diabetes.

Methods: Fourty patients whose therapies were modified by adding either pioglitazone (group 1) or rosiglitazone (group 2) in addition to their previous therapies were observed for three months. Blood samples were taken before and after the treatment. Cardiovascular risk markers such as asymmetric dimethylarginine (ADMA), hsCRP, homocysteine and lipid peroxidation and product malondialdehyde (MDA) were measured in the samples.

Results: In none of the groups serum ADMA and homocysteine levels showed a significant difference after the therapy ($p>0.05$). In both groups, the levels of hsCRP were significantly lowered ($p<0.05$). Although there was a non-significant decrease of MDA levels in group 1 it was significant in group 2 ($p>0.05$ and $p<0.01$ respectively).

Conclusion: The results of this study showed that the use of pioglitazone and rosiglitazone in patients with type 2 diabetes reduced the level of hsCRP, a arteriosclerotic risk factor, and especially the use of rosiglitazone decreased the oxidative stress. However, both drugs had no positive effect on serum levels of ADMA and homocysteine.

C-73

Vitamin D Testing outcomes in a Large Rural Medical Center and considerations of the Institute of Medicine Report

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The objective of this study was to review our experience with Vitamin D testing and to examine the potential impact that adopting the recent Institute of Medicine report recommendations would have on the outcomes. Immunological 25-hydroxyvitamin D testing (DiaSorin) was initiated in our laboratory 18 months ago. The laboratory performs testing for the Geisinger Health System in central PA, which has an ethnically stable, rural population. Since then we have performed approximately 75,000 tests. Reference ranges were established in consultation with staff rheumatologists who recommended the four-tiered system in Table 1. Outcomes are tallied monthly, to track analytical performance and population-based changes with 2010 results shown below. Tests/month (value, SD) was4,308 (350), with outcomes being: Deficient 20.5% (6.2), Insufficient 31.6% (2.3), sufficient 47.8% (6.7), and high 0.2% (0.1).

Table 1.	Deficient < 20 ng/mL	Insufficient 20 to 30 ng/mL	Sufficient 30 to 100 ng/mL	High > 100 ng/mL
Adults (>18 yo)	11,194 (21.9%)	15,918 (31.1%)	23,889 (46.7%)	109 (0.2%)
Women	8,110	11,847	18,585	82
Men	3,084	4,071	5,304	27
Adolescent (12 to 18)	159 (21.3%)	254 (34.0%)	329 (44.1%)	4 (0.5%)
Girls	85	148	180	1
Boys	74	106	149	3
Children (< 12 yo)	46 (13.3%)	104 (30.1%)	195 (56.5%)	0
Girls	17	40	97	0
Boys	29	64	98	0

The data show progression of deficiency with age, although the same trend does not carry through for all categories. 53% of patients with low, i.e., deficient or insufficient, values is consistent with reported urban population-based inadequate Vit D levels. The recent IOM Report on Dietary Reference Intakes for Calcium and Vitamin D suggested, based on a literature review, that relative deficiency of Vit D has been overdiagnosed. The IOM also recommends a four-tiered model with Deficiency at <12 ng/mL, Inadequacy 12- 20 ng/mL, Sufficiency 20- 50 ng/mL, and a zone of Concern > 50 ng/mL. Switching to the IOM range would reduce low values in our lab from 53% to 21.9%, yet, without evidence-based clinical data, the failure to offer supplementation at levels between 20 and 30 could incur liability as an arbitrary change in practice. Vitamin D, while relatively well-studied in bone and calcium metabolism, is a ubiquitous hormone with unelucidated actions in other pathways. Thus, we have decided to retain current reference ranges, given that Vit D supplementation has very little intrinsic risk.

C-74

Relationship between gamma-glutamyltranspeptidase and indices of metabolic syndrome in young women with excessive body weight

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Background: The mechanism of relationship between obesity, insulin resistance and gamma-glutamyltranspeptidase activity (GGTP) has not been fully clarified. We investigated the association between GGTP and components of metabolic syndrome in young overweight and obese women.

Methods: GGTP, fasting glucose, insulin and lipids were determined (ARCHITECT ci8200) in blood samples obtained from overweight and obese (n=98;BMI≥25kg/m^2) women aged 25-40 yrs and age-matched healthy controls (n=39; BMI<25kg/m^2). Anthropometric measurements and blood pressure (BP) were determined. Insulin resistance was assessed using HOMA-IR. In 59 subjects metabolic syndrome (MetS) was diagnosed (Intl Diab Federation criteria 2005).

Results: GGTP was elevated (>39 U/L) in 13% women with excessive BMI and 22% with MetS. Median GGTP (17U/L) was higher in overweight and obese compared to controls (10 U/L ; $p<0,0002$) and higher in women with MetS compared to these without MetS (20U/L vs 13U/L; $p<0,00001$). In obese and women with MetS, GGTP positively correlated with waist circumference, systolic and diastolic BP, insulin and HOMA-IR. In obese women with insulin resistance GGTP was significantly higher than in obese with normal insulin sensitivity (22 vs 17 U/L; $p<0,02$). Similarly,

GGTP was higher in these with MetS and insulin resistance (23 vs 17 U/L; p<0,02). Moreover, GGTP was an independent predictor of diastolic BP and HOMA-IR (β=0,35; p= 0,002 and β= 0,23; p= 0,04) in obese and also an independent predictor of diastolic BP in MetS (β= 0,30; p= 0,02).

Conclusion: High normal GGTP activity can be useful for selecting obese women at high risk for insulin resistance and may be considered a predictive factor of metabolic syndrome.

C-75

Value of ischemia-modified albumin in the management of chronic liver disease

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Background: Ischemia-modified albumin (IMA) is used as a cardiac or intestinal ischemic marker. A recent study indicates that the ratio of IMA and albumin - IMAR correlated with the severity of decompensated cirrhosis of liver.

Methods: We studied the role of IMAR with standard liver function tests including bilirubin, albumin, Child-Turcotte-Pugh (CTP) score, model for end-stage liver disease (MELD) score, international normalized ratio (INR) and indocyanine green (ICG) in assessment of patients with chronic liver disease. Blood samples from healthy volunteer (n = 51), patients with chronic hepatitis (n = 14), and cirrhosis of liver (n = 21) were included.

Results: IMA in patients with cirrhosis was significantly higher, compared to that in healthy subjects [mean (SD): 0.71 (0.10) vs. 0.48 (0.05), p<0.0001], but was similar to that in patients with chronic hepatitis [0.69 (0.14)]. IMAR in patients with cirrhosis was significantly higher, compared to that in patients with chronic hepatitis [mean (SD): 0.206 (0.054) vs. 0.160 (0.055), p<0.01], and compared to that in healthy subjects [0.100 (0.014), p<0.0001]. In patients with chronic liver disease, IMAR was significantly correlated with bilirubin (r=0.493, p<0.01), INR (r=0.605, p<0.005), MELD score (r=0.374, p<0.05) and ICG (r=0.712, p<0.0001).

Conclusion: Our data suggest that IMAR may be a supplemental test to currently used liver function tests.

C-76

Incidance of familial mediterranean fever gene mutations in children in Van region and its relationship with cytokines

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Background: Familial Mediterranean Fever (FMF) is an autosomal recessive disease, and it is characterized by recurrent inflammatory attacks of the serous membranes which cause periodic abdominal pain, fever and joint pain. Even if the mutations in the gene of MEFV have been shown to be responsible for the disease, the physiopathology of the disease seems more complex than it has been known. It has been considered that various cytokines have also played a role in the pathogenesis of the disease. The aim of this study was to screen MEFV gene for 12 mutations in children with FMF diagnosis and to evaluate the role of cytokines in the development of the disease by comparing cytokine levels in controls and in active and passive periods of the disease.

Methods:The study included 157 patients aged 5-15 years. The patients were divided into two groups as active (n = 81) and passive (n = 76) according to their clinical findings. In addition, 30 children were included in the study as a control group. Mutations of MEFV gene in patients were examined by analysis of reverse hybridization. In addition, the levels of IL-1β, IL-6, IL-8, IL-10, TNF-α and CRP were measured both in patient and control groups.

Results:A total of 66,87% of the patients had mutation, of which 42,7% had heterozygous, 11,5% compound heterozygous and 12,7% homozygous mutations. The most frequently observed mutations were E148Q heterozygous (22,92%), M694V homozygous (10,82%) and M694V heterozygous (8,28%). The alleles which were most detected were M694V (40,0%), E148Q (32,41%) and V726A (11,72%). Furthermore, while the level of IL-1β in the active group was found higher than that of the control group, the levels of IL-8, TNF-α and CRP were higher both in the active and in the passive group than those the controls (p <0.05). While the levels of IL-6 both in the active and in the passive group were higher than those of the control group, the level of active group was also significantly higher than that of the passive group (p <0,001).

Conclusion: The results of this study have supported the heterogeneity of the mutation of MEFV gene in patients with FMF and have shown that our patients have a wide range of mutations. In addition, it is thought that, the levels of IL-8, TNF-α, CRP and especially IL-6 could be used in the diagnosis of acute attack and monitoring the response to the treatment. However, increased cytokine levels in the passive period have supported the view that the subclinical inflammation has continued in these patients.

C-77

Improved Architect Lh Assay For Determination Of Concentration Of Luteinizing Hormone In Human Serum And Plasma

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Background: Determination of the concentration of human luteinizing hormone (LH, Lutropin) is instrumental for the predication of ovulation, in the evaluation of infertility, and in the diagnosis of pituitary and gonadal disorders. The ARCHITECT LH assay is a chemiluminescent microparticle assay (CIMA) for the quantitative determination of human luteinizing hormone (LH) in human serum and plasma. A reformulated assay was launched in 2011 allowing the customer to discontinue current practice of matching reagent, calibrators and controls. The new assay uses a 6-point Standard Calibration curve which is referenced to the WHO Luteinizing Hormone Human Pituitary 2nd International Standard 80/552 and can be run with any commercially available control.

Methods: Precision and Lot-to-Lot reproducibility of the ARCHITECT LH assay was determined across the measuring range based on guidance from CLSI protocol EP5-A2 using serum and plasma based panels. All panels were assayed on 2 reagent lots and 2 instruments, in replicates of 3 at two separate times per day over a 20 day period. Correlation with the preceding ARCHITECT LH assay was evaluated (CLSI EP9-A2-IR) on 107 unique specimens across the range of 0 to 250 mIU/mL using the Passing-Bablok regression method. Sensitivity (e.g. Limit of Quantitation (LoQ, CLSI EP17-A), Limit of Blank (LoB) and Limit of Detection (LoD)), Linearity (CLSI EP6-A) and accuracy by recovery were determined. Expected values were established for normal males (n=199), normal cycling females (n=64) and post-menopausal females (n=124).

Results: The total (within laboratory) imprecision (%CV) of the assay was determined using samples with LH concentrations across the claimed measuring interval (0.09 mIU/mL to 250.00 mIU/mL) and ranged from 2.4% to 8.9%. Analytical sensitivity (LoQ) across 2 instruments and 2 reagent lots was determined as 0.09 mIU/mL (LoB=0.01 and LoD=0.03 mIU/mL). Correlation slope to the preceding ARCHITECT LH assay was 1.04 (Y=1.04x - 0.27) with a correlation coefficient of r=0.99. The assay demonstrated linearity within the range of LoQ to 250.00 mIU/mL with an absolute deviation from linearity of <=1 mIU/mL for samples within LOQ and 10 mIU/mL, <=11% for samples within 10 and 70 mIU/mL, and <=15% for samples above 70 mIU/mL. Mean recovery determined on 15 specimen spiked with known LH concentrations was 101.2%.

Conclusion: The reformulated ARCHITECT LH assay showed excellent precision and sensitivity as well as good correlation with the preceding ARCHITCET LH assay. High lot-to-lot reproducibility allows more flexibility in managing inventory; matching of reagents and calibrators is no longer required. The ARCHITECT LH assay is a valuable tool in clinical laboratories for the accurate and precise determination of human luteinizing hormone (LH, Lutropin) in human serum and plasma.

C-78

Neutrophil Gelatinase-Associated Lipocalin as a biomarker for Acute Kidney Injury during and after Liver Transplantation

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Backgrounds: Acute kidney injury (AKI) is a common complication in patients undergoing liver transplantation and a significant prognostic factor for their long-

term outcome. The diagnosis of AKI is based on an increase in serum creatinine (Cr) concentrations; however, serum Cr levels do not increase until approximately half of the kidney function is impaired. Recently, most promising biomarker for AKI, neutrophil gelatinase-associated lipocalin (NGAL) has been used for the early detection of AKI. In this study, we evaluated the urine and plasma levels of NGAL as biomarker for AKI during and after living-related liver transplantation.

Materials and Methods: We prospectively enrolled 19 adult patients with living-related liver transplantation from April to June 2010. Serial blood and urine samples (baseline and at 2, 4, 6, 10, 16, 24, 30, 40, and 48 h after reperfusion) were collected. We also reviewed the patients' electronic medical records. The concentrations of NGAL in plasma and urine were measured using a The NAGA test (Bio-Porto Diagnostics, DenmarK) and the ARCHITECT Urine NGAL assay (Abbott Diagnostics, USA), respectively. Urine NGAL levels were corrected with urine Cr to compensate dilution and/or concentration effect according to urinary output. AKI was defined by Risk-Injury-Failure-Loss-End stage kidney disease (RIFLE) criteria as a 50% or greater increase in serum Cr from baseline. Currently, there is no established diagnostic criteria for AKI according to NGAL level, so in this study we defined AKI as 50% or greater increase in plasma NGAL and urine NGAL/urine Cr from baseline. And we compared time to increase of more than 50% from baseline after reperfusion.

Results: The median age and gender ratio (male:female) were 52 years (range, 39-65 years) and 3.75:1, respectively. Eleven out of 19 patients were diagnosed as AKI according to RIFLE criteria. Urine NGAL/urine Cr was increased immediately after reperfusion and peaked 4 h later. The increment in urine NGAL/urine Cr preceded the 50% elevation in serum Cr by 19.0 h (range 0-43 h, $p = 0.011$) after reperfusion. And also plasma NGAL elevated 13.0 h earlier than serum Cr, however, it was marginally statistically significant ($p = 0.075$).

Conclusions: The urine NGAL level seems to be compensated with urine Cr. And urine NGAL/urine Cr could be used as a biomarker for the early detection of AKI in patients undergoing adult LRLT.

C-79

Is Pancreatic Gland Affected In Patients With Septic Shock?

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Background. Hyperamylasemia has been observed anecdotally during the course of severe sepsis or septic shock.

Aim. To investigate the possibility of pancreatic involvement in patients with septic shock using serum pancreatic enzyme determinations and imaging techniques.

Methods. Twenty-one consecutive patients with septic shock and 21 healthy subjects were enrolled as controls in the study. The serum activity of pancreatic amylase and lipase were assayed initially in all subjects and 24 and 48 hours after the initial observation in patients with septic shock. All septic shock patients also underwent radiological examination to detect pancreatic abnormalities. Serum amylase and lipase activities were assayed in all patients using commercially available kits (Amylase, Roche Diagnostics, Milan, Italy, reference range 17-115 U/L, the withinrun %CV (n = 20) was 1.6 at 38.5 U/L and 1.5 at 225 U/L and the between-run %CV (n = 20) was 1.4 at 38.6 and 1.2 at 280 U/L; lipase, Roche Diagnostics, reference range 13-60 U/L, the withinrun %CV (n = 20) was 1.16 at 34 U/L and 0.72 at 71 U/L and the between-run %CV (n = 20) was 0.65 at 35 and 0.87 at 63 U/L). Results. The serum activity of pancreatic amylase were significantly higher in patients with septic shock than in the control subjects during the study period (initial observation P=0.003; 24 hours after initial observation P<0.001; 48 hours after initial observation P<0.001), while the serum activity of lipase were similar to those of the control subjects (initial observation P=0.762; 24 hours after initial observation P=0.667; 48 hours after initial observation P=0.938). Amylase and lipase serum activity did not significantly changed throughout the study period in patients with septic shock. Two patients (9.5%) one with upper gastrointestinal tract injury due to the ingestion of caustic substances and the other with infection of an aortic prosthesis, had serum activity of pancreatic amylase more than three times the upper reference limit while none of the patients studied had serum lipase activity more than three times the upper reference limit. Taking into account those patients who died during hospitalization and the survivors, no significant differences in the serum activity of amylase and lipase were found between survivors and non-survivors in the three days of the study. None of the patients with pancreatic hyperenzymemia had clinical signs or morphological alterations compatible with acute pancreatitis.

Conclusions. The presence of pancreatic hyperenzymemia in septic shock patients is not a biochemical manifestation of acute pancreatic damage, and the management of these patients should be guided by the clinical situation and not merely by evaluation of the biochemical results.

C-80

Prevalence of decreased Glomerular Filtration Rate (< 60 mL/min/1.73 m2) in genersal population of Spain as estimated by different prediction equations

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Background. Chronic kidney disease (CKD) is a significant public health problem whose diagnosis and staging relies upon Glomerular filtration rate (GFR)-estimating equations, used to determine the prevalence of CKD in population-based studies. However, it has been suggested that since the commonly used GFR equations were originally developed from samples of patients with CKD, they may yield a misleading estimatation of GFR in healthy populations. The aim of our study was to compare the CKD-EPI and MAYO Clinic quadratic (MCQ) equations to the MDRD equations [MDRD4-IDMS, MDRD6] in the general population of Asturias, north coast of Spain.

Methods. We enrolled (simple random sampling) 960 adults in this study [sex: 542 females (56.45 %); age: median, 50 y; range, 18-89 y]. Creatinine was measured by an alkaline picrate reaction method (ROCHE Diagnostics) calibrated to be traceable to isotope-dilution mass spectrometry (IDMS).

Results. Prevalences of CKD stage 3 in men were: MDRD4-IDMS, 8.6%; MDRD6, 5%; CKD-EPI, 7.7%; MCQ, 2.4%. Prevalence of CKD stage 3 in women (%) were: MDRD4-IDMS, 22.2% (88.3% 3a: GFR=45-59 mL/min/1.73m2); MDRD6, 10.5%; CKD-EPI, 12.9%; MCQ, 2.0%. We found that the MDRD4-IDMS, the current standard to estimate GFR, appeared to overestimate the prevalence of CKD in general population, specially in the female population.

Conclusion. These results support the fact that the new CKD-EPI equation, and specially the MCQ, reclasify to stage 2 (higher GFR) an important number of women clasified as CKD stage 3a by MDRD-IDMS .

C-82

To Study the pathological mechanisms of hepatorenal syndrome

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Objective: Hepatorenal syndrome (HRS) is caused by severe renal vasoconstriction without structural changes in the kidneys consequent upon splanchnic and peripheral vasodilatation, resulting in decreasing effective arterial volume and renal hypoperfusion. Plasma exchange(PE) is a specialized form of dialysis that clears free and albumin-bound substances, including vasodilators, and therefore can potentially reduce systemic vasodilatation in type 1 HRS in acute-on-chronic hepatic failure(AoCHF) patients with ascites. This study was designed to evaluate the efficacy of PE in improving systemic and renal hemodynamics in AoCHF with ascites and type 1 HRS and to explore the pathogenetic mechanism of HRS.

Methods: A pilot study was carried out in an academic teaching hospital. The study group comprised twenty patients with AoCHF , ascites and type 1 HRS . All patients received 6 days of 3 times of PE dialysis. The main outcome measures were Pre-PE, during PE and post- PE measurements of blood pressure, heart rate, 24h urinary volume, liver function indicators, renal function indicators, serum nitric oxide(NO), cardiac troponin T(cTnT),brain natriuretic peptide (BNP), aldosterone , interleukin -6(IL-6),tumor necrosis factor-α(TNF-α)and plasma ammonia, measured before plasma exchange, in three plasma exchange process and after plasma exchange.

Results: NO level of HRS patients before PE was 113.7 ±26.3umol/L, one day after three times' PE was 78.3 ±24.7 umol/L, three days after three times' PE was 85.3 ±29.8 umol/L, NO levels after PE were lower than that before PE (P<0.5). Creatine level of HRS patients before PE was 191.0±43.7umol/L, one day after three times' PE was 142.8±42.6umol/L, lower than that before PE (P<0.5). After three days by three times' PE, creatine level was 221.9±105.2umol/L, higher than that before PE (P<0.5). Before, during-and post-PE treatment, the levels of sodium was lower than the normal range(normal range: 135-145mmol/L), the level of aldosterone was high than the normal range(normal range:10.0-27.0ng/L), the level of cTnT was high than the normal range(normal range:<14ng/L) and the level of BNP was higher than the

normal range(normal range:<366ng/L).MELD, bilirubin, urea, cysteine proteinase inhibitor C and ammonia levels were decreased during PE, but increased post-PE. Systolic pressure and 24 h urinary volume was decreased gradually.

Conclusion: In patients with AoCHF , ascites and type 1 HRS , PE is ineffective in improving systemic haemodynamics and renal function .The reduction of NO levels did not improve splanchnic and peripheral vasodilatation. Serum NO was not related to hepatorenal syndrome. Hyponatremia and cardiac dysfunction could be the important in the occurence of HRS .

C-85

Selected Laboratory Test Volumes in the Out-Patient Medicare Population, 2000-2009

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Background: Changes in clinical laboratory testing practices in the US can help to assess the impact of testing guidelines, and provide information for health-care and public health policy decisions. In 1996, laboratory test utilization was evaluated in a random, stratified sample of US clinical laboratories (Arch Pathol Lab Med 2000;124:1201-1208), providing volumes for the most commonly ordered laboratory tests; however, there has been no report of longitudinal trends in national laboratory test utilization. We present an overview of laboratory test reimbursement for the out-patient Medicare population between 2000 and 2009.

Methods: Laboratory test reimbursement volumes were obtained from the Centers for Medicare & Medicaid Services (CMS) Medicare Part B data, and were normalized by the number of Medicare enrollees each year. The ratio of the most recent (2009) divided by the oldest (usually 2000) test reimbursement volume per Medicare enrollees (“volume ratio”) during this decade was used as a measure of trends in test utilization. Laboratory tests were selected based on reimbursement claim frequency (at least once in 2009 per 1000 Medicare enrollee). Another criterion for test inclusion was the presence of a pronounced increase or decrease in utilization. A few tests were listed under more than one Current Procedural Terminology (CPT) codes in which cases, reimbursement volumes were combined.

Results: The greatest volume ratio during the 2000-2009 period occurred for the following tests: methadone (303×), opiates (266×), amphetamines (217×), vitamin D (62.0×), human papilloma virus (HPV) DNA (61.7×), benzodiazepines (25.0×), urine creatinine (10.6×) and myoglobin (10.1×). The lowest volume ratio was for digoxin (0.37×). Of interest were increases in the volume ratio for the following analytes: natriuretic peptide (6.82× since 2003), troponin (4.54×), microalbumin (4.54×), high-sensitivity C-reactive protein (hs-CRP) (4.34×), allergen-specific IgE (3.98×), creatine kinase MB (CK-MB) (3.14×), homocysteine (2.17×), glycated hemoglobin (1.92×), and thyroid-stimulating hormone (1.69×). Also of interest were increases in the use of the following test panels: renal (3.73×), comprehensive metabolic (2.05×), lipid (1.53×), hepatic (1.09×), and basic metabolic (1.07×).

Conclusion: These data show trends that may be related to (1) increased surveillance for drugs of abuse or therapeutic drugs, (2) increased utilization of new tests recommended for use in recent national guidelines [e.g., vitamin D, HPV DNA, myoglobin, natriuretic peptide, troponin, hs-CRP, and glycated hemoglobin], and (3) decreased utilization of a few tests such as digoxin. Despite recent recommendations against using CK-MB for evaluation of risk of cardiovascular events, there was a modestly increasing use of this test in the past decade. These data will be useful to policy makers, health systems researchers, laboratory managers and industry scientists to address and anticipate trends in utilization of laboratory tests in the evaluation of the health of the Medicare population in out-patient care settings.

C-86

Auto-antibodies against disulphide isomerase ER-60 as a possible diagnostic marker in male immunological infertility

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Inflammation and infection of the male genital tract have been reported in up to 15% of cases as main aetiologies of male fertility disturbances. In the majority of these patients, however, diagnosis is hampered by an asymptomatic course of the disease, especially subacute or chronic inflammatory conditions in the testis and/or epididymis remain obscure. Definitive diagnosis of suspected chronic testicular inflammation is based only on invasive testicular biopsy. By proteomic approach using 2D-SDS-PAGE and immunoblotting followed by mass spectrometry, we have identified three proteins (ER-60, transferrin, TCP-1) as immunodominant antigens recognized by auto-antibodies in sera from patients with testicular inflammation. Of note, ER-60 was also identified as an auto-antigen in a rodent model of experimental testicular inflammation.

To establish an immunodiagnostic assay highly purified recombinant human ER-60 (recognized by 92% of sera from patients with testicular inflammation) was selected and used for an ELISA development. Sera from the following groups were screened: healthy normozoospermic men (n=21, control group 1); male blood donors (n=20, control group 2); patients with impaired semen quality according to WHO reference values, without symptoms of genital tract infection/inflammation (n=18, group 3); patients similar to group 3, but with symptoms of genital tract infection/inflammation (n=33, group 4); patients with chronic testicular inflammation confirmed by testicular biopsy (n=14, group 5); patients after pharmacotherapy of genital tract infection/inflammation (n=18, group 6); patients with acute epididymo-orchitis (n=21, group 7).

Significantly increased titers of auto-antibodies against ER-60 were found in the sera from infertile men in group 4 ($p<0.001$) and group 5 ($p<0.01$) as compared to normozoospermic men or healthy men with unknown fertility status (control groups 1 and 2). Compared to group 4, significantly lower levels of anti-ER60 antibodies were measured in the sera from patients after the use of anti-inflammatory pharmacotherapy ($p<0.05$). Anti-ER60 antibody titers in serum samples from other investigated groups did not significantly differ from those of the control groups.

Our preliminary results show that determination of ER-60 auto-antibodies in male serum may be a promising marker for the diagnosis of asymptomatic inflammatory processes in the testis and/or excurrent ducts.

C-87

Investigation of differences of certain disinfectant resistant genes qac*G,H,J* between general population and medical staff

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Background and objective:Several genes increasing tolerance to disinfectants have been described. However, the presence of the recently identified *qacG, H* and *J* have not been demonstrated in human isolates. This text aimed to compare certain disinfectant resistant genes *qacG, H* and *J* between staphylococci isolated from medical staff and general population,to investigate minimum inhibitory concentrations of benzalkonium chloride and chlorhexidine in isolates harboring *qacG, H* and *J* genes and to compare antibiotic resistance patterns between *qac* positive and negative strains.

Methods and Results: 300 medical staff and 800 members of the general population participated in this project, and a total of 865 staphylococci strains were isolated from these subjects. *S.aureus* were identified by the femention of mannitol, positive plasma-coagulase, production of acetoin, absence of β-galactosidase and resistance to polymyxin B. Conventional PCR was required to detect *qac* genes in *S.aureus* and coagulase negative staphylococci. Only nine strains were confirmed to be carrying a *qacG,H* or *J* gene. Of the four strains containing the *qacG* gene, one harbored *qacG* and *qacA/B* and another one hosted *qacG* and *smr* concomitantly. Only one strain harbored the *qacH* gene and four strains were positive for *qacJ*. Eight of these nine strains were *S.aureus* and one *qacJ* isolate was identified as *S.epidermidis*. Minimum inhibitory concentrations (MIC) were determined by broth dilution and the mean MICs of benzalkonium chloride and chlorhexidine in *qacG*, *qacH* and *qacJ* groups were 6 and 2.5 mg/l, 4 and 2 mg/l, 5 and 2 mg/l respectively. All *qacG,H* and *J* strains were susceptible to Quinupristin/Dalfopristin, Linezolid, Ciprofloxacin, Imipenem and Vancomycin. Resistance to Fusidic acid(33.3%), Clindamycin(55.6%), Tetracycline(44.4%) and Gentamycin(33.3%) was observed, and there were significant differences between *qacG,H,J* positive and *qac* negative strains, which indicate the presence of *qacG,H* and *J* genes is probably associated with resistant mechanisms of these antibiotics.

Conclusion: The prevalence of *qacG*, *qacH* and *qacJ* genes are low in both general population and medical staff. However, attention should be paid for the elevated MICs of disinfectants of these *qac* positive strains, which indicate improper use of disinfectants can increase numbers of resistance microorganisms.

C-88

Discussion of using CKD-EPI equation for the calculation of estimated glomerular filtration rate among mild CKD patients in Southern China

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Background: The new chronic kidney disease epidemiology collaboration (CKD-EPI) equation is introduced as a more accurate GFR estimating equation in mild renal injury recently. However, it has been suggested to result in higher estimated glomerular filtration rate (eGFR) than the abbreviated equation Modification of Diet in Renal Disease (abbreviated MDRD) equation. The results of applicability evaluation of CKD-EPI equation were not consistent in Chinese populations hence this study is aim to evaluate the CKD-EPI equation in mild chronic kidney disease (CKD StageI-II) in Southern China.

Methods: 421 CKD patients whose residual GFR (rGFR) were more than 60 mL/min/1.73 m^2 in Southern China were included in the study (213 males and 208 females). Serum creatinine values were determined using the Jaffe method, and the plasma clearance of ^{99m}Tc-DTPA was used to measure rGFR. A comparison between CKD-EPI equation and abbreviated MDRD equation was done. And the results of bias, accuracy and Bland-Altman analysis were calculated and comprised then.

Results: The mean of rGFR (^{99m}Tc-DTPA clearance) was 78.2ml/min/1.73m^2. And the bias, accuracy of 30% and 50% of CKD-EPI equation were -2.7, 88.2%, 93.4% respectively while that for the abbreviated MDRD equation were -8.7, 78.7%, 90.1%. Bland-Altman plots of CKD-EPI equation showed a greater dispersion of values and a less satisfactory correlation when rGFR were more than 75 mL/min/1.73 m^2. There might be a possible overestimate of the eGFR when CKD-EPI equation was used.

Conclusion: In this study, the CKD-EPI equation appeared to be smaller bias and more accurate when GFR in mild CKD patients was estimated in Southern China. But it may result in a higher eGFR at the same time.

C-89

A comparison of GFR estimation equations based on serum cystatin C and serum creatinine in CKD patients in Southern China

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Background: Serum cystatin C (s-CysC) has been reported as an improved potential alternative filtration marker to serum creatinine (s-Cr). Therefore, s-CysC-based prediction equations were expected to be more precise or accurate for GFR estimation. This study aims to develop and evaluate the s-CysC-based prediction equation for different stages of chronic kidney disease (CKD) in Southern China.

Methods: S-CysC and s-Cr were measured in 242 CKD patients with different degrees of renal dysfunction. The plasma clearance of ^{99m}Tc-DTPA was used to measure residual GFR (rGFR). And these 242 patients were divided into two groups. Group 1 was used to calculate GFR prediction equation by using multiple linear regression analysis while Group 2 was used to verify the equation. Then all of these equations together with the abbreviated MDRD equation and CKD-EPI equation were tested in the Group 2.

Results: In Group 2, the mean of rGFR (^{99m}Tc-DTPA clearance) was 67.9ml/min/1.73m^2 while that of s-CysC was 1.21mg/l and mean s-Cr was 1.39 mg/dl. And the bias for the s-CysC-based equations was in the range of -4.6 to 1.2ml/min/1.73m^2, while that for the s-Cr-based equations was in the range of -4.4 to 4.2 ml/min/1.73m^2. Moreover, when the rGFR of patients was higher than 60mL/min/1.73 m^2, the accuracy of 30% of s-CysC-based prediction equations and s-Cr-based prediction equations were ranged from 80.4 to 88.2% and 73.7-85.9%, respectively. But the accuracy would decrease, ranging from 54 to 68.9% for s-CysC-based equations , and 51 to 62.2% for s-Cr-based equations if the rGFR was lower than 60mL/min/1.73 m^2.

Conclusion: S-CysC-based prediction equations are more accurate than abbreviated MDRD equation, while CKD-EPI equation is not. Therefore, both s-CysC-based prediction equations and CKD-EPI equation may have advantages in the earlier detection of renal impairment.

C-91

Reference intervals of and factors contributing to serum Cystatin C

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Background: The objective of this study was to describe the reference range of serum cystatin C (Cys-C) and identify factors associated with serum Cys-C or its variability, including age, gender, Creatinine (CREA), BUN and Uric Acid.

Methods: Serum Cys-C, CREA, BUN and Uric acid were measured in 4517 participants aged 8 to 89 yr attending West China Hospital of Sichuan University in China for routine health checks. Serum Cys-C samples were analyzed using an Latex Enhanced Imnunotuibidimetric Method. CREA were tested by jaffe method, BUN and Uric acid by Kinetic UV assays. All analyses work on automated clinical chemistry analyzers.

Results: The predominant characteristic of CyS-C distribution is that Cys-C concentration in age>=60 years group were relatively highest (P<0.05). The differences of Cys-C concentration between males and females existed when subjects were aged from 20 to 60 years (P<0.05). In a multiple model adjusted only for gender and age, compared with age((β=0.003), gender(β=0.007) has stronger effect on Cys-C levels. The clinical variables, comprising of age, gender, CREA, BUN and Uric acid, involved in the fully adjusted equation accounted for 37.6% of variation of Cys-C, while factors, comprising of age and gender accounted for 10.6% of variation of Cys-C.

Conclusion: 95% reference intervals of healthy population were partitioned into three categories only by age, 0.61~1.13mg/L for all subjects aged from 8 to 19 years; 0.74~1.13mg/L for all subjects aged >60 years;0.60~1.06mg/L for males and females aged 20-60 years.Serum cystatin C is significantly related to gender, age, uric acid, CREA and BUN. Besides, there are still more factors contributing to variants of Cys-C levels.

Table 1 Age-varying characteristics of Cys-C , CREA, BUN and Uric Acid

Age groups (years)	n(%)	Age(years) Mean±SD	CYS-C (mg/L)* mean±SD (Range)	CREA(umol/L)* mean±SD (Range)	BUN(mmol/L)* mean±SD (Range)	URIC# mean±SD (Range)
<=19	67	17.1±2.4	0.872 ±0.132* (0.58,1.09)	66.7±13.5* (39.3,99.70)	4.9±1.26 (2.44,7.14)	320.7±66.3 (184.0,487.0)
20~29	674	25.6±2.6	0.806±0.118 (0.52,1.09)	73.0±14.9 (40.6,121.90)	5.01±1.1 (2.74,7.92)	309.9±73.5 (166.0,524.0)
30~39	1258	35.0±2.8	0.814±0.116 (0.51,1.09)	74.6±14.5 (41,118.6)	5.12±1.10 (2.80,10.58)	313±76.7 (158.0,609.0)
40~49	1297	44.0±2.7	0.837±0.121 (0.51,1.18)	74.7±14.4 (38.0,130.1)	5.26±1.15 (2.86,9.53)	311.5±75.7 (151.0,587.0)
50~59	769	54.3±2.5	0.887±0.108 (0.54,1.17)	74.8±14.1 (38.4,127.1)	5.5±1.17 (3.15,8.56)	312.9±72.3 (160.0,551.0)
>=60	452	66±5.2	0.939±0.101* (0.63,1.19)	75.30±13.6 (46.3,112.5)	5.6±1.21 (3.16,11.30)	308.0±65.7 (132.0,485.0))
total	4517	42±12.5	0.845±0.122 (0.51,1.19)	74.4±14.4 (39.3,130.1)	5.2±1.15 (2.44,11.30)	311.7±74.0 (132.0,609.0)

*P< 0.05; means of Cys-C, CREA and BUN in age groups are different repectively.

#P>0.05

C-92

Cardiac Troponin T elevations following noncardiac surgery

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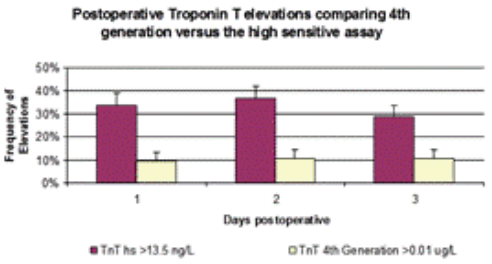
Background: Globally 200-million adults undergo major noncardiac surgery annually, and in excess of 5-million of these patients will suffer a major vascular complication, of which myocardial infarction (MI) is the most common. Because the majority of perioperative MIs occur without ischemic symptoms monitoring cardiac troponin (cTn) measurements following surgery is helpful to avoid missing MIs. Our objective in this study was to measure cTnT using both the 4th generation and high sensitive (hs-cTnT) assays in the first 3 days following surgery to assess the prevalence of injury in this setting.

Methods: Patients (n=299) enrolled in the bio-bank sub-study of the VISION Study (a prospective cohort study of adults undergoing noncardiac surgery) that had

serum in storage (below -80C) on days 1,2,3 following surgery were selected for measurement with the hs-cTnT assay. As part of clinical care, patients also had the 4th generation cTnT assay measured on the same 3 days following surgery (day-1;n=280; day-2;n=272; day-3;n=275). Testing for the hs-cTnT assay in this study cohort was performed over 1 month on the Elecsys 2010 platform (low serum pool (mean=12 ng/L; n=32) CV=15%). This study received ethics approval.

Results: The average age of the population was 65 years (SD=11), and 52% were male. Postoperatively, using the 4th generation assay the prevalence of injury (i.e., >0.01 ug/L) was 10% (95%CI:7-14%) on day 1; 11% (95%CI:8-15%) on day 2; and 11% (95%CI:7-15%) on day 3. By comparison, the prevalence of injury using the hs-cTnT assay and the 99th percentile from a healthy population (i.e., >13.5 ng/L) was 34% (95%CI:29-39%); 37% (95%CI:32-42%) and 29% (95%CI:24-34%); respectively (Figure).

Conclusion: The prevalence of elevations above the 99th percentile using the hs-cTnT assay is significantly higher than the 4th generation assay. Outcome-based studies are needed to establish cutoffs for the hs-cTnT assay in patients undergoing noncardiac surgery.



C-94

CYP2D6*18 polymorphism on the efficacy of Donepezil in Alzheimer's disease patients

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Alzheimer's disease (AD) is a neurological condition that affects patients mostly over the age of 65. The lack of accurate diagnostic markers for early prediction and effective therapy are the two-most important problems to halt AD progression efficiently. Donepezil is an effective symptomatic treatment for patients with mild to moderate AD metabolized by hepatic isoenzymes CYP2D6.The present study was undertaken to evaluate the CYP2D6*18 genetic polymorphism on the efficacy of donepezil in patients with AD.

Forty five patients with AD were selected according to DSM IV guidelines. Patients were treated with donepezil 10 mg/daily for 3 months. The analysis of CYP2D6*18 polymorphism were done using polymerase chain reaction - restriction fragment polymorphisms (PCR-RFLP) method in a blinded fashion. Plasma donepezil concentration was determined using HPLC method. All subjects underwent a standardized clinical evaluation which included medical history and cognitive function assessment [Mini-Mental State Examination (MMSE). The approval of the study for experiments using human subjects was obtained from the local ethics committees on human experimentation. Written informed consent for research was obtained from each patient or from the relatives or a legal guardian in the case of critically disabled patients with dementia. Patients were excluded from the study if they were unwilling or unable to fulfill the requirements of the study, had clinically significant and unstable medical illness.

At 3 month follow up 29 out of 45 patients were responders and 16 were non responders to donepezil treatment. CYP2D6*18 was shown to be in hardy-weinberg equillibrium and showed significant genotypic association between responders and non-responders to donepezil therapy. CYP2D6*18 Genotypic frequency: P = 0.01; χ2-test= 9.17, Allelic frequency: P= 0.06; OR = 1.09 (0.54-2.2). The average donezepil level in responders was found to be 15.39 ± 7.88 ng/ml and in non responders 2.874 ± 1.53 ng/ml receiving a donepezil dose of 10 mg/daily and the Average Mini mental state Examination score for responders was 24 and for non-responder it was found to be < 20. The single nucleotide polymorphism CYP2D6*18 in the CYP2D6 gene may influence the clinical efficacy of donepezil in patients with mild to moderate AD. The analysis of CYP2D6 genotypes may be useful in identifying subgroups of patients with AD who have different clinical responses to donepezil.

C-95

Evaluating the clinical utility of gender-specific clinical laboratory reference ranges in transgender patients

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Background: Clinical practice guidelines for the treatment of transgender persons were recently published and call for routine laboratory monitoring of patients on cross-sex hormone therapy. However, many of the recommended tests have gender-specific reference ranges leaving clinicians with the dilemma of having to decide what levels are "normal" for each individual patient. The goal of this study is to identify consistent changes in analyte levels with hormone therapy, which could indicate whether changes in these analytes from pre-therapeutic levels should be considered indicative of potential adverse effects or whether it may simply be a part of the desired physiological changes induced by the therapy.

Methods: IRB approval was obtained through Emory University. Twenty self-identified male to female transgender patients on hormone therapy for >6months were enrolled and laboratory data was abstracted from the medical records. Preliminary analyte reference ranges generated from data from these patients was compared to normal range data from 25 male and 25 female non-transgender subjects.

Results: The preliminary range for hematocrit resembled the normal female reference range and was significantly different from the normal male range ($p<0.01$). Conversely, the creatinine range was similar to the male range, but significantly different from the female range ($p<0.01$). While LDL cholesterol was significantly different than both the male ($p<0.0001$) and the female ($p<0.001$) normal ranges. All other analytes including hemoglobin, sodium, potassium, albumin, blood urea nitrogen (BUN), alkaline phosphatase (ALP), alanine transferase (ALT), aspartate transferase (AST), and total cholesterol had notable overlap between the normal male and female ranges.

Conclusion: Use of correct gender-specific reference ranges plays an important role in the interpretation of laboratory results, allowing for more accurate identification of patients in need follow-up testing and care. Preliminary data suggests that there may be a difference in analyte reference ranges for transgender patients receiving hormone therapy compared to non-transgender patients. More extensive studies are needed to generate reliable statistical data confirming these differences.

C-96

Evaluation for renal markers in type 2 diabetic patients

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Background: Diabetic nephropathy is the major microvascular complication that develops in 30-40% of diabetic patients and is associated with a high risk of other vascular complications. It can be progress to renal failure and death, and is initially characterized by microalbuminuria. Although less type 2 diabetic patients develop nephropathy than type 1, diabetic nephropathy of type 2 diabetic patients is getting more important due to the rapidly increasing number of these patients. However, currently used renal markers to assess kidney function are largely insufficient. Thus, there have been extensive efforts to find out a superior indicator of impaired kidney function. Recently, several candidates such as Neutrophil Gelatinase-Associated Lipocalin (NGAL) and N-acetyl-beta-D-glucosaminidase (NAG) were investigated to detect kidney damage earlier than traditional renal markers. In this study, we planned to evaluate several renal markers and clarify relationships of these markers with other parameters in type 2 diabetes mellitus (DM).

Methods: A total of 210 patients with type 2 DM and 46 control individuals were enrolled for this study between March 2010 and October 2010 in KyungHee University Hospital at Gangdong. Each renal markers such as were tested by Commercial Enzyme Linked ImmunoSorbent Assay for urinary neutrophil gelatinase-associated lipocalin (NGAL) (R&D systems, Minneapolis, USA), N-acetylglucosaminidase (NAG) (Nittobo, Tokyo, Japan), cystatin C (Roche, Mannheim, Germany) and adiponectin (R&D systems). Total patients were subsequently classified by a kind of accompanying complications, a degree of albuminuria and glomerular filtration rate (GFR).

Results: The median age of total patients was 55.79 years (range 34~81 years) and the male to female ratio was 1.47. The means of body weight, body mass index and hemoglobin A1c were 68.07 kg, 25.58 kg/m^2 and 7.08%, respectively. In independent t-test, only adiponectin showed significantly increased value in DM patients group than controls (P=0.007). Moreover, as patients group was subclassified with the degree of albuminuria, only adiponectin showed significant difference among

normoalbuminuria, microalbuminuria and macroalbuminuria. When patients group was divided subgroups according to whether had complications or not, adiponectin and cystatin C presented significant differences that were to be increased in patients group accompanying complications (P=0.005 and P=0.02, respectively). Confined to patients with renal complications, NGAL was significantly increased in type 2 DM with nephropathy than in type 2 DM with other complications (P=0.005. In correlation study, there was no significant association among renal markers.

Conclusions: Adiponectin has been focused in many studies about metabolic syndrome like DM. In our study, adiponectin presented the best significance between control group and patients group as well as among normoalbuminuria, microalbuminuria and macroalbuminuria. In the DM patients accompanying complications, adiponectin and cystatin C were increased regardless of the kind of complications. Because cystatin C is also an inflammatory marker, it is needed to rule out the inflammatory effect in patients group. Although NGAL is a verified marker of acute kidney disease, it showed the possibility to be used in chronic renal disease such as DM nephropathy.

C-97

Hypoalbuminema is the Most Important Risk Factor of Early Ascites Formation in Patients with Liver Cirrhosis

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Objective: To study the effect of hypoalbuminema on formation of early ascites in patients with liver cirrhosis.

Methods: Between January 2008 and October 2010, we recruited 118 newly diagnosed patients with liver cirrhosis admitted to West China Hospital of Sichuan University, investigated their clinical data, obtained fasting blood samples for standard hematological (including hemoglobin, platelets, white blood cell), biochemical (including liver, renal function indicators, blood ammonia, and electrolytes), coagulation (including prothrombin time and fibrinogen) tests as well as nitric oxide and aldosterone, measured the blood pressure, heart rate in the second morning of hospitalization and recorded 24-hour urine volume. Ascites was diagnosed by B-ultrasound, and the patients were divided into two groups, ascites group and ascites-without group. All the parameters were compared between the two groups.

Results: 38 patients (32.2% of the cohort) were diagnosed with ascites on the first day of admission. The majority of our patients presented with ascites were due to alcoholic liver cirrhosis (50.0%) or Chronic hepatitis B (34.2%), with 65.8% classified to Child-Pugh scores C. Serum albumin levels and 24-hour urine volume levels in patients with ascites (27.7±5.9g/L and 1184±605ml) showed a significantly (P<0.05) reduction in comparison to those without (30.2±6.1g/L and 1473±839ml);alkaline phosphatase, γ-glutamyltransferase and aldosterone levels (167±147U/L, 131±173U/L and 18.74±14.18ng/dl) presented a significant (P<0.05) increase in comparison to those without (119±80U/L, 76±93U/L and 10.99±7.17ng/dl) while other parameters did not differ and were in normal range.

Conclusion: Cooperating with portal hypertension, hypoalbuminema, neither NO overproduction mentioned in peripheral arterial vasodilation theory nor sodium and water retention emphasized in Guidelines on the Management of Ascites in Cirrhosis, Britain, 2006, is considered to be the most crucial factor for early ascites formation in patients with liver cirrhosis as Figure.1 shows.

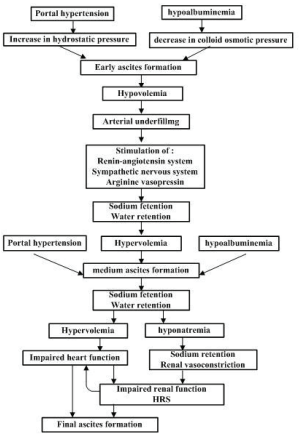

Fig.1 Pathophysiology of Ascites and Hepatorenal Syndrome Hypothesis

C-99

Partial pressure of NH_3 in cirrhotic patients with and without hepatic encephalopathy

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Objectives Ammonia is considered to be the major cause of neurotoxicity. Since unionized ammonia (NH_3) is the only form of ammonia that is able to freely pass through the blood-brain barrier and cause cerebral dysfunction, we tested the hypothesis that the concentration of unionized ammonia is correlated with the severity of hepatic encephalopathy.

Methods One hundred fifty-six patients with cirrhosis (74 with hepatic encephalopathy and 82 without) were enrolled, and underwent clinical examination and blood testing. Twenty five patients with hepatic encephalopathy were participated the two days' follow-up events, ammonia and pH determinations were repeated after two days' treatment. pNH_3 was calculated according to Manning's nomogram. The differences in venous ammonia, pNH_3, and pH between patients with and without encephalopathy were analyzed, and the area under the ROC curve of blood ammonia and partial pNH_3 was compared.

Results In cirrhosis patients with hepatic encephalopathy, pH, pNH_3 and venous ammonia levels were all higher than those in patients without hepatic encephalopathy, and alkalosis was more common in patients with hepatic encephalopathy. Both venous ammonia and pNH_3 were significantly correlated to the clinical degree of hepatic encephalopathy; however, the r was smaller for venous ammonia (r=0.63) than for pNH_3 (r=0.68). Among 20 patients follow-up events, median levels of pH, pNH_3 and venous ammonia decreased; however, venous ammonia levels were unchanged or higher in some patients after resolution of hepatic encephalopathy.

Conclusions Although the correlations of pNH_3 and venous ammonia with the clinical grade of hepatic encephalopathy were similar, pNH_3 was superior to total venous ammonia in identifying those patients with hepatic encephalopathy, and in evaluating neurotoxicity. This study supports the idea that pH-dependant pNH_3 and pH could be useful diagnostic and prognostic tools in cirrhosis patients with hepatic encephalopathy.

C-100

Prevalence Of Hepatitis B Surface Antigen (Hbsag) In Pregnant Women Attending Antenatal Clinic (Anc) In Wenchi

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Background: Hepatitis can be severe in babies. It can threaten their lives. Even babies who appear well may be at risk for serious health problems. Infected newborns have a high risk (up to 90%) of becoming carriers. This study was conducted to assess the prevalence of hepatitis B surface antigen (HBsAg) in pregnant women attending antenatal clinic (ANC) in Wenchi. The results will better inform the Ghana Health Service in implementing the necessary measures to curb the hepatitis B menace.

Methods: The unlinked anonymous method was used to collect blood samples from 269 pregnant women within the reproductive age. The samples were allowed to clot and centrifuged at 3000 rpm for 5 minutes. The serum was then screened for the presence of HBsAg using ACON®, USA chromatographic rapid test strip.

Results: Percentage positive rate in the various age groups were 15-19(14.7%), 20-24(14.8%), 25-29(21.9%), 30-34(5.6%), 35-39(15.6%), 40-44(15.4%), 45-49(0%). The overall percentage of hepatitis B was 14.8 with majority of infections occurring in the sexually active group of 21-29 being 51.4%.

Conclusions: The finding of 14.8% pregnant women seropositive for HBsAg is significant since about 10-20% of infected women will transmit the virus to their babies. Even though the CDC recommends immunoprophylaxis together with vaccination for babies born to hepatitis B positive mothers, this is not done in most district hospitals in Ghana. Efforts should be made at providing Hepatitis B Immunoglobulin (HBIG) in our hospitals to help reduce mother to child transmission. Mass screening and vaccination of adolescents of premarital age will also have a significant impact on the fight against hepatitis B infection.

C-101

Evaluation on short- term prognosis of hepatitis B patients with ACLF using MELD, MELD-Na and Imeld

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Background: Acute-on-chronic liver failure (ACLF) refers to an acute deterioration in liver function. In China, hepatitis B viruses (HBV) infection accounts for about 85% of ACLF. The aim of this study was to compare MELD scoring system, MELD-Na scoring system and iMELD scoring system in survival prediction of hepatitis B patients with acute-on-chronic liver failure.

Methods: 200 hepatitis B patients with acute-on-chronic liver failure were divided into survival and death group, and evaluated by MELD, MELD-Na and iMELD. The area under receiver operating characteristic (ROC) curve was used to compare MELD, MELD-Na and iMELD.

Results: The MELD, MELD-Na and iMELD score of survival group was 22.34±4.36, 23.26±5.45 and 37.59±6.97 respectively, and those for the death group were 27.76±7.28, 30.11±10.19 and 46.65±11.0. The differences were of statistical significance (P<0.01).The area under curve (AUC) generated by the ROC curves was 0.726 (95%CI 0.659~0.787) for MELD, 0.722 (95%CI 0.654~0.783) for MELD-Na and 0.747 (95%CI 0.681~0.806) for iMELD, and there was no significant difference between them.

Conclusion: MELD scoring system, MELD-Na scoring system and iMELD scoring system can all predict the short-term prognosis of hepatitis B patients with acute-on-chronic liver failure. There were no significant statistic differences in predictive values of three systems .The mortality increases with the MELD, MELD-Na and iMELD score increasing. If more accurate prognosis is needed, we must combined with clinical symptoms and pathogenic conditions.

C-104

Clinicopathological evaluation of apoptotic cytokeratin 18 in sera and liver tissue from patients with chronic hepatitis C

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Background: Cytokeratin 18 (CK-18) is released from hepatocytes during apoptosis. Recent studies have indicated that serum levels of CK-18 could be a clinically useful biomarker of early liver injury.

Objective: The aim of this study was to correlate CK-18 in chronic Hepatitis C patients with the severity of hepatic inflammation, fibrosis and ALT level.

Methods: Eighty patients were included; 69 with chronic hepatitis C , 11 with liver cirrhosis post hepatitis C and 15 healthy individuals.

Liver biopsies were used for histological analysis as well as for immunohistochemical detection of apoptosis marker (caspase generated CK-18 fragments). CK-18 fragment was quantified in paired serum by Elisa technique and on biopsy samples using immunohistochemical technique with monoclonal antibody for CK-18.

Results:

	Chronic HCV group (n=69) Mean (Standard deviation)	Cirrhosis group (n=11) Mean (Standard deviation)	Control group (n=15) Mean (Standard deviation)
Serum level of CK-18	27.59 (13.47)	61.73 (28.06)	17.40 (4.56)
Liver tissue expression of CK-18	203.19 (231.08)	638.63 (464.71)	57.40 (13.77)

The serum level of CK-18 and liver tissue expression of CK-18 of the control group was significantly less than chronic HCV and cirrhotic groups ($p<0.05$, $p<0.01$ respectively). Serum level of CK-18 and liver tissue expression of CK-18 is less in chronic HCV than in liver cirrhosis ($p<0.01$). The serum level of CK-18 increases significantly with the severity of liver inflammation and the stages of fibrosis in chronic HCV group and cirrhotic group ($p<0.05$).

Conclusion: Serum level of CK-18 plays a role in prediction of disease progression in chronic hepatitis C patients . Highest serum CK-18 neoepitope levels were observed in patients with cirrhosis.

Serum level of CK-18 as well as hepatic expression of CK-18 are related to the degree of disease activity, which may point out to the possibility of using serum CK-18 levels as well as the expression scores of hepatic CK-18 as efficient tools for monitoring the disease activity in chronic HCV and liver cirrhosis patients.

C-105

Evaluation of the AMPAR Peptide and AMPAR Antibody biomarkers for mild traumatic brain injury

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Background: Mild traumatic brain injury (mTBI) remains a serious health concern, and TBI is one of the leading causes of death and disability, especially among young adults. Although available imaging technique (CT, MRI) have dramatically increased the certainty of diagnosis in more sever cases, mild injuries cannot be readily detected using current techniques. A large majority of mTBI patients, both symptomatic and asymptomatic have normal CT and structural MRI scans. An objective simple testing for brain-specific biomarker of mTBI that can be performed in any clinical laboratory may be extremely helpful for diagnosis. Mild TBI affects electrical and chemical circuits, leading to edema-activating necrosis. AMPA receptors are key components that control these processes. Excessive amounts of glutamate activate AMPA receptors, triggering an excessive influx of calcium, leading to overexpression of glutamate receptors in the extra-synaptic region. Degradation of receptors by thrombin-activated serine proteases results in peptide fragments entering the bloodstream via the compromised blood-brain barrier. AMPAR peptide serves as a tissue-based evidence of neuronal dysfunction due to mTBI. The objective of the study is to compare AMPAR peptide and AMPAR antibody levels in mild TBI patients and healthy control group.

Methods: Serum and plasma samples were drawn from adult patients (age >18 years old) with symptoms suggestive of mTBI. The diagnosis was based on clinical symptoms, history of head injury, and Glasgow Coma Scale (GCS). Neurological examination and CT scans were performed for all mTBI patients. Healthy volunteers were recruited among apparently healthy males and females of matched age. An AMPAR peptide assay based on magnetic-particle (MP) ELISA and AMPAR antibody ELISA assays (GRACE-Peptide assay and GRACE-Antibody assay, GRACE Laboratories, LLC, Atlanta, GA) were used.

Results: AMPA Peptide levels were assessed in 49 mTBI patients (38 male, 11 female, mean age 41), presented within 9 hours to 5 days after the injury, and in 9 healthy volunteers (5 male, 5 female, mean age 36). Levels of antibodies to AMPAR peptide were measured in 61 mTBI patients (44 male, 17 female, mean age 38) and in 11 healthy volunteers (6 male, 5 female, mean age 34). All of the mTBI patients had normal CT scans. Our data shows that AMPAR peptide levels were significantly higher in mTBI group in comparison with control group (mean±SD: 1.5±0.73 versus 0.250±0.18, $p<0.05$). No significant difference was observed between antibody levels in two groups.

Conclusion: Our study demonstrates that AMPAR Peptide assay can increase diagnostic certainty of mTBI in conjunction with neurologic assessment and neuropsychological testing. Additionally, peptide levels should be assessed in the earlier stages of mTBI (less than 9 hours), as it might have a short-half-life in the bloodstream. Conversely, antibody levels should be measured later after trauma due to delay in immune response.

C-106

Increased Microalbumin in Urine and Decreased Serum High Molecular Weight-adiponectin in Hypertensive Patients with Metabolic Syndrome

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Objective: Hypertension and metabolic syndrome (MS) are known independent risk factors for kidney damage and the development of cardiovascular disease. Little is known about the combined impact on kidney damage of the two risk factors. This study was to investigate microalbuminuria (determined by ACR, urinary albumin to creatinine ratio) and serum high molecular weight-adiponectin (HMW-ADP) levels in hypertensive patients with MS and their correlation with hypertension and MS.

Subjects and Methods: Of the total 68 patients with hypertension enrolled, 35 patients were with essential hypertension and 33 patients were with essential hypertension and MS. Fasting blood glucose, total cholesterol, high-density lipoprotein cholesterol (HDL-C), triglycerides (TG), serum and urine creatinine, urea, and blood uric acid

were assayed on the Synchron LX20 (Beckman, Palo Alto, CA). Serum low-density lipoprotein cholesterol (LDL-C) was calculated based on Friedewald equation. Serum HMW-ADP was determined using commercial ELISA kit. Urinary albumin was measured by an immunonephelometric assay (Siemens Healthcare Diagnostics).

Results: The body mass index, waist circumference, fasting blood glucose, TG, LDL-C, and uric acid were highly increased in patients with essential hypertension and MS compared to patients with only essential hypertension. Serum HDL-C levels were lower in patients with essential hypertension and MS than that in patients with essential hypertension. In comparison with patients with essential hypertension only, urinary ACR was significantly increased (66.38 ± 28.64 vs. 39.15 ± 28.54 mg/g, $P < 0.01$) whereas serum HMW-ADP levels were significantly decreased (14.15 ± 4.02 vs. 17.69 ± 4.54 mg/L, $P < 0.01$) in patients with essential hypertension and MS. Pearson regression analysis revealed that serum HMW-ADP was negatively correlated with body mass index, waist circumference, blood pressure, fasting blood glucose, LDL-C but positively correlated with HDL-C. However, these correlations were no longer existed after multiple regression analysis, and only LDL-C was negatively correlated with HMW-ADP levels. Binary logistic regression analysis showed that fasting blood glucose, TG, LDL-C, ACR, and low HDL-C and HMW-ADP were associated with the development of MS in hypertensive patients ($P < 0.05$ - 0.01).

Conclusions: In addition to the traditional risk factors, increased ACR in patients with essential hypertension and MS compared with patients with only essential hypertension indicates that MS aggravates kidney damage of hypertensive patients. Low levels of HMW-ADP could be a potential underlying mechanism promoting microalbuminuria in hypertensive patients with MS.

C-107

Study on the mechanism of plasma ammonia elevation in patients with cirrhosis

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Objective: To study the relationship between plasma ammonia and the status of the liver, renal, and gastrointestinal tract, to research the mechanism of plasma ammonia elevation in patients with cirrhosis, and to define the affections and individual method of lowering ammonia.

Methods: The study population enrolled 2743 patients who had liver dysfunction, including hepatic cancer, hepatic encephalopathy, liver cirrhosis, liver transplantation, drug-induced hepatitis, hepatitis B, severe hepatitis. Pathology groups were 100 patients who had just cirrhosis, 100 patients who had hepatorenal syndrome, and 95 patients who had hepatointestinal syndrome. Pathology control groups were 80 patients who had just renal dysfunction, 113 patients who had just gastrointestinal hemorrhage, 50 patients who had just intestinal obstruction. Normal control group consisted of 80 healthy people. Carbamoyl phosphate synthetase I (CPS I), ornithine carbamoyl transferase (OCT), liver function indicators, and ammonia were measured. The difference in ammonia, CPS I, OCT between patients who had cirrhosis and who did not, and the correlation between CPS I, OCT and ammonia, liver function indicators were analyzed. We compared the ammonia level in patients who had HRS and renal dysfunction to normal controls, and analyzed the correlation of serum urea, creatinine with ammonia among 40 patients. We also analyzed the difference of ammonia among patients who had HIS, gastrointestinal hemorrhage, intestinal obstruction, and compared the ammonia before and after intestinal acidification treatment in patients who had HIS.

Results: Ammonia levels among patients who had hepatic cancer, hepatic encephalopathy, liver cirrhosis, etc, were higher than those among healthy people ($P<0.05$), ammonia level among patients who had HE was the highest. CPS I and OCT among patients who just had cirrhosis were (172.30±52.22) IU/L and (339.00±229.80)×10IU/L, respectively, which were lower than normal controls,(262.30±109.20)IU/L and (499.70±229.80)×10IU/L, respectively ($P<0.05$), and CPS I, OCT levels were negatively correlated with ammonia. Ammonia among patients who had HRS was (64.80±47.25)umol/L, which was higher than patients who just had renal dysfunction (26.59±14.34)umol/L, and healthy people (21.42±7.93) umol/L ($P<0.01$), but there were no significant difference when comparing patients who just had renal dysfunction and healthy people ($P>0.05$). Serum urea, creatinine among patients who had HRS was positively correlated with ammonia in Followed Study. Ammonia level among HIS patients was (74.75±36.00)umol/L, significant different from patients who had just gastrointestinal hemorrhage (24.85±14.79) umol/L, and intestinal obstruction (24.90±11.02)umol/L ($P<0.05$), but the ammonia levels among patients who had just gastrointestinal hemorrhage or intestinal obstruction were not significantly different to healthy people($P>0.05$).

Conclusions: Several factors, such as intestinal, liver, renal status may increase ammonia level, HE is essentially intestinal-liver-renal-brain syndrome.

C-108

Development of an algorithm using the APRI (AST, platelet ratio index) and ELF™ (Enhanced Liver Fibrosis) Tests to detect mild (F0-F1) and significant (F2-F4) fibrosis due to chronic hepatitis C

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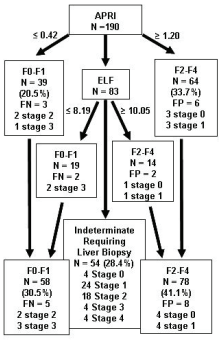
Background: The assessment of liver fibrosis in patients with hepatitis C virus (HCV) is important for prognosis. While considered the reference standard for assessing liver fibrosis, liver biopsy is invasive and associated with sampling and inter-observer variability. We have previously found the APRI to be an accurate test for separating mild from significant fibrosis in HCV-infected populations, either alone or combined with other markers. The objective of our study was to develop an algorithm using APRI and ELF™ biochemical tests to detect mild and significant liver fibrosis, limiting the need for liver biopsies.

Methods: We completed a prospective study of 191 patients in which blood draws and liver biopsies were performed on the same visit. We calculated sensitivity, specificity, negative and positive predictive values, and AUROC values for both tests. These data were used to generate a clinically useful algorithm for differentiating mild and significant fibrosis

Results: The distribution by stage was: F0:21(11%), F1:68(35.6%), F2:46(24.1%), F3:24(12.6%), F4:32(16.7%). The AUROC for the APRI and ELF™ tests were 0.865 and 0.833, respectively (p=0.312). The clinical sensitivity and specificity of the APRI and ELF™ tests in separating mild from significant fibrosis was 83.2% and 80.0%, and 72.3% and 83.3%, respectively. Using APRI as the primary test, followed by ELF, the algorithm would have been able to decrease the number of biopsies needed by 71.6% leaving 54(28.4%) in the intermediate zone.

Conclusion: This and other studies have provided sufficient evidence that the APRI used individually or in combination with other non-invasive markers (e.g., ELF), is able to accurately separate mild from significant fibrosis potentially decreasing the number of liver biopsies. However, with AUROC values approaching those of the reference standard, we must accept that patients falling in the intermediate zone using non-invasive tests will still require liver biopsies for accurate fibrosis staging.



C-109

Prevalence of Metabolic Syndrome in Chronic Kidney Disease

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Background:Over caloric nutrition and sedentary lifestyle, the root causes of metabolic syndrome (MetS), are now common even in the developing countries. There are several studies which have linked MetS with Chronic Kidney Disease (CKD). The components of MetS are the established risk factors of CKD. Therefore MetS and interplay of various components of MetS may have deleterious effect on patients with CKD. The objective of this study was to find out the prevalence of MetS as well as prevalence of various components of MetS in the patients with CKD.

Methods: This hospital based cross-sectional study was conducted in the Department of Clinical Biochemistry in collaboration with Department of Internal Medicine (nephrology unit), Tribhuvan University Teaching Hospital, Institute of Medicine (TUTH, IOM), Nepal from 2008 February to 2009 August. One hundred and sixty

confirmed CKD diagnosed patients were included in this study. CKD was defined as either an eGFR of <60 mL/min per 1.73m^2 body surface area or urinary albumin-creatinine ratio of greater than 30 mg/gram for more than three months as per national kidney foundation (NKF) guidelines. Anthropometric measurements like height, weight, waist circumference (WC) of subjects were noted in a semi-structured pro-forma. Fasting blood sample was collected for the estimation of glucose (FBG), triglyceride (TG) and HDL-cholesterol (HDL-C). Spot urine sample was collected to measure microalbumin and creatinine. CKD patients were diagnosed as having the metabolic syndrome by using the modified NCEP ATP III criteria. According to the NCEP report, participants who had three or more of the following criteria were defined as having the MetS: 1) abdominal obesity WC >102 cm in men and >88 cm in women, 2) TG ≥1.7 mmol/l, 3) HDL-C <1.03 mmol/l in men and <1.29 mmol/l in women, 4) systolic blood pressure ≥130 mmHg or a diastolic blood pressure ≥85 mmHg), and 5) FBG≥5.6 mmol/l. The participants who currently reported using anti-hypertensive or anti-diabetic medication were counted as having high blood pressure or diabetes, respectively.

Results: Sixty (37.5%) of the CKD patients had the MetS according to modified NCEP ATPIII criteria. The prevalence of hypertension, high FBG, high TG, low HDL-C and high WC in CKD patients was 70.0%, 22.5%, 46.25%, 61.25% and 18.75% respectively. Among the five components of the metabolic syndrome, WC has the highest positive predictive value (73.34%) for CKD and was followed by FBG level (72.23%), TG level (70.27%), decreased HDL-C level (55.1%), and BP (51.78%).

Conclusion: MetS, as defined by the modified NCEP ATPIII criteria occurs in one-third of CKD patients. This study shows the higher prevalence of hypertension, dyslipidemia and high FBG in CKD patients.

C-112

Accurate Estimation of Glomerular Filtration Rate: Analysis of 6034 Chinese Individuals

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Background: Serum creatinine and cystatin C concentrations are commonly used as a measure of renal function and are endogenous markers of glomerular filtration rate (GFR). In clinic practice, estimated equations of GFR are over 25, and no one can be equally applicable to all populations,and equally applicable all situations in a population. The aim of the present study was to evaluate common four equations which based on combined of Cystatin C and creatinine with sex, age, and race ($eGFR_4$), Cystatin C wit ($eGFR_3$) and without sex, age and race ($eGFR_2$) , and creatinine with sex, age and race ($eGFR_1$) in a Chinese cohort study.

Methods: This study is carried out in 6034 Chinese individuals in Wuhan, China. First, use univariate correlation between $eGFR_{1,2,3,4}$, and biological parameters. Second, use non parametric regression to evaluate nature log transformed $eGFR_{1,2,3,4}$ with log transformed blood urea nitrogen (BUN), age and sex. Then, based on turn points to split into subsets, and use a linear regression to fit eGFR with BUN in each subset. Finally, use root mean square error (RMSE) in linear fit, and residual standard error (RSE) in non parametric regression to evaluate $eGFR_{1,2,3,4}$.

Results: 58% male in study with mean age 56.55 ± 17.28 years. Partial correlation (sex, and age) between BUN and eGFRs was negative and great than 0.5. Non parametric regression showed two lines, one falls into normal BUN (BUN < 7 mmol/L) and other falls into abnormal BUN (BUN ≥ 7 mmol/L). The RMSE and RSE order from smallest was $eGFR_3$, $eGFR_2$, $eGFR_4$, $eGFR_1$ in abnormal BUN subset, and accurate order was $eGFR_4$ > $eGFR_1$ > $eGFR_3$ > $eGFR_2$ in a normal BUN subset.

Conclusion: eGFR estimation based on one equation cannot be equally applicable to all situations in Chinese population: For a normal BUN, superiority of eGFR based on an equation which combined serum Cystatin C and Creatinine with age, sex, and race. For abnormal BUN, GFR based on Cystatin C with age, sex, and race seems more be the most of accurate. In elderly, eGFR based on Cystatin C is first selection.

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C-113

Prognostic Significance of Neutrophil Gelatinase-Associated Lipocalin in ST-Segment Elevation Myocardial İnfarction

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Background: Neutrophil gelatinase-associated lipocalin (NGAL) is a promising biomarker for acute kidney injury. Recently it was concluded that NGAL may be used beyond the boundaries of renal physiopathology. It was found to be an important factor indirectly contributing to the inflammatory processes. Little is known regarding its predictive role in ST-segment elevation myocardial infarction (STEMI). This study investigated the prognostic value of NGAL in patients with STEMI was evaluated in the present study.

Methods:106 consecutive patients who underwent percutaneous coronary intervention (PCI) for STEMI and 50 healthy age and sex matched volunteers were included into the study. According to median NGAL level, patients were classified into high and low NGAL group.

Results: Neutrophil gelatinase-associated lipocalin levels were higher in patients with STEMI than in the healthy group subjects. In-hospital and one-year mortality rates were found to be significantly greater in patients with high NGAL group, compared to low NGAL group. In addition, in-hospital and one-year MACE rates were significantly greater in high NGAL group, compared to low NGAL group.

Conclusion: High NGAL level may be associated with poor prognosis after PCI in patients with STEMI. This relation may be the cause of increased inflammation and proteolytic activity that NGAL establishes. However further studies with larger number of patients and longer follow-up are required to evaluate the usefulness of plasma NGAL level for predicting prognosis of STEMI.

C-114

Study on the relationship between severe hyponatremia and hepatorenal syndrome in patients with cirrhosis

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Objective: To investigate the relationship between severe hyponatremia and hepatorenal syndrome in cirrhosis patients.

Methods: Fifty two cirrhosis patients with severe hyponatremia(Na<125.0mmol/L) and 100 cases of cirrosis with normal serum sodium were recruited from January 2008 to November 2010, the clinical features, electrolytes, liver function, kidney function ,ALD , NO and the incidence of HRS were compared.

Results: The average level of serum sodium in the cirrhosis patients with hyponatremia was 122.2 mmol/L, and in the control group the average serum sodium was 139.8mmol/L. The HRS incidence rate of hyponatremia group was 26.9%, which was significantly higher than those in the control group (3.4%)(P<0.05). The average level of serum ALD in hyponatremia group was 48.21±29.85ng/L, significantly higher than the control group 23.81±17.29ng/L (P<0.05). Some of the laboratory data are shown in table 1.

Conclusion: Cirrhosis patients with hyponatremia have dilutional low sodium, the body would activate the renin-angiotensin-aldosterone system to secrete high level of aldosterone. The high levels of aldosterone will reduce the glomerular filtration, resulting in function impairment. These results show that cirrhotic severe hyponatremia may play an important role in the development of HRS.

Figure1 Comparison of the partial data between the cirrosis with severe hyponatremia

tests	BUN (mmol/L)	Crea (mmol/L)	ALD (ng/L)	NO (ummol/L)	HRS incidence rate
hyponatremia group	11±7.3*	134±139*	48.21±29.85*	4.46±2.88*	26.9%*
Control group	5.9±6.7	78.4±31.27	23.81±17.29	3.74±2.61	3.4%

* P<0.05 comparison with control group

C-115

Performance Validation of the iChem®Velocity for use in Urinalysis Testing

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Background: The iChem®Velocity Automated Urine Chemistry System is an automated urine chemistry system for the in vitro measurement of the following analytes in urine: Bilirubin, Urobiliniogen, Ketone (Acetoacetic Acid), Ascorbic Acid, Glucose, Protein (Albumin), Blood, pH, Nitrite and Leukocytes esterase, specific gravity, color and clarity. Validation of the system was performed to show comparability to instruments currently in use in the United States as well as other countries.

Objective: To evaluate the performance of the iChemVelocity for the analysis of urine chemistries against other instruments used in the United States. This unit can be used as a stand-alone unit or as a complement to the IQ200.

Methods: Studies were performed using negative urine spiked with an analyte to reach specific concentrations. The solutions were then run on three iChemVelocity instruments to determine limit of detection (LOD) and linearity. Precision studies which consisted of within-run precision, instrument to instrument precision, strip lot to strip lot precision and day to day precision were also performed with the spiked negative urine. After completion of the above testing, 1594 human urine specimens were evaluated. The urine sample results ranged from completely negative to those exhibiting a positive result for all ranges of each of the ten chemical analytes tested by the system. The specimens were then tested on three iChemVelocity instruments and against one of five instruments: the iChem®100 Urine Chemistry Analyzer, the Arkray AUTION MAX™ AX-4280 Automated Urine Chemistry Analyzer, the Synchron CX4 Clinical Analyzer, a pH meter and a refractometer. The latter was used for specific gravity. The results were tabulated and compared to show concordance between the instruments.

Results: For each analyte, the limits of detection were set by determining the lowest concentration that would yield > 67% positive results. The acceptance criteria for linearity and precision were set as follows: equal to or greater than (≥) 90% agreement to color pad with no deviation greater than plus/minus one color pad. One hundred percent agreement at plus/minus one color pad was required. Each iChemVelocity reagent strip analyte exhibited expected linearity. Each iChemVelocity chemistry pad met acceptance criteria for all precision studies. The acceptance criteria for the concordance study were as follows: 80% of samples must display exact agreement to the predicate device. All samples must display 100% agreement at +/- (plus/minus) one color pad. The concordance study results against the predicate device for the specific analyte with the iChemVelocity showed an exact agreement of 94.1%, with a 100% agreement among positive samples within one color pad and an exact agreement of 97.8% with a 100% agreement among negative samples within one color pad.

Conclusion: The iChemVelocity has excellent performance and is comparable to other instruments being utilized in the United States for the in-vitro measurements of analytes in human urine.

C-116

Low serum creatinine is associated with fasting plasma glucose in a non-diabetic population

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Background: Serum creatinine is regards as a biochemical measure of muscle mass volume in individuals with normal renal function. A recent study has reported that a low serum creatinine can be a predictor of type 2 diabetes mellitus, suggesting the impaired insulin action on plasma glucose via a low muscle mass. The aim of this study was to investigate the association between serum creatinine and fasting plasma glucose (FPG) levels in the general Japanese population.

Methods: The smoking habits, exercise habits, body mass index (BMI), blood pressure, serum cholesterol, FPG and serum creatinine levels were analyzed among non-diabetic and non-medicated subjects (3366 males and 2347 females, mean age of 51 years). Non-diabetic subjects were defined as those with < 7.00 mmol/L of FPG, and subjects with > 1.2 mg/dL of creatinine were not included in this study. The creatinine concentrations were divided into the quartile ranges (Q1-Q4 from the lowest to highest) in the present analysis.

Results: In men, the mean FPG levels were 5.35 mmol/L in the group of subjects with the Q1 (< 0.73 mg/dL) of creatinine, 5.27 in the Q2 group (0.73-0.79) , 5.30 in the Q3 group (0.80-0.87) and 5.29 in the Q4 group (> 0.87), respectively (trend $p < 0.05$). In women, the mean FPG levels were 5.35 mmol/L in the group of subjects with the Q1 (< 0.55 mg/dL) of creatinine, 5.27 in the Q2 group (0.55-0.60), 5.30 in the Q3 group (0.61-0.67) and 5.29 in the Q4 group (> 0.67), respectively (trend $p < 0.01$). The trend of the highest FPG levels in the Q1 group remained significant, even though the other confounders including BMI and exercise habits were adjusted in men ($p < 0.05$) and in women ($p < 0.05$).

Conclusion: Serum creatinine, when the concentrations are low, may be a marker reflective to the regulation of glucose concentrations even in non-diabetic subjects, regardless of genders. Further studies are needed to determine the clinical application of serum creatinine in glucose and diabetes practice.

C-117

Magnesium supplementation on blood pressure, intracellular ions level and insulin resistance in hypertensive patients using diuretics

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Background: Alterations in intracellular homeostasis of magnesium, can contribute to the development of hypertension and insulin resistance. Long term use of diuretics can induce a depletion of intracellular magnesium. The objective of the study is evaluate the effect of oral supplementation of magnesium on blood pressure, intra-erythrocyte concentration of magnesium and sodium, and in insulin resistance of primary hypertensive patients treated with thiazidic diuretic.

Methods: Randomized, double blind clinical trial. Thirty nine primary hypertensive patients stage 1 were distributed in 2 groups. One group received thiazidic diuretic (25mg) and magnesium (240mg of element magnesium) (Mg group) and the other group received thiazidic diuretic (25mg) and placebo during 16 weeks (Placebo group). The intra-erythrocyte concentration of magnesium was made by atomic absorption.

Results: After 16 weeks using diuretic plus magnesium or diuretic plus placebo we observed significant reduction in blood pressure evaluated by auscultatory method and by ambulatory blood pressure monitoring during 24 hour in the two groups; although there was no significant difference between the two groups. The intracellular concentration of magnesium did not change significantly in the Mg group (-3.8±2.9mEq/L/cel), however in the placebo group the reduction was significant (-16.3±4,1mEq/L/cel;p=0.001). The amount of the intra-erythrocyte sodium was significantly reduced in both groups, without statistical significance between the two groups. At the final visit the sensibility to insulin evaluated by HOMA was not changed significantly in both groups.

Conclusion: This trial demonstrated that the thiazidic diuretic reduced significantly and equally the blood pressure in the two groups of treatment. In spite of the significant reduction in the level of intracellular magnesium in the placebo group any hypotensive effect of this treatment was registered.

Wednesday AM, July 27

Poster Session: 10:00 am - 12:30 pm
Lipids/Lipoproteins

C-118

Relationship between oxidized lipoprotein(a) and carotid atherosclerosis in asymptomatic subjects: a comparison with native Lp(a)

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Background: Oxidized lipoproteins play important roles in the atherosclerotic process even in asymptomatic subjects. The biochemical measurements of oxidized lipoproteins have therefore gained a large amount of attention in order to assess the pathophysiology of atherosclerotic formation. Oxidized lipoprotein(a) (oxLp(a)) is suggested to be a more potent marker of atherogenesis than native Lp(a), a cardiovascular disease-relevant lipoprotein. However, limited clinical data are available regarding the oxLp(a) in atherosclerosis. The aim of this study was to investigate the association between levels of serum oxLp(a) and carotid artery intima-media thickness (CIMT) in comparison to the serum Lp(a) levels.

Methods: In 137 non-medicated and non-smoking subjects (61 males and 76 females, mean age of 64 years), the atheroscrerosis-related variables including Lp(a) and oxLp(a) were measured. The serum oxLp(a) level was quantified using a sandwich ELISA system which contained the oxLp (a)-specific monoclonal antibody. The CIMT level was measured on the bilateral carotid arteries using B-mode ultrasonic imaging with a 10-MHz linear transducer.

Results: The mean level of variables was as follows: 130 mmHg of systolic blood pressure (SBP), 3.00 mmol/L of serum total cholesterol, 1.33 mmol/L of triglyceride, 1.40 mmol/L of high-density lipoprotein cholesterol, 0.12 mmol/L of Lp(a), 0.06 nmol/L of oxLp(a), 0.7 mm of CIMT. A simple linear regression analysis revealed CIMT to be significantly and positively correlated with age ($r = 0.43$, $p < 0.01$), SBP ($r = 0.28$, $p < 0.01$), and (log-)oxLp(a) ($r = 0.23$, $p < 0.01$), respectively. In a multiple linear regression analysis, adjusted for age, sex, SBP, lipids and glucose, oxLp(a) continued to show a significantly positive and independent correlation with CIMT ($\beta = 0.21$, $p < 0.01$). Although the similar analyses were conducted for Lp(a), (log-)Lp(a) did not show any relative significant correlation to CIMT.

Conclusion: The serum oxLp(a) was a significant positive indicator of carotid atherosclerosis, in comparison to Lp(a), even in asymptomatic subjects. This finding may be important for obtaining a better understanding of the different atherogenic roles played by oxLp(a) in comparison to Lp(a), and also considering the relevance of measuring the oxLp(a) in atherosclerosis practice.

C-120

Novel homogeneous assay for LDL-apolipoprotein B (LDL particle number)

J. L. Toher, C. A. Happe, J. H. Contois. *Maine Standards Company, LLC, Windham, ME,*

Background: There is great interest in measuring low density lipoprotein (LDL) particle concentration in blood as a means to monitor therapy and estimate risk for future coronary heart disease. There remains a need for a simple and reliable method to measure LDL particle number in routine chemistry laboratories. Because there is one molecule of apoB per LDL particle, measurement of LDL-apoB indicates the total number of LDL particles in the circulation. Ultracentrifugation can be used to separate LDL from other lipoproteins prior to apoB measurement, and NMR can measure LDL particles directly. This homogeneous, immunoturbidimetric assay specifically measures LDL-associated apoB while excluding VLDL-apoB (d<1.006 g/mL). In conjunction with our total apoB assay (ApoB-T) this would allow for quantitation of VLDL-apoB by difference.

Methods: IRB approval was obtained and sera were obtained from 16 volunteers who provided informed consent. LDL and VLDL were separated by ultracentrifugation. The assay uses two reagents, a diluent (R1) and diluted antiserum (R2). Proprietary additives are used to selectively block the interaction of VLDL with apoB antibodies. Sample is incubated with R1 and a blank measurement is taken; then R2 is added and final absorbance is read at endpoint (340 nm) at 37°C.

Results: Total imprecision for the LDL-apoB assay was assessed with two levels of QC, 1346 and 3003 nmol/L (69 and 154 mg/dL) over 8 runs in 4 days with 2 recalibrations and was 4.8% and 5.4% respectively. The limit of the blank was 0 nmol/L (0 mg/dL), and the limit of detection (LoD), the lowest concentration that can be differentiated from the blank, was 117 nmol/L (6 mg/dL). The limit of quantitation, defined as the concentration associated with 20% imprecision, was 195 nmol/L (10 mg/dL). Linearity, assessed by linear regression of observed (y) and expected (x) concentrations derived from dilution of concentrated LDL, was 0-3900 nmol/L (0-200 mg/dL). Method comparison of 16 patient sera, measured in triplicate, for LDL-apoB in sera (y) and apoB in isolated LDL fractions measured by our total apoB assay (x), gave the following regression equation: y =0.92x + 7.6, r = 0.95. VLDL fractions, also measured with the LDL-apoB produced results < LoD, indicating that VLDL-apoB was not measured. No interference was seen with hemolysis, lipemia, total protein or EDTA.

Conclusion: We describe a homogeneous immunoturbidimetric assay for LDL-apoB (LDL particle number) that is applicable for use on most automated chemistry analyzers. Future validation studies with a larger sample size are needed. Also planned are studies to establish calibrator traceability to SP3-08 and to assess specimen stability, reagent stability, and reference intervals.

C-121

Establishment of practical procedure for measurement of total glycerides by Isotope Dilution/Gas Chromatography/Mass Spectrometry at the Osaka Medical Center for Health Science and Promotion (CRMLN lipid reference laboratory)

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Background: Traceability of triglycerides (TG) (glycerol-blanked triglycerides) measurements can be verified by comparing manufacturers' and clinical laboratories' methods with the chromotropic acid reference measurement procedure (RMP) in the CDC/CRMLN certification program. CDC previously presented a RMP for total glycerides using Isotope Dilution/Gas Chromatography/Mass Spectrometry (GC-IDMS)[1] as substitute for the chromotropic acid method. We have also completed to set up a practical measurement system for total glycerides using GC-IDMS for purpose of more accurate TG standardization in Japan.

Methods: Sample preparation is started from a preliminary dilution with tris-HCl buffer (50mM, pH7.4). Aliquots of diluted serum, calibration standards that have been contained with the internal standard 1,3-$^{13}C_2$-glycerol are hydrolyzed with ethanolic potassium hydroxide (EtOH/KOH) at 60 ^{0}C for 1 hr followed by evaporation under vacuum. The residue containing free and hydrolyzed glycerol was derivatized with acetic anhydride in the presence of pyridine. The derivatized product triacetin was then extracted with ethyl acetate and the organic layer was removed and dried under nitrogen at 60 ^{0}C. A magnetic sector-type mass spectrometer (JMS GC mateII) is operated in electron impact ionization mode and mass ion fragments m/z 116 and m/z 118 corresponding to the native and labeled triacetin fragment ions, respectively, are used for selective ion monitoring.

Results: Two quality control pools (Q27, Q28) from CDC were analyzed in quadruplicate in 20 analytical runs. The data were compared with the assigned values of CDC. As a result, r^2 for the standard calibration range (0-100mg/dL) was 0.9999. The within run (n=4) CV and the among run (20 assays) CV ranged from 0.22% - 1.77% and 0.76% - 1.02%, respectively. The average bias from CDC was -0.37% and -0.68%, respectively.

Conclusion: These results demonstrate sufficient precision and accuracy that is required for the method to be considered as a potential RMP.

1. S.H. Edwards, S.D. Pyatt, S.L. Stribling, K.D. Dobbin, M.M. Kimberly, G.L. Myers. Measurement of total glycerides in serum with gas chromatography-isotope dilution mass spectrometry. Clin Chem 2009; 55(S6), A85 [Abstract]

C-122

Apolipoprotein B/A1 ratio as risk factor for stroke

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Backgrounds: According to current knowledge, apoB/A1 ratio is like to be risk factor in coronary artery disease. There is evidence form case-control studies that apoB/A1

ratio may be a superior to LDL and HDL cholesterol in discriminating coronary artery disease case subject from control subject. However, relationship between apoB/A1 ratio and stroke is undefined. The main object of this study is to determine whether the risk of stroke is related to levels of apoB/A1 and correlation between lipid profiles.

Materials and Methods: The study group included 643 patients (Men: 372, Women 271) diagnosed stroke at Ewha Womans University of Mokdong Hospital between January 2008 to December 2010. The control groups were composed of 378 patients (Men: 139, Women 239) who diagnosed other neurological disease such as dementia, migraine. The correlation between lipid profiles and odds ratio of 10 preliminary risk factors (Total cholesterol, Triglyceride, LDL, HDL, apoA1, apoB, apoB/A1 ratio, Non HDL, Total cholesterol/HDL ratio, LDL/HDL ratio) of stroke were analyzed. The results were compared with the risks of stroke and control group.

Results: ApoB/A1 ratio was significantly increased in case patients compared with control subjects. Multivariate logistic regression analysis identified decrease of apoB/A1 ratio (odds ratio (OR), 1.583; 95% CI 1.105-2.269) as significantly associated with stroke. Individual apoA1 (OR, 1.303; 95% CI 0.967-1.755) and apoB (OR, 1.397; 95% CI 0.773-2.523) were also not significantly associated with stroke. Correlation analyses of cholesterol indices and apolipoprotein revealed that apoB/A1 ratio was strongly correlated with LDL/HDL ratio(r= 0.849) and moderately correlated with Total cholesterol/HDL ratio(r=0.713) and apoB(r=0.639).

Conclusions: Increase of apoB/A1 ratio is associated with an increase risk of stroke. Use of apoB/A1 ratio is efficient as conventional lipids, for the identification of subjects at increased risk of stroke. So apoB/A1 ratio to standard lipid profile testing could improve the evaluation of risk factors of stroke.

Keywords: *apolipoprotein B/A*1 *ratio(apoB/A*1*), stroke , Odds ratio*

C-123

Elevated small, dense low-density lipoprotein cholesterol (sdLDL-C) is associated with the severity of coronary artery disease (CAD)

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Background: Small, dense low-density lipoprotein (sdLDL) is one of the subclasses of low-density lipoprotein (LDL). Lately, sdLDL has been suggested to be a biomarker for the prediction of coronary artery disease (CAD) due to its high atherogenic characteristics. We sought to determine the association between the severity of coronary atherosclerosis assessed by invasive coronary X-ray angiography (XRA) and sdLDL-C levels.

Methods: We conducted a retrospective, case-control study in subjects who underwent XRA for clinical indications. Subjects on lipid-modifying medications, and those with previous history of coronary revascularization or diabetes were excluded. Cases were defined as 50% or more and controls as less than 50% luminal diameter stenosis based on the worst stenosis. Serum samples were used for all laboratory measurements. SdLDL-C levels were determined by a homogeneous assay. The distribution of sdLDL-C and other lipid parameters was compared between CAD cases and controls by two-sided, unpaired Student's t-test. The odds ratio to develop CAD was compared based on a tertile analysis. The relationship between the presence of CAD and various risk factors was analyzed by logistic regression evaluating the significance by Chi-square test.

Results: The study population was consisted of 184 patients (96 cases and 88 controls). Significant difference between cases and controls was observed in age, gender proportion and distribution of triglycerides and sdLDL-C. Tertile analysis of all subjects based on sdLDL-C levels (7.4-19.5, 19.5-37.0, 37.0-113.3 mg/dL) showed that the odds ratio increased significantly with increasing sdLDL-C levels (0.66, 0.97, 2.11, $p<0.05$). Only age, gender, and sdLDL-C remained independent predictors of CAD in the multinomial regression analysis starting with traditional risk factors and lipid markers.

Conclusion: In this study, sdLDL-C levels were significantly higher in subjects with CAD, and were associated with CAD severity, implying that sdLDL-C is a risk factor for CAD. Longitudinal studies will enable further analyses.

Characteristics of various variables in CAD and non-CAD group

Variables	All subjects	CAD cases	Controls (non-CAD)	p value for CAD vs non-CAD
age (yrs)	60.3 ± 11.5	64.7 ± 1.1	55.6 ± 1.1	0.0001*
gender (%) male / female	53.3 / 46.7	64.6 / 35.4	40.9 / 59.1	0.0010*
hypertension (%)	63.0	64.6	61.4	0.3823
smoking (%)	54.9	58.5	51.1	0.1976
total cholesterol (mg/dL)	177.8 ± 38.6	180.6 ± 3.9	174.8 ± 4.1	0.3065
triglycerides (mg/dL)	118.2 ± 86.0	134.5 ± 8.6	100.5 ± 9.0	0.0071*
LDL-C (mg/dL)	113.3 ± 30.7	115.1 ± 3.1	111.4 ± 3.3	0.4204
HDL-C (mg/dL)	45.5 ± 12.6	44.0 ± 1.3	47.2 ± 1.3	0.0810
sdLDL-C (mg/dL)	32.4 ± 19.9	37.1 ± 2.0	27.3 ± 2.1	0.0008*

Data are shown as meas ± SD except those presented in %. * $p<0.05$

C-125

A rapid measurement system for LDL particle size using polyacrylamide slab gel electrophoresis

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Background: Evidence that a significant contribution of small dense low-density lipoprotein (sdLDL) to atherogenic processes has been accumulating. Determination of LDL size is thus essential in lipid analysis in connection with atherosclerosis. Ultracentrifugation, gradient gel electrophoresis (GGE), nuclear magnetic resonance and gel permeation high-performance liquid chromatography (HPLC) have been applied to separate and quantify the sdLDL. Although GGE is most commonly used in research laboratories, it is time-consuming (approximately 24 hours) and difficult to make a stable gradient gel. Simple, prompt and reliable methods to determine LDL size are essential in both clinical settings and lipid research. *Aims-* Although non-gradient polyacrylamide gel electrophoresis (NPAGE) is thought unsuitable for LDL size measurements, the advantages are gel casting simplicity and rapid performance. We explored ideal NPAGE conditions to identify average LDL diameter within 70 min.

Methods: First, we determined the most suitable polyacrylamide gel concentration, gel buffer composition, and the NPAGE running buffer system. Second, LDL partcle size determined by NPAGE in human blood samples was compared with that determined by HPLC. For the HPLC analysis, whole serum was injected into a Superose 6 10/300GL column (GE Healthcare), and detected by an on-line enzymatic reaction.

Results: *NPAGE system-* Based on the experiments of varying NPAGE conditions, derived suitable characteristics of the gel and the system for LDL size determination were as follows: A homogeneous 3.5% separating gel in the gel-casting plate (120 x 100 x 1 mm, ATTO) with pouring the gel solution consisted of 0.375M Tris-HCl (pH 8.8), 3.5% acrylamide/bisacrylamide (5%C), 0.07% ammonium peroxodisulfate and 0.1% tetramethylethylenediamine. Applied samples (10 μL/well) were as follow: The 3 μL of serum was mixed with 3 μL of 1% Sudan Black B (dissolved in ethylene glycol) and 6 μL of 20% sucrose. Electrophoresis was run at 75V for 10min, and 125V for 60min, in 0.025M Tris-0.192M glycine buffer. After electrophoresis, the gel was analyzed by Image J software (NIH). The NPAGE method produced within-gel imprecision values (coefficients of variation, CV) below 1.24% (n=5) and between-gel CV below 1.38% (n=5). *Comparison of LDL sizes-* LDL sizes obtained by the NPAGE correlated well with those by the HPLC (r=0.777, n=45). No significant difference was observed between LDL sizes determined by the NPAGE and the HPLC, judged by Student's t-test (26.07±1.41 nm vs. 25.68 ±0.80 nm, n=45, p=0.112). LDL sizes determined by NPAGE significantly correlated with HDL-cholesterol (r=0.568, $p<0.01$) and triglycerides levels (r=-0.754, $p<0.01$), consistent with results reported previously. In contrast, migration distance on agarose gel electrophoresis, which is mainly affected by the lipoprotein particle charge, showed no significant correlation with LDL sizes determined by the NPAGE (r=0.034, p=0.826, n=45), indicating electrophoretic mobility on the NPAGE is less sensitive to the particle charge.

Conclusion: The present results disclosed that the NPAGE system with conditions established appropriately is a simple, rapid and reliable method for LDL size determination. The present NPAGE method is thus useful in both clinical settings and lipid research.

C-126

Validation of new immunoturbidimetric assays for apolipoproteins AI and B

J. L. Toher, C. A. Happe, J. H. Contois. *Maine Standards Company, LLC, Windham, ME,*

Background: Apolipoprotein AI (apoAI) is essential for the formation of HDL particles and the transport of cholesterol from peripheral cells to the liver for excretion. Apolipoprotein B (apoB) is present in VLDL, IDL, LDL, and Lp(a), and measurement reflects the total number of atherogenic particles in the circulation. There is increasing recognition of the importance of apolipoprotein measurement in prevention of cardiovascular disease, in diagnosing lipoprotein disorders, and in monitoring therapy. Therefore, we have developed reliable immunoturbidimetric assays for apoAI and apoB.

Methods: IRB approval was obtained and sera were obtained from 16 volunteers who provided informed consent. Both assays use two reagents, a diluent (R1) and diluted antiserum (R2). Sample is incubated with R1 and a blank measurement is taken; then R2 is added and final absorbance is read at endpoint (340 nm) at 37°C.

Results: For the apoB assay, total imprecision, assessed with three levels of QC (79, 96 and 158 mg/dL), was 2.4%, 3.5%, and 1.9%, respectively, over 3 days with 6 recalibrations. The limit of the blank (LoB) was 1 mg/dL, and the limit of detection (LoD), the lowest concentration that can be differentiated from the blank, was 2 mg/dL. The limit of quantitation (LoQ), defined as the concentration associated with 20% imprecision, was 3 mg/dL. Linearity, assessed by linear regression of observed (y) and expected (x) concentrations derived from dilution of concentrated LDL, was 0-800 mg/dL. Method comparison, using 16 sera measured in triplicate with both the apoB (y) and Roche Integra apoB (x) assays, gave the following regression equation: y =1.00x + 17.3, r= 0.94: bias is explained in part by the greater recovery of VLDL-apoB by the apoB assay (Clin Chem 2010; 56 (Suppl); A185) and by a mean -7% bias for the Roche Integra apoB assay (CDC Lipid Standardization Program, LSP). We measured sera supplied for the LSP in duplicate with the apoB assay (y) and compared results to CDC-assigned values (x), which gave the following regression equation: y =0.90x + 13.0, r= 0.98. For the apoAI assay total imprecision, assessed with three levels of QC (115, 133 and 164 mg/dL), was 2.8%, 3.0%, and 3.1%, respectively, over 3 days with 6 recalibrations. The LoB, LoD and LoQ were 0 mg/dL, 1 mg/dL and 3 mg/dL, respectively. Linearity, derived from dilution of concentrated HDL, was 0-530 mg/dL. Method comparison, using 16 sera measured in triplicate with both the apoAI (y) and Roche Integra apoAI (x) assays, gave the following regression equation: y =1.03x - 2.8, r= 0.98. No hook effect was evident for either assay until >800 mg/dL. For both assays, no interference was seen with hemolysis, lipemia, icterus or EDTA.

Conclusion: We describe reliable and accurate immunoturbidimetric assays for apoAI and apoB. Further studies are needed to assess method comparison with a larger sample size, to assess reagent stability, and to certify traceability of calibrators to international reference materials.

C-127

Usability of the laboratory report and knowledge of lipid-lowering in out-patients from dyslipidemia-related departments

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Background: The lipid profile is a group of tests that are often ordered together to determine risk of coronary heart disease. They are tests that have been shown to be good indicators of whether someone is likely to have a heart attack or stroke caused by blockage of blood vessels or hardening of the arteries (atherosclerois). Our aims were to investigate the usability of laboratory test report from the angle of patients and understand to what degree the patients master the knowledge of lipid-lowering.

Methods: A total of 508 outpatients, selected from a Grade III-A general hospital, were queried by a questionnaire, their medical records and test reports were reviewed and their heights and weights were measured. In the study, 431 of them fulfilled the inclusion criteria and their information about lipid lowering treatment and treatment compliance were collected.

Results: Of the 508 subjects, 90.2% (458/508) read the report seriously; however, only 47.4% (240/508) took drugs according to the doctor's prescription even if the tests were "normal". Of the 431 lipid-lowering therapy related patients, only 26.4% (112/431) chose right in their cardiovascular risk classification, and less than 37.1% (160/431) agreed that "different people had different lipid lowering target". Of the 381 patients who needed the lipid-lowering treatment, 71.7% (273/381) recognized the need for treatment, but 98.7% (376/381) answered a wrong target for treatment; 60.9% (232/381) recognized that the reference values given in the laboratory test reports should be the target for treatment. Of the 246 patients under the lipid-lowering treatment, 35.4% (87/246)had reached their treatment goals, and only 52.0% (128/246) had a good compliance.

Conclusion: Most patients read and trusted the laboratory test reports. However, dyslipidemia patients scarcely knew their lipid lowering treatment goals and their cardiovascular risk levels. The compliance of patients was poor, and the goal attainment was low. The laboratory medicine department should find out a simple and intuitional way to change the current situation.

C-128

Potential Role of Adiponectin in Development of Metabolic Syndrome in Type 2 Diabetics (obese vs non-obese)

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Background: Diabetes is one of the most challenging health problems of 21st century and India leads the world with largest number of diabetic subjects. Obese type 2 Diabetes Mellitus (DM) patients are characterized by increase in adipose tissue mass. Adiponectin produced by adipose tissue exhibits various anti-inflammatory, antiatherogenic properties and prevents insulin resistance. The study was planned to elucidate the relationship of adiponectin levels in non-obese type-2 diabetics, as limited studies are available.

Methods: Fifty type-2 Diabetes Mellitus were studied with twenty, age and body mass index (BMI) matched nondiabetic controls with informed consent. All patients were divided into three groups viz. Low, Normal and High BMI (body mass index) with <19, 19-25 and >25 kg/m^2 respectively. Both insulin and adiponectin were measured by sandwich ELISA technique. Statistical analysis was performed by using SPSS software version-12.

Results: We found 15 % low BMI, 35% normal BMI and 50% high BMI diabetics. The mean insulin levels were low (2.73 µU/ml) whereas adiponectin was high (15.29 µg/ml) in low BMI diabetics compared to other groups. Statistically significant inverse correlation ($p=0.041$) was found between adiponectin and insulin levels in high BMI diabetics only. Adiponectin was found to be directly correlated with HDL-C ($r= +0.67$, $p<0.01$) whereas Non-HDL-C, triglyceride level ($r= -0.13$, $p>0.05$) and waist circumference ($r= -0.55$, $p<0.01$) have shown inverse correlation with adiponectin. Adiponectin levels were significantly higher in diabetic patients present with nephropathy ($p=0.007$) and retinopathy ($p=0.023$) compared to diabetics without it.

Conclusion: These observations suggest high adiponectin levels correlate well with its antiatherogenic lipid profile action. Therefore, it prevents insulin resistance. Thus, it suggests that adiponectin may be missing link in the etiopathogenesis of diabetes mellitus.

C-129

Specific detection of apolipoprotein E4 in biological samples with a biochip immunoassay

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Background. Apolipoprotein E (ApoE) is a plasma protein that participates in different biological processes such as plasma lipoprotein metabolism, cell growth, intracellular cholesterol utilization, immunoregulation, neuronal growth and repair. ApoE is encoded by the three alleles ApoE-ε2, ApoE-ε3, and ApoE-ε4, determining the three major isoforms, namely ApoE2, ApoE3 and ApoE4, in six phenotypes. Human apolipoprotein E4 (ApoE4) is one of 3 major isoforms of apolipoprotein E (ApoE). Genetically, the ApoE-ε4 allele is associated with both familial late-onset and sporadic Alzheimer's disease (AD), which accounts for more than 95% of AD cases. Therefore it is renowned as the best known genetic risk factor for AD. In addition, several studies have linked the ε4 allele with greater risk of cardiovascular disease and with significantly greater progression of disability in multiple sclerosis, though not susceptibility to it.

Relevance. We report the development of a biochip assay for the specific detection of ApoE4 in biological samples, which will facilitate the diagnosis of Alzheimer's disease and the monitoring of its progression.

Methodology. The biochip represents the solid-phase where the capture molecules

are immobilized and stabilized and the vessel for the immunoreactions. A sandwich chemiluminescent immunoassay is employed for the detection. The assay was applied to the semi-automated Evidence Investigator analyser. The system incorporates the software to process, report and archive the data generated.

Results. The assay has a measuring range of 0-400ng/ml and exhibits <1% cross-reactivity with ApoE2 and <1% crossreactivity with ApoE3. Within-run precision was <6% for standard level concentrations.

Conclusion. Results indicate that this biochip assay is highly specific for the detection of ApoE4 genotypes, which can be applied to the diagnosis and monitoring of AD and other disease states.

C-130

N-homocysteinylation of apolipoprotein AI and its effect on antioxidant ability of HDL

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Background: Hyperhomocyteinemia has been recognized as a risk factor for various human diseases including atherosclerotic vascular diseases. A portion of homocysteine (Hcy) exists as an N-linked form to the ε-amino group of protein lysine residues, consequently induces denaturation and/or dysfunction of the proteins. Paraoxonase 1(PON1), carried on high-density lipoprotein (HDL) in the circulation, is considered to hydrolyze Hcy-thiolactone (HcyT), the toxic metabolite of Hcy, and protects proteins against N-homocysteinylation in vivo. We recently developed a novel detection and quantification method for N-homocysteinylated apolipoprotein AI (N-Hcy-apoAI) and proved its existence in normal human serum. The aims of this study are to estimate the effects of (i) HcyT concentration and (ii) PON1 activity on N-Homocyteinylation of apoAI, and (iii) N-Homocyteinylation on the antioxidant ability of HDL.

Methods: N-Hcy-apoAI in human serum was identified and quantified by the previously reported method. Briefly, serum incubated with or without cysteamine was supplied to isoelectric focusing followed by an immunoblot using an anti-apoAI antibody. Human apoAI dose not react with cysteamine due to the absence of a cysteine residue in its molecule. In contrast, N-Hcy-apoAI has free -SH group(s) derived from homocysteine. Thus, cysteamine treatment alters the isoelectric point of N-Hcy-apoAI but not intact apoAI. For quantification of N-Hcy-apoAI, the bands of the apoAI isoforms were digitally scanned and the ratio of N-Hcy-apoAI to total apoAI was calculated with a CS Analyzer (ATTO, Japan). To determine the effect of HcyT concentration and PON1 on N-homocysteinylation of apoAI, HDL was incubated with various concentration of HcyT at 37°C overnight in the absence or presence of EDTA-2Na, inhibitor of PON1. PON1 activity was measured by the previously described method using paraoxon as the substrate. The antioxidant abilities of N-Hcy-HDL and intact HDL were estimated by those effects on LDL oxidation induced by $CuSO_4$, which is detected by an increasing absorbance at 234 nm due to the conjugated diene formation. The absorbance at 234 nm was recorded at 10-min intervals and the lag time, corresponding to the consumption of protective endogenous antioxidants, was calculated as described previously.

Results: The fold changes in relative amount of N-Hcy-apoAI compared to untreated HDL was increased in HcyT concentration dependent manner, however, the statistically significant increase was only observed by the incubation with 0.5 mmol/L of HcyT and over in the present N-homocysteinylation and detection methods. After the HDL with 205 U/L of PON1 activity was N-homocysteinylated by HcyT in the absence and presence of EDTA-2Na, the ratio of N-Hcy-apoAI to total apoAI was 9.7% and 21.2%, respectively. The lag time calculated by the diene formation curves induced by LDL oxidation was statistically prolonged by adding intact HDL. In contrast, no influence was observed by adding N-homocysteinylated HDL, indicating that N-homocysteinylation blocked antioxidant ability of HDL.

Conclusion: N-homocyteinylation of apoAI reduced antioxidation ability of HDL. The other hand, PON1 protects HDL against N-homocyteinylation induced by HcyT. These results suggested the availability of N-Hcy-apoAI as a potential biomarker for atherosclerosis.

C-131

Stability and commutability of a lipid quality control with apolipoproteins

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Background: The measurement of apolipoproteins is becoming increasingly important in coronary heart disease risk assessment. Our goal was to validate apolipoprotein AI (apoAI) and apolipoprotein B (apoB) as additional components of our stable liquid quality control (QC) material that includes total cholesterol (CHOL), triglycerides (TRIG), LDL-C, and HDL-C. The lipids and apolipoproteins are derived from intact human lipoproteins in a human serum protein base.

Methods: In Study 1, our two-level QC was tested daily under open-vial conditions with storage at 2°C to 8°C, and performance was compared to Biorad Lipids Control. Stability was determined according to CLSI EP25-A guidelines with the maximum allowable change from baseline defined as ≤10%. For Study 2, commutability was assessed according to CLSI C53-P proposed guidelines by assaying 16 fresh patient sera samples in triplicate along with two lots of our two-level QC on multiple platforms (Roche Integra 400, Beckman AU 680, Roche Cobas c501, Beckman DxC 800, Beckman Immage 800 (apoAI and apoB only), and Ortho Clinical Diagnostics Vitros 5,1 FS) for CHOL, TRIG, LDL-C, HDL-C, apoAI, and apoB. IRB approval and informed consent were obtained. All results are in mg/dL.

Results: The two-level QC material showed exceptional open-vial refrigerated stability on multiple platforms: at least 60 days for both apoAI and apoB. The QC material also showed excellent precision comparable to Biorad Lipids Control. For apoAI, the mean (± SD) for QC Level 1 after 60 days was 105.9 ± 5.0, 102.8 ± 2.4, 97.0 ± 2.7, and 107.8 ± 3.3 on the Integra, Vitros, Immage, and Cobas respectively. Similarly, for apoB, QC Level 1 was 66.6 ± 2.5, 75.3 ± 2.8, 66.8 ± 1.9, and 70.3 ± 1.3, respectively. Mean (± SD) apoAI for QC Level 2 on the Integra, Vitros, Immage, and Cobas was 163.0 ± 6.1, 153.9 ± 2.8, 142.2 ± 3.1, and 169.8 ± 4.2, respectively, while Level 2 QC for apoB was 116.1 ± 3.4, 128.3 ± 4.1, 107.1 ± 3.2, and 118.5 ± 1.7, respectively. The two-level QC material was commutable with human sera for all assays and on all platforms, except for LDL-C, HDL-C, apoAI, and apoB on the Beckman AU and DxC.

Conclusion: This QC material was stable and precise, and because the material is made with human serum components, we are seeing commutability with human serum for most analytes and platforms.

C-132

Investigation of genetic variants predicting cardiovascular events in hyperlipidemic smokers

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Background: Aim of the study was to investigate genetic variants predicting cardiovascular events in patients with dyslipidaemia and compare the relationship between genetic variants and smoking as common risk factor.

Methods: 108 hyperlipidemic smokers (42 males, 66 females, mean age 57 years) and control group of 63 non-smokers (10 males, 53 females, mean age 52 years) with normal lipid profile were analyzed for the presence of eight mutations and polymorphisms (eNOS -786T→C and G894T; LTA C804A; ACE ins/del; Hpa1 a/b; betaFBG -455G→A; ApoB R3500Q; APOE E2/E3/E4) using ViennaLab CVD Strip essay based on single multiplex polymerase chain reaction (PCR) followed by the reverse hybridization of biotinylated amplification products and detection by visible enzymatic color reaction.. Value of odds ratio with chi square test were used to determine the association of genetic variants with smoking as common risk factor. Value of $p<0.05$ was considered significant.

Results: ACE deletions are most frequent variants found in dyslipidemic smokers (51% of all patients). eNOS G894T mutation shows strongest relationship with smoking (odds ratio = 1.62, 95% confidence interval = 1.01-2.58, p = 0.044)
Conclusion: Testing for presence of polymorphisms and mutations will help in diagnostic and therapeutic medical care of patients at high cardiovascular risk. Complex multigenic approach play important role in investigation and management of dyslipidemic patients.

C-133

Serum lipids and anti-oxidized LDL antibody in Nepalese preeclamptic subjects

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Background: Preeclampsia is pregnancy specific complication characterized by hypertension, proteinuria, edema, and activation of the homeostatic system. Women with a history of preeclampsia are at higher risk for cardiovascular disease (CVD) in later life. Hyperlipidemia is a prominent feature of preeclampsia and abnormalities

have been detected several months prior to clinical detection, implicating these changes in the disease process. Oxidative stress plays a pivotal role in the pathogenesis of preeclampsia. Deficiency in antioxidant level might further enhance the oxidation of Low density lipoprotein (LDL). Hyperlipidemia and increase oxidized LDL may put preeclamptic subject at higher risk for future coronary events. Assessment of cardiovascular risk in Nepalese preeclamptic subject has not been done yet.

Present study was designed for assessing cardiovascular risk in Nepalese preeclamptic women by using serum lipids and antibodies to oxidized LDL (oxLDL Antibodies).

Methods: This study comprises 54 preeclamptic individuals of mean gestational weeks of 32.01±3.08, with mean age of 26.4±3.23. Out of which 41 had mild preeclampsia and 13 had severe preeclampsia. Sixty age and gestational week matched pregnant control were selected among women who visited obstetrics OPD for routine ante-natal check-up. Seventy nine healthy control female volunteers from 21-35 years of age were also recruited. Preeclampsia was defined as per Australasian Society Consensus Statement research definition.

Blood samples were analyzed for total cholesterol (TC), HDL-cholesterol (HDL-C), direct LDL-cholesterol (LDL-C), triglyceride (Tg), and oxLDL Antibodies. LDL-C/HDL-C ratio, TC/HDL-C ratio and non-HDL-C were also calculated. Data were analyzed using SPSS for window.

Results: Preeclampsia was characterized by dyslipidemia and increase level of oxLDL antibodies ($p<0.05$). Mean level of TC, Tg, LDL-C, HDL-C and oxLDL antibodies in preeclampsia was 6.03±0.97 mmol/L, 3.06±0.46 mmol/L, 3.44±1.13 mmol/L, 1.19±0.40 mmol/L, and 55.79±28.85 U/L (vs 5.56±0.65, 2.46±0.39, 3.10±0.78, 1.33±0.36 and 22.30±8.40 in pregnant control; and 4.1±0.50, 1.39±0.58, 2.49±0.50, 1.06±0.19 and 20.83±6.95 in non-pregnant control) respectively.

No significant difference in mean of TC, Tg, HDL-C was seen between mild and severe preeclampsia. In-contrast, mean of LDL-C ($p=0.02$), LDL-C/HDL-C ($p=0.03$), TC/HDL-C ($p=0.029$), non-HDL-C ($p=0.016$) and oxLDL antibodies ($p=0.048$) were significantly higher in severe preeclampsia.

Area under the Receiver Operating Characteristics (ROC) curve, plotted to find out the diagnostic efficiency of oxLDL antibodies was 0.910 at 95% CI. ($p<0.001$). ROC curve identified that with cut-off point of 28.0 U/L, the sensitivity and specificity of oxLDL antibodies to diagnose preeclampsia was respectively 90% and 85%, with positive and negative predictive value of 84.4% and 91.07% respectively.

Conclusion: Clustering of cardiac risk factors was associated with preeclampsia. Established risk factors including TC, LDL-C, non-HDL-C and Tg were significantly higher in preeclampsia. The emerging risk factor oxLDL antibodies was also significantly higher in preeclampsia. Increase levels of these biomarkers add risk for CVD. oxLDL antibodies with cut-off of 28.0 U/L can be used for differentiating preeclampsia from normal pregnancy.

C-134

Development and Validation of an LC-MS/MS Assay for Quantitation of Urinary Prostaglandins

A. J. Lueke, A. K. Saenger. *Mayo Clinic Rochester, Rochester, MN,*

Introduction: Prostaglandins (PG) and thromboxanes are produced and mediated via the cyclooxygenase pathway and are of broad interest in the pathobiology of cardiovascular diseases. Isoprostanes are PG-like compounds produced by the free radical-catalyzed perioxidation of arachidonic acid (AA) independent of the cyclooxygenase pathway and thus provide an *in vivo* assessment of lipid peroxidation or "oxidative stress". Mechanistically prostaglandins are generated only from free AA while isoprostanes are primarily formed esterified to phospholipases. Various modalities currently exist to measure small subsets of these biomarkers, although isomer cross-reactivity remains a major issue. We describe a novel liquid chromatography tandem mass spectrometry (LC-MS/MS) method to specifically quantitate urinary 11-dehydro-thromboxane B_2 (11-dTxB_2), prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), and 15-F_{2t}-IsoP (8-iso-$PGF_{2\alpha}$) as markers of platelet activation/aspirin responsiveness, vascular regulation, and lipid peroxidation. This method allows for online Turboflow extraction of urine specimens and is ideal for large studies involving urine thromboxanes and prostaglandins.

Methods: Urine was acidified (pH 2.0) and internal standard (three deuterium labeled IS, concentration = 25 ng/mL) was added to aliquots in a 96 well Teflon filter plate. Samples were vacuum filtered into a 96 well collection plate before injection of 45 µL onto an autosampler and retention on a Turboflow MAX mixed-mode anion exchange column (0.5 x 50 mm). Following elution with methanol/0.1% formic acid, prostaglandins were further chromatographically separated on an analytical column (Waters Xbridge, C18 reversed phase, 2.1 x 50mm, 60°C) using a H_2O/MeOH gradient for 6 minutes, using 0.1% ammonium hydroxide as the modifier. Analytes were monitored in negative MRM mode (AB Sciex API 5000 MS/MS) using the following transitions: m/z 367/161, 367/305 (11-dTxB_2) and m/z 371/165, 371/309 (d_4-11-dTxB_2), respectively. PGF_{2a} and 8-iso-PGF_{2a} were monitored using a transition of m/z 353/193 (analyte) and m/z 357/197 (internal standard); separation was achieved chromatographically. Total analysis time was 10 minutes 35 seconds, with a 3.5 minute window on the mass spectrometer.

Results: Our results demonstrate linearity between 0 - 5000 pg/mL (11-dTxB2), 0 - 10,000 pg/mL (PGF2a), and 0 - 2000 pg/mL (8-iso-PGF2a). (intercept = 0.0003, R2 = 0.9999). Intra-and inter-assay CVs were 6-8% and 8-10% respectively across all analytes. Sensitivity was 86 pg/mL (11-dTxB2), 345 pg/mL (PGF2a), and 31 pg/mL (8-iso-PGF2a) determined in the lowest level native urine sample run (n= 40). Recovery of a spike into native urine was within 15% of expected for all three analytes.

Conclusion: We have developed a sensitive, precise, and rapid LC-MS/MS assay to measure urinary 11-dTxB2, PGF2a, and 8-iso-PGF2a which is suitable for large scale studies involving a broad range of cardiovascular entities.

C-135

Relationship between TRIB1 gene s17321515 polymorphisms and type 2 diabetes mellitus in Han nationality from northern China

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Background:TRIB1 gene rs17321515 single-nucleotide polymorphism (SNP) has been associated with variation in low density lipoprotein cholesterol high density lipoprotein cholesterol and triglycerides (TG) concentrations. This effect has never been studied in patients with type 2 diabetes mellitus (T2DM) in China. Our aims were to assess the association of the rs17321515 (TRIB1) SNP with plasma lipids concentrations and to explore the interaction between this SNP and some classic risk factors in patients with T2DM in Han nationality from Northern China.

Methods: Genotypes of TRIB1 rs17321515 polymorphisms were analyzed by using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) in 148 unrelated healthy individuals, 98 patients diagnosed of T2DM, and 76 patients of T2DM combined coronary heart disease (CHD). The association of TRIB1 gene rs17321515 SNP with levels of hemoglobin A1c(HbA1c), serum lipids, and glucose was also assessed.

Results: Neither the frequencies of genotypes nor frequencies of alleles of rs17321515 polymorphisms were statistically different among DM patients, DM+CHD patients and controls ($P>0.05$). The genotype frequencies of AA, AG, GG were 0.151, 0.500, 0.349, respectively; the allele frequencies of A and G were 0.401, 0.599, respectively. There was no significant difference in the frequencies of genotypes or alleles of rs17321515 polymorphisms between Han nationality from Northern China and other populations abroad ($\chi^2=3.543$,$P=0.471$). In the T2DM+CHD group, TG levels of G allele gene carriers were statistically higher than that of AA genotype ($P<0.05$). No relationship between gender and TRIB1 rs17321515 polymorphisms was found. Logistic regression analysis showed that A allele was the risk factor for T2DM with CHD (OR=2.212, $P=0.041$).

Conclusion: The rs17321515 polymorphisms of TRIB1 gene showed no significant correlation with T2DM, but showed the association with T2DM combined CHD. A allele of TRIB1 gene rs17321515 may be the genetic risk factor of T2DM with CHD in Han nationality from Northern China.

C-136

Differences in reactivity toward abnormal lipoproteins between six homogenous assays for LDL-cholesterol in sera of patients with cholestasis

H. Sugiuchi[1], T. Irie[2], K. Matsushima[1], H. Nishimura[3], M. Manabe[3], K. Ikeda[3], Y. Ando[3], Y. Ishitsuka[2], M. Irikura[2]. *[1]Kumamoto Health Science University, Kumamoto, Japan, [2]Graduate School of Pharmaceutical Sciences, Kumamoto University, Kumamoto, Japan, [3]Graduate School of Medical Sciences, Kumamoto University, Kumamoto, Japan,*

Background: Abnormal lipoproteins, such as lipoprotein X and abnormal LDL, frequently appear in sera of patients with cholestasis. Because the density of the abnormal lipoproteins is equivalent to that of the normal LDL, the abnormal lipoproteins are interpreted as LDL on the β-quantification reference method (BQ method). This makes it necessary to identify the accuracy of homogeneous assays that use different principles to measure LDL-cholesterol (LDL-C) with respect to sera containing the abnormal lipoproteins.

Methods: The concentrations of LDL-C in the sera of patients with cholestasis or hypercholesterolemia were measured by the modified BQ method and 6 homogenous assays: Liquid selective detergent method (Method A), selective solubilization method (Method B), elimination method (Method C), enzyme selective protecting method (Method D), calixarene complex method (Method E), and phosphate complex inhibition method (Method F). The concentrations of Lp-X in sera from the cholestatic patients were analyzed using the precipitation method. The fractions with densities of 1.006-1.063 kg/L (LDL fractions) were isolated from sera of the cholestatic patients, and the chemical composition of lipids and apoproteins and the reactivity of the homogeneous assays in each of the fractions were analyzed by gel filtration HPLC.

Results: A good correlation exits between the modified BQ method and each of the homogeneous assays in sera of the hypercholesterolemic patients (average difference: -0.429-0.165 mmol/L, 2SD: 0.371-0.840 mmol/L). In sera of the cholestatic patients, each of the homogeneous assays, except for Method A, was negatively biased compared to the modified BQ method, the magnitude of the bias increasing along with increasing concentrations of Lp-X. Gel filtration analyses revealed that the LDL fractions from sera of the cholestatic patients contained large amounts of Lp-X; the lipid composition in Lp-X was almost same regardless of the background of the patients. In the Methods A and C, with increasing concentrations of Lp-X in each of the Lp-X fraction, relative reactivity of cholesterol in the corresponding fraction increased, probably due to insufficient deletion of Lp-X by reaction reagents during the first process of the analytical procedure employed. In contrast, the Methods B and F were less reactive toward the Lp-X fraction.

In addition to Lp-X, the LDL fractions from sera of cholestatic patients contained abnormal LDL that showed an increased ratio of phospholipids and triglycerides to total cholesterol ((PL+TG)/TC ratio) compared to the normal LDL; the degree of the abnormality in lipid composition and content inof the abnormal LDL varied with the background of the patients. Each of the homogenous assays showed decreased reactivity of cholesterol toward the abnormal LDL fraction along with increasing the (PL+TG)/TC ratio.

Conclusion: We demonstrated that in the modified BQ method and some of the LDL-C homogeneous assays, non-atherogenic Lp-X in sera of the patients with cholestasis was measured as LDL. In order to avoid confusion in clinical settings, it is important to thoroughly understand the measuring principles and reaction characteristics in the assays used, when analyzing cholestatic samples.

C-139

The presence of postprandial remnant lipoproteins in the fasting plasma with TG levels below 150 mg/dL

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Background: Remnant-like lipoprotein particles (RLP) have been measured by cholesterol as RLP-C for CHD risk assessment. However, RLP-TG is found to a better marker to detect the presence of postprandial remnant lipoproteins in the fasting plasma with TG levels below 150 mg/dL.

Methods: Serum RLP-TG levels in the fasting and postprandial state were determined in health-check population, cardiovascular disease and oral fat load cases. Serum TC, TG, HDL-C, LDL-C and RLP-C concentrations were also determined in the same cases.

Results: RLP-TG levels were shown to be significantly higher in cases with diabetes, metabolic syndrome, cardiovascular disease as RLP-C levels. Cut-off value (75 percentile) of RLP-TG determined in the fasting normal control cases in Japanese population was 13 mg/dl in men and 10 mg/dL in women. Ninety fifth percentile of RLP-TG with TG levels below 150 mg/dL was 20 mg/dL. In patients with diabetes, metabolic syndrome, cardiovascular disease, RLP-TG levels below 150 mg/dL were significantly higher than those in normal control subjects. When fasting TG levels were below 150 mg/dl, the frequency of the cases with RLP-TG>20 mg/dL in normal control group was 4.8% and that in disease cases were significantly higher (more than 10% respectively). RLP-TG levels increased significantly after oral fat load in postprandial plasma with TG levels above 100 m/dL.

Conclusion; The frequency of higher RLP-TG levels above 20 mg/dL in the fasting state with TG< 150 mg/dL was significantly higher in the disease cases than in normal controls. Especially RLP-TG levels started to increase significantly above TG 100mg/dL after oral fat loard. These results revealed that cut-off value of TG below 150 mg/dL are limited only to the normal controls. To detect the cases with RLP-TG above 20 mg/dL in the fasting state may provide the new insight of abnormal postprandial remnant metabolism.

C-140

Effect of ezetimibe on components of HDL subclass in patients with end-stage renal disease

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Background: The aim of this study was to investigate the effects of Ezetimibe monotherapy on HDL subspecies and serum amyloid A(SAA), an apolipoprotein mainly bound and transported by HDL particles, in patients with end-stage renal disease (ERSD).

Methods: 26 ERSD patients receiving hemodialysis (HD) were given ezetimibe (10 mg/d) for 6 - 8 weeks. HDL3 was separated from serum by a single precipitation method using heparin, $MnCl_2$ and dextran sulfate. The components of HDL3 were measured after separation. HDL3-C was measured by a homogeneous HDL-C assay (Denka Seiken). HDL2 was estimated by subtracting HDL3 from total HDL. SAA was measured by ELISA method (Carlsbad). Apolipoprotein (apo) A1, apo B was measured by immunoturbidometric assay (Sekisui Chemical).

Results: Ezetimibe significantly reduced Remnant-like particle (RLP)-C, low-density lipoprotein (LDL)-C, and apo B without affecting triglyceride, HDL-C and LCAT activities. HDL2-C levels were lower and HDL3-C was substantially lower in the HD patients than in the controls. Ezetimibe increased HDL2-apoAI but decreased HDL3-apoAI without affecting serum apoAI or AII. HDL-SAA was 5-fold higher in HD patients than in control. Ezetimibe decreased HDL-SAA by 43 %, and this inhibitory effect was exclusively attributable to a 72% reduction in HDL3-SAA in response to the ezetimibe treatment. The reduction of HDL3-SAA was significantly associated with increased HDL2-apo AI and reduced HDL3-apo AI.

Conclusion: Ezetimibe treatment decreased "inflammatory" (SAA-containing) HDL3, and may thus have restored the anti-atherogenic function of HDL particles in ESRD patients.

C-144

Comparative test between dosed and estimated LDL cholesterol to determine the best type of Friedewald's formula different Triglycerides ranges

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Background: The LDL-cholesterol (LDL) is useful in the diagnosis of dyslipidemia and a risk factor for coronary artery atherosclerosis. Its laboratorial realization is performed directly (LDLd) or is calculated through the Friedewald's formula (LDLe). The latter uses the values of total cholesterol (ColT), HDL-cholesterol (HDL) and triglycerides (TG). However the equation has only been useful in TG levels <400 mg/dL; and in its formula (LDL= TG / k + HDL - ColT) the multiplication constant (k) of the TG varies between 1/8 and 1/5. Although the latter is mostly used, it is still being questioned. This study was designed to obtain a better constant (k) and to determine if there is a different cut level for TG than 400mg/dL.

Methods: 87 patients between the ages of 15 and 90 were evaluated, among which 37 from the feminine gender and 50 from the masculine gender. TG, HDL, ColT and LDLd were dosed through the enzimatic method (Roche - Hitachi Modular P). The statistical analysis through linear correspondence in the Excel program (Microsoft). Apolipoprotein B(ApoB) and Apolipoprotein A-1(ApoA-1) were measured by nephelometry method (Siemens - BNII). ApoB and ApoA-1 were normal in all patients.

Results: The samples were evaluated and set in TG levels in the following groups: 29 patients with TG >400mg/dL (group 1), 14 patients with TG between 200-400mg/dL (group 2), 44 patients with TG up to 199 mg/dL (group 3). The constants (k) in the values of 1/5(k1/5) and 1/8(k1/8) were applied in the different ranges of TG, and the LDLe was determined by the Friedewald's formula, and correlated with the LDLd, this one being considered the pattern. The values obtained for the constant k1/8 were: Group 1, 0.39; Group 2, 0.95; and Group 3, 0,99. For the constant k1/5 were: Group 1, 0,90; Group 2, 0,97; and group 3, 0,99.

Conclusion: The constant k1/5 is the best one for all TG ranges, including the range where the patients presented TG above 400mg/dL, with correlation coefficient of 0,90. The TG cut point to determine the LDLe remains 400mg/Dl for the constant k1/8.

Wednesday PM, July 27

Poster Session: 2:00 pm - 4:30 pm
Point-of-Care Testing

D-01

Under-estimation of Plasma Creatinine using iSTAT Point-of-Care Analyzer: Impact on Carboplatin Dosing in an Academic Cancer Center

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Background: Chemotherapeutic agents, specifically carboplatin, are frequently dosed using equations which rely on Creatinine (Creat) measurements from point of care (POC) test systems or automated chemistry analyzers. As manufacturers converted to IDMS-traceable Creatinine methods, an increased incidence of carboplatin-related adverse events has been reported. The FDA, ASCO and NCI issued recommendations for capping carboplatin doses using a maximum GFR of 125 ml/min. The aim of the current study was to (1) correlate the POC whole blood Creat measurements on the i-STAT1 Portable Clinical Analyzer (iSTAT) to plasma Creat measurements on the Siemens Dimension Vista chemistry analyzer (Vista) in oncology patients with normal to low abnormal creatinine and (2) to evaluate the potential impact on carboplatin dosing using results from both methods.

Methods: Samples for routine Creat measurement were obtained from outpatients in a cancer clinic prior to therapy. Whole blood samples were initially tested using the iSTAT enzymatic Creat method. These samples were subsequently centrifuged and Creat measured on the plasma samples by an IDMS-traceable enzymatic Creat method (Vista). Creatinine clearance (CrCl) was calculated using the Cockroft-Gault equation. Estimated glomerular filtration rate (eGFR) was calculated using the Modified Diet in Renal Disease (MDRD) equation for IDMS-traceable Creatinine measurements. The Calvert formula was used to estimate the total carboplatin dose [Total dose (mg) = (target AUC) x (CrCl + 25)]. The potential impact on the CrCl/eGFR and carboplatin dose was modeled using a non-African American female aged 55y, weight 60kg, with target AUC of 5. Correlations of Creat results, eGFR, and carboplatin dose by iSTAT vs VISTA were assessed by linear regression and Bland-Altman analysis.

Results: Thirty-three patient samples were obtained for comparison. Results ranged from 0.46 to 1.94 mg/dL (Vista) and 0.20 to 1.90 mg/dL (iSTAT). Linear regression between (iSTAT) and (Vista) Creat levels resulted in the following relationships: Slope 1.092 intercept -0.096, Standard Error of the Estimate 0.144, Correlation Coefficient 0.926, and mean Bias -0.02. iSTAT Creat results below the lower limit of the reference range of 0.60 mg/dL averaged 0.19 mg/dL lower than Vista Creat in n=7 events (range -0.03 to -0.32 mg/dL). CrCl and eGFR were overestimated with a potential increase in carboplatin dose by an average 455 mg using CrCl (600 mg using MDRD) without FDA dose capping (range -38 to +926 mg CrCl; -47 to 1232 mg MDRD). Implementation of the 750 mg maximum dose cap for a target AUC of 5mg resulted in overestimation of the carboplatin dose based on iSTAT measurement by an average 103 mg using CrCl (82 mg using MDRD) (range 46 to 139 mg CrCl; 13 to 125 mg MDRD) in n=4 events.

Conclusion: The iSTAT whole blood Creat demonstrated acceptable overall correlation to Vista plasma creatinine patients with normal renal function, but was noted to underestimate Creat at and below the lower limit of the reference range. Even with use of the recommended eGFR cap, this difference could lead to overestimation of the carboplatin dose in patients with normal renal function using the iSTAT analyzer.

D-02

Operational characteristics of AmniSure vaginal fluid alpha microglobulin-1 test for detection of rupture of fetal membranes

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Background: The AmniSure alpha microglobulin-1 test (AMG) is intended to detect AMG in vaginal fluid as a marker for rupture of fetal membranes (ROM) during pregnancy. Accurate diagnosis of premature ROM is helpful to treat patients prophylactically against infection. Here we report our investigations of operational characteristics of the test.

Methods: AmniSure (AmniSure LLC, Cambridge, MA) is a qualitative, single-use disposable strip immunoassay for AMG in format equivalent to conventional point-of-care pregnancy tests for hCG (colloid-label sandwich immunoassay). For patient testing, primary sample is a vaginal swab, which is eluted into approximately 0.5 mL of prealiquotted kit diluent for testing. The test strip is inserted into diluent for 10-15 min before reading of test and control lines. Positive controls are provided by manufacturer as prepackaged lyophilized AMG, which is reconstituted in 1 mL saline; this solution is tested directly by insertion of test strips into the reconstitution vials. For investigation, we prepared controls according to package insert instructions, and also prepared samples and normal saline dilutions of amniotic fluid, whole blood, plasma, urine, and 22% bovine albumin solution (BAS, Immucor, Norcross, GA); these samples were tested either directly as per controls (strip placed directly in sample) or indirectly per patient sample testing (strip placed in diluent after swab transfer).

Results: Reconstituted controls (10 ng/mL) were positive on immediate testing. The reconstituted control solutions were found not to be stable, producing negative results after 23 hours refrigeration or freezing. The positive controls were near the limit of detection for the assay (reported to be 5 ng/mL), as 1/3 dilution of freshly reconstituted controls in saline produced negative test results. Indirect sampling of amniotic fluid samples were strong positives at up to 1/100 dilutions which remained strong positives after freezing. Direct sampling of urine from a pregnant female (hCG=45,000 mIU/L) was negative; however, direct test strip sampling of serum from pregnant female (hCG=280,000 mIU/L) was a strong positive, as were male serum samples and 22% BAS. Dilutions of male serum were positive by direct sampling for as low as 1/16 dilution in saline. However, neither serum nor BAS samples tested positive by indirect testing. Whole blood was found to be positive by both direct and indirect sampling. Measurement of swab capacity for saline was found to be approximately 170 uL, such that dilution of samples after swab elution into prealiquotted kit diluent is at least 1/5 assuming 100% recovery from the swab. The difference between direct and indirect sampling of serum samples indicated, however, that recovery of AMG from swabs for these samples was less than 50%.

Conclusions: Reconstituted positive control solutions were found to be unstable on storage. Fluids other than amniotic fluids were strong positives on direct sampling; however, there were no instances of positives from indirect sampling of fluids other than amniotic fluid and whole blood. Barring bloody samples, positive patient test results by indirect sampling are likely to be due to presence of amniotic fluid, consistent with the manufacturer's description and intent of the test.

D-03

Evaluation of the HemoCue Hb 201 DM using capillary compared to venous whole blood samples

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Objective: In our practice we use HemoCue Hb 201 DM at the point of care with samples obtained by venipuncture and capillary fingerstick sampling. Initial validations were completed with samples obtained by venipuncture. In response to requests from additional clinical areas to use the HemoCue with capillary fingerstick sampling, we studied the reliability of the HemoCue using both venipuncture and capillary fingerstick samples.

Methods: Twenty five volunteers had 2 capillary punctures performed for hemoglobin measurements on the HemoCue Hb 201 DM (HemoCue AB, Ängelholm Sweden), and venipuncture performed for EDTA whole blood hemoglobin measurement on the HemoCue and the Sysmex XT-2000(Sysmex Corporation, Kobe Japan) used as a reference method. The first capillary puncture was completed by 2 technicians experienced in both capillary puncture and use of the HemoCue. A second capillary puncture (for a total of 50 capillary punctures) was completed by five different technicians inexperienced in both capillary puncture and use of the HemoCue device; the goal of this study design was to determine whether user experience impacted bias and variability between capillary HemoCue and venous reference (Sysmex) hemoglobin values. The HemoCue Hb 201 DM system utilizes an azidemethemoglobin method for spectrophometric measurement of hemoglobin. The Sysmex XT-2000 is an automated hematology analyzer that measures hemoglobin spectrophotometrically using a sodium laurel sulfate hemoglobin method.

Results: Mean bias (SD) for results obtained by capillary fingerstick was 0.5 ± 0.7 g/dL. Mean bias (SD) for venous HemoCue samples was 0.5 ± 0.2 g/dL. Only 17 of 50 (34%) capillary vs. 16 of 25 (64%) venous whole blood HemoCue results were within 0.5 g/dL of the reference result; while 37 of 50 (74%) capillary vs. 25 of 25 (100%) venous HemoCue results were within 1.0 g/dL of the reference value. Mean bias and standard deviation between fingerstick capillary and reference hemoglobin samples

performed by experienced vs. inexperienced collectors were identical, indicating that user experience was not a significant factor in bias and variability observed with capillary HemoCue measurement.

Conclusions: Mean bias does not differ between capillary and EDTA whole blood samples analyzed on the HemoCue Hb 201 DM analyzer. However variability (as measured by standard deviation) is significantly greater when capillary samples are used, such that a significant number of samples differ from reference hemoglobin by more than 1.0 g/dL. This variability may limit the utility of capillary sampling when precise estimates of hemoglobin are needed, and does not appear to be related to user experience.

D-04

New POCT system "Banalyst® M" using a microfluidic chip achieves simultaneous rapid diagnosis of HbAlc and Glu

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Background: Banalyst® M, a new microblood analyzing system for POCT, comprises a μTAS (Micro Total Analysis System) chip (l30 mm, w62 mm, t12 mm) and a compact desktop measuring instrument, and measures and mixes samples in a chip with micro channels. Unlike the Banalyst reported at the same academic conference, Banalyst® M measures maximum 5 items by one chip. "Banalyst M HbA1c/Glu" recently developed measures hemoglobin A1c (HbA1c) and glucose (Glu) simultaneously from only a 10 μL whole blood sample. Using this chip, system was evaluated at Kyushu University Hospital. The results are reported as follows

Methods: In the system, the solution inside the chip is moved by the centrifugal force generated by rotation of the stage inside the analyzer and solution travel directions are controlled by switching directions of the chip on the stage, thereby treating, measuring, and mixing solutions. In the HbA1c/Glu chip, the whole blood is centrifuged and separated into blood cells and plasma. The blood cells are transferred to the HbA1c measurement unit and the plasma to the Glu measurement unit, respectively. In the chip, liquid reagents of HbA1c (latex agglutination) and Glu (GDH method) are packed in advance, specimens separated and reagents successively mixed, and finally, absorbance measured. All operations are finished in about 14 minutes.

Examinations and **Results:** (*HbA1c: NGSP%) [HbA1c] *Reproducibility:* Patient whole bloods of two concentrations were measured. In the normal concentration region, CV 1.6% (mean 4.8%, n=5); in the high-concentration region, CV 0.9% (mean 8.6%, n=5). *Linearity:* Two Samples of close concentrations of Hb (HbA1c: 4.8 % and 13.1%) were mixed in 10 stages and measured. Linearity was confirmed at 4.8 to 13.1%. *Correlativity:* Correlation formula between Banalyst M(y) and H7700(x) (latex agglutination, Hitachi High-Technologies Corporation): y = 1.022 x -0.224 and correlation factor: r = 0.995 (n=101, (y) whole blood, (x) blood cell). Between Banalyst M(y) and HLC-723G7(x) (HPLC method, TOSOH Corporation): y = 1.008 x -0.123, r = 0.993 (n=101, (y) whole blood, (x) blood cell). [Glu] *Reproducibility:* Patient whole bloods of two concentrations were measured. In the normal concentration region, CV 4.4% (mean 4.4 mmol/L, n=5); in the high-concentration region, CV 2.7% (mean 20.5 mmol/L, n=5). *Linearity:* 111.0 mmol/L sample was diluted with physiological saline in 10 stages and measured. The linearity was confirmed up to 55.5 mmol/L. *Correlativity:* Correlation formula between Banalyst M(y) and H7700(x) (GDH method, Hitachi High-Technologies Corporation): y = 1.062 x -8.258 and correlation factor: r = 0.987 (n=101, (y) whole blood, (x) serum).

Conclusion: Favorable fundamental characteristics were obtained. Unlike many POCT systems, Banalyst M adopts liquid reagents used with general automatic analyzer. This enables Banalyst to achieve good correlation with general automatic analyzer. Banalyst measures HbA1c and Glu using 10 μL whole blood and provides the measurement results in about 14 minutes after blood collection. This is why Banalyst is thought useful for diagnosis and control of diabetes in outpatient settings. It is expected that changing the reagent in the chip will allow other items to be measured.

D-05

Automatic Urine Strip Identification and Strip Quality Check on the CLINITEK Advantus® Analyzer

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Introduction and Objectives: The CLINITEK Advantus analyzers (Siemens Healthcare Diagnostics, Tarrytown, NY, US) incorporate new automated checks (Auto-Checks) which deliver quality results to healthcare professionals. The analyzers are designed to meet the needs of the central laboratory, emergency room environments, physician offices, and outpatient clinics performing medium- to high-volume urinalysis testing. These automated quality checks are performed on each test strip, and the analyzer automatically identifies the Siemens urine strip type and improves workflow by eliminating operator intervention to select strip type. In addition to this feature, a novel strip-quality check for humidity-compromised strips was implemented. Urine strip quality is essential for obtaining accurate urinalysis test results, and improper handling of strips can lead to false results.

Materials and Methods: A study was performed to verify correct identification of the 18 strip types included in the current analyzer menu. To simulate a user setting, a total of 10,901 individual strips were tested by multiple operators with solutions representative of possible problematic clinical samples, as well as normal samples. A negative test solution; a high-protein, dark-colored solution representing approximately 5% of clinical samples; and high-analyte solutions for nitrite, urobilinogen, protein, and pH were all implemented in this study. For analyzer evaluation, a process for addition of multiple strip types was conducted in which one replicate of a strip type was tested and rapidly followed by one replicate of a different strip type until all strip types were tested. In addition, a systematic process for addition of a single strip type was conducted in which the same strip type was tested repeatedly. A second study analyzed Siemens' MULTISTIX® 10SG urine reagent strips exposed to high humidity. Strips were exposed to 80% humidity in an environmental chamber for periods of 10, 20, 30, and 40 minutes. Along with a set of non-exposed strips, all strips were tested on the analyzer with test solutions containing different concentrations of leukocyte esterase. Clinical results for each strip at each exposure time were recorded.

Results: The first study, designed to challenge the auto identification feature of the CLINITEK Advantus analyzer, demonstrated a rate of misidentification of <0.01%. The second study demonstrated the analyzer's ability to detect strips compromised after 20 minutes of exposure to high humidity. Moreover, a compromised strip error was incurred in >99% of strips exposed to humidity for 30 minutes or longer. These strips that were detected would have otherwise generated a positive leukocyte clinical result for a leukocyte-negative test solution.

Conclusion: Two new features have been developed for the CLINITEK Advantus analyzer to deliver quality results to healthcare professionals in the US. The automatic identification of urine test-strip types provides the user the freedom to automatically alter strip types during urinalysis testing without additional user intervention, saving time and minimizing errors. The strip-quality check provides a high detection rate for humidity-compromised strips that could otherwise yield false results.

D-06

Novel Cellular Hemoglobin A1c Control - A Commutable Control Across HPLC, Immunoassay and Boronate Affinity Methods

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Background: Diabetes is one of the leading causes of premature death in the present world. Research showed that diabetes is most effectively managed by healthy lifestyle and routine monitoring of glycemic status. Hemoglobin A1c is an index of diabetes with several advantages over the traditional glucose based diabetes indices.[1] A1c tests are independent of external patient conditions such as smoking or fasting; are quick test and provide long term glycemic information. The A1c instruments are required to be calibrated and checked using A1c controls. Commercial A1c controls are noncellular which do not test the entire analytical system of the instrument, including the lysing step. Lysate based A1c controls are also reported to give erroneous results.[2] Streck A1c-Cellular® is the only cellular control which has intact RBC. Therefore, Streck A1c control tests the entire assay procedure. Streck A1c control is also commutable across different A1c analyzers of all methodologies. The importance of commutable calibrators is emphasized in order to harmonize the clinical instruments.[3] The aim of the present study is to compare the performance of Streck A1c controls with the other commercial controls and establish its commutability across different A1c methodologies.

Methods: Bio-Rad Lyphochec®, Bio-Rad Liquichek™ and MAS® Diabetes controls are compared with Streck A1c-Cellular Control. G8 A1c analyzer (Tosoh BioScience), DCA Vantage™ (Siemens), Cholestech GDX™ analyzer (Cholestech) and *Ultra*[2] Dual Assay (Primus diagnostic) instruments were used for analysis. Methods and calculations were performed based on CLSI guidelines.

Results: The mean S-A1c of the level-1 Streck A1c control is ~6±1% compared to 5.8-6% for the other controls. The mean S-A1c of the level-2 Streck A1c control is ~11±1% as compared to ~10±1% for the diabetic level of Bio-Rad and MAS controls. The CV values of the Streck controls are 2±1% which compare to the competitor controls and are within the NGSP recommendations. The Streck A1c-Cellular® A1c

values are consistent across three methodologies of A1c analyzers. The SD for Streck controls are 0.6±0.1% across different methodologies. Under the microscope the Streck controls clearly show the presence of intact RBCs, whereas the other controls do not show presence of any cellular component.

Conclusion: The Streck A1c-Cellular controls are reliable A1c controls with CV values within NGSP and IFCC recommendations. The Streck A1c-Cellular controls are comparable with the other commercial controls in terms of A1c values. The Streck A1c-Cellular® control can be used with all A1c methodologies. Thus can be used for harmonization of various A1c instruments. Furthermore, Streck A1c-Cellular control is the only cellular control with intact human RBC.

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D-07

Transcutaneous Bilirubin Comparison Studies: BiliChek Versus Drager Methods

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Backround: Bilirubin measurement is required for all newborns for the prevention of kernicterus. Our hospital has been utilizing a non-invasive point-of-care (POC) transcutaneous bilirubin (TcB) meter, the Bilichek system by Philips, and found the system provided reliable results while reducing the need for bilirubin testing in our core laboratory. The instantaneous results allow a discharge protocol of 24-hours for all well newborns. Recently, we initiated an evaluation of a more cost effective Drager Jaundice Meter (Model JM-103) which eliminates the extra expense of the disposable shield.

Methods: Comparison studies were performed to establish the accuracy and precision of the new Drager meter. Initially, seventy-three newborns were tested for TcB using the BiliCheck and Drager devices as well as serum bilirubin testing in the core laboratory on our Beckman Coulter DxC800 chemistry analyzers. All sternum TcB measurements performed by our trained nursing personnel following manufactures' recommendations. After observation of a significant positive bias on the Drager meter in three darkly pigmented newborns, we initiated an additional 47 patient TcB comparison study focused on the effect of skin color.

Results: As shown in Table 1, both the BiliChek and Drager TcB methods showed reasonable correlation with serum bilirubin levels. However, we observed a more consistent scatter about the regression line on the BiliChek compared to the Drager meter (Standard error of estimate Syx = 1.30 vs. 1.71). The Drager meter showed increased deviation in the 8 to 10 mg/dL range, overlapping our cutoff value of 10 mg/dL. While the the initial study showed significant bias of the Drager meter (8.4, 9.3, 9.2 mg/dL serum versus 13.3, 14.5, 14.1 Drager, respectively), followup skin color studies failed to confirm this observed bias.

Conclusion: Our studies showed the Drager meter compared favorably with the BiliChek system regardless of skin color.

Table 1. Comparison Studies Summary

Study 1 and 2	n	Skin Color	Slope	y-Inter	R2	Std Error
BiliChek vs Serum Study 1	65	all	0.99	1.35	0.78	1.3
Drager vs Serum Study 1	73	all	1.03	0.56	0.73	1.7
Drager vs BiliChek Study 1	74	all	0.90	0.40	0.67	1.79
BiliChek vs Serum Study 2	47	all	1.01	0.69	0.78	1.24
Drager vs Serum Study 2	47	all	1.06	-0.55	0.80	1.24
BiliChek vs Serum Study 2	24	light	0.92	0.91	0.73	1.17
Drager vs Serum Study 2	24	light	1.02	-0.67	0.76	1.16
BiliChek vs Serum Study 2	23	dark	1.10	0.54	0.85	1.20
Drager vs Serum Study 2	23	dark	1.13	-0.50	0.86	1.19

D-09

Main Laboratory vs. Point of Care Laboratory Testing in the Emergency Department: Impact on Patient Flow and Meaningful Use.

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Background: The study investigates the impact of a point of care laboratory in the emergency department on patient care. It also looks at potential financial implications on the hospital in terms of the governmental initiative of meaningful use.

Methods: Two emergency departments located 10 miles apart and part of an integrated health network serve as the basis for this comparative study. One hospital has a POC laboratory in the ED and is staffed by lab personnel who are responsible for sample analysis and result reporting. At the second site samples are sent via a pneumatic tube system to the main lab. The network's electronic medical record system was used to perform a retrospective review during a 2-week period for three chief complaints: chest pain and/or shortness of breath and/or weakness. The POC laboratory uses the Abbott iStat to perform the Basic Metabolic Panel (CPT code 80048) and blood gases and a Biosite Triage Meter to perform cardiac markers (troponin I, CKMB, and BNP). The main laboratory uses a Beckman DxC 600 for the Basic Metabolic Panel, a Radiometer ABL 835 for blood gases, and Beckman Access2 to perform cardiac markers.

Results: The mean turnaround time from placement of the order to result reporting was 20 and 36 minutes, respectively, for the POC lab and the main lab. The 16-minute reduction in lab reporting time translated into a reduction in patient disposition or time to hospital admission (81 minutes using the POC lab vs. 97 minutes using the main lab). The ED data is being collected and submitted for the meaningful use governmental initiative. The guidelines have yet to be set; however, lab turnaround times and time to decision making will likely be under consideration for meaningful use. Anecdotally, the ED POC laboratory program has demonstrated improved communication between the laboratory and the ED, with a reduction in mislabled, lost, and improperly collected samples.

Conclusion: The POC lab improved patient flow through the ED, eliminated many potential delays caused by sample quality issues, and will make it easier for the ED to meet meaningful use guidelines. These effects are seen with the most significant impact for the critically ill patient.:

D-10

Validation of a new POC-system for the detection of drugs-of-abuse in oral fluids

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Background: The detection of licit and illicit drugs in oral fluids represents a novel procedure for forensic and medical applications. Dräger DrugTest®5000 is a new point of care (POC) system for the rapid detection of drugs of abuse in oral fluids. The system comprises a test-cassette integrating an oral fluid collector and immunochromatographic assays as well as a portable analyzer for optoelectronic signal detection and data management. The objective of this study was to validate Dräger DrugTest 5000 using oral fluid specimens from patients enrolled in methadone substitution programs.

Methods: Patients were asked to provide two oral fluid samples on a voluntary basis. The first sample was collected and analyzed on the site by means of Dräger DrugTest 5000 and the second sample by means of Dräger DCD® 5000 for confirmatory analysis. Confirmation samples were analyzed by GC-MS or HPLC-MS-MS for cocaine, opiates, cannabinoids, benzodiazepines and methadone as well as for the major metabolites of these drugs.

Results: Based on 100 oral fluid sample pairs clinical sensitivity and specificity data (see table) were calculated using the cut-off concentrations of the POC device: tetrahydrocannabinol (5 ng/ml), morphine (20 ng/ml), cocaine (20 ng/ml), diazepam (15 ng/ml) and methadone (20 ng/ml). Compared to confirmatory analysis clinical sensitivity and specificity of the POC system ranged from 70% to 99% and 94% to 99%, respectively. NPV and PPV ranged from 88% to 98% and 94% to 99%, respectively.

Conclusion: The results of this clinical validation study indicate that the Dräger DrugTest 5000 system is a reliable method for the detection of drugs-of-abuse in oral fluid samples. Compared to urine based POC devices the sampling procedure is hygienic, less invasive and can be monitored more easily.

Clinical data

Drug Class	Sensitivity	Specificity	NPV	PPV
cannabis	70%	99%	88%	99%
opiates	87%	98%	94%	94%
cocaine	91%	99%	98%	95%
benzodiazepines	90%	99%	98%	95%
methadon	99%	94%	97%	97%

D-11

Implementing Heart Disease Screening using a Point of Care Cholesterol Assay and Health Risk Assessment on the American Heart Association (AHA) National "Go Red for Women" Day

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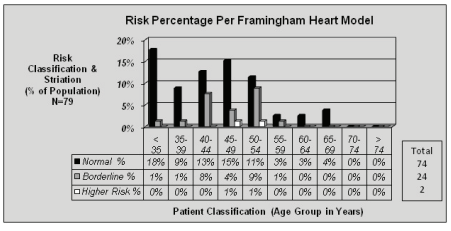
Point of Care Testing (POCT) is readily adaptable for health care screening programs. The CardioChek® lipid panel test yields quantifiable cholesterol fraction assessment using either fingerstick or venous blood. Results when imputed into either the AHA Framingham Heart Model or National Cholesterol Education Program (NCEP) Model can determine the relative heart disease (HD) risk. During the recent national AHA sponsored "Go Red for Women" Day in Indianapolis IN, CardioChek lipid panel (Cholesterol-HDL- Triglyceride) was used to screen 97 volunteer donors from the approx 1000 attendees. In prior studies the lipid panel repetitively shows an average precision of 4-6% and a bias against a reference of 2-5%. Using the lipid assay only (N=97), plus measures of blood pressure, height, weight, and medical history including diabetes and smoking (N=79), we calculated the heart disease risk based on the two models. The population was predominantly female (96%). Using a Framingham HD risk calculation the 10 yr risk arbitrarily categorized as normal (10yr; <5%); borderline (10 yr; 5-10%); High (10 yr; >10%) is shown in the table. Using NCEP Guidelines which only consider either total cholesterol (TC) or calculated Low Density Lipoprotein (cLDL) the normal/borderline/high risk categorization in percent was 46/34/20 for TC and 76/15/9 for cLDL.

This demonstrates the value of POCT in large scale screening of apparently healthy individuals to identify HD risk. Donors can be counseled regarding their HD risk and advised of appropriate follow-up with their personal physician. The HD risk categorization also serves to illustrate that these assessment tools may yield differing results depending on the health data which is available. The POCT technology is a valuable tool in providing immediate feedback to individuals in the AHA mission to create awareness to heart disease, in this case especially for women for whom HD is a leading cause of death.



D-12

Acceptance of a New Lot of i-STAT® cartridges. A Practical Example with the Assay for pCO2

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Background: Upon receipt of a new lot of i-STAT cartridges, the Laboratorian should ensure that the results of assays performed with each lot on patient specimens are interchangeable to give seamless patient care.

Methods: The performance of the pCO2 test was determined by assaying patient specimens with the new and the old lot of i-STAT cartridges (G3+,CG4+,CG8+, eleven lots) received over three months .The acceptance criterion target value +/- 5mmHg was adopted. Previous methods comparison studies using patient specimens estimated that for pCO2 values between 30-75mmHg the maximum standard deviation was 2.2mmHg. Consequently, for the paired t-Test the number of patient specimens to be assyed to achieve a power = 0.95 was calculated setting alpha=0.05, factor effect (difference) =5mmHg, standard deviation of paired differences (s)=2.2; power analysis determined n= 5. Five patient specimens were assayed in parallel and within two minutes with the old and the new lot of cartridges.The observations were accessioned in Minitab® (Version 16, Minitab Inc.) statistical software and analyzed with descriptive statistics, capability indexes, paired t-Test and statistical graphic representations.

Results: Summary statistics for the absolute differences between lots: mean=-.05,s=1.6,min.=-5.2, Q1=-1.3, Median=-0.4,Q3=0.5, max.=2.8. Both the histogram and the normal probability plot of the absolute differences showed a skewed distribution toward negative values (skewness=-0.6). The chart of the mean differences by each comparison showed that for the comparisons of lots #3-4 and #4-5 the respective mean differences (-2.4 and -2.7mmHg) exceeded the 95% LCI and individual observations exceeded -5.0mmHg. The capability indexes for LSL=-5 and USL=5: Cp=1.49, CpK=1.32, Pp=1.14, PpK=1.01, indicated that the estimated performance was at 1 sigma. The plot of the absolute differences by the value of pCO2, as determined with the old lot of cartridges, showed that the variability increased for increasing values of pCO2. Consequently, for paired t-Test the observations were grouped to obtain homogeneity of the variance. For groups 31-40mmHg (n=6,s=0.75), 41-50 (n=14,s=0.8),51-60mmHg (n=24,s=1.6), 71-80mmHg (n=5,s=3.1) there were no statistically significant differences(P>0.05). For the group 61-70mmHg (n=21,s=1.6) the observed paired differences were in the interval -5.1mmHg and 1.7mmHg and were statistically significant (P=0.008). For the group 71-80mmHg power analysis showed that for s=3.1 eight observations were required to have a power=0.95 in detecting a difference =5mmHg.

Conclusion: This study showed that for pCO2, assayed on patient specimens with i-STAT cartridges of different lots, the overall performance was at 1 sigma for the acceptance criterion target value +/- 5mmHg. An increase in variance was detected for increasing values of pCO2. Consequently, while for pCO2 values in the interval 30-70mmHg five paired observations could detect differences of 5mmHg with a power=0.95, for pCO2 values greater than 70mmHg eigth paired observations were required to detect a difference of 5mmHg with the same power. These findings indicate that an acceptance program should employ patient specimens within an interval with homogeneous standard deviation. This study was limited by the sample size and the number of lots of i-STAT cartridges.However, this study alerts the Laboratorian that for the critical pCO2 values>60mmHg the variability between lots may not allow their interchangeable use for patient care.

D-13

Critical Evaluation of the Effect of Haematocrit Fluctuation on Blood Glucose determination by Point of Care Blood Gluc0se Meters

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Introduction. Marked fluctuations in Haematocrit are complications of Critical Illness, Trauma and Surgery resulting from blood loss, fluid resuscitation, blood transfusion, inappropriate fluid therapy or severe dehydration. Haematocrit values outside the manufacturer's specified limits are contraindications to the use of POC blood glucose test systems. Within this study Roche Accu-Chek Inform II, Nova Stat Strip and Abbott PXP were critically compared to reference Roche cobas b221 blood gas methodology routinely used in our institution.

Methods. 30 random heparinised venous blood samples were obtained from patients with Haematocrit values outside the manufacturer specified limits. All samples were analysed using all POC devices and reference cobas b221 methodology

Results. Haematocrit values 18.64 ±5.16 (below manufacturers specified limits) glucose concentration:

1. 7.8 ± 2.77 mmol/l cobas b 221,
2. 8.14 ± 2.87 mmol/l Accu-Chek Inform II (positive bias 0.34 ± 0.19).
3. 8.07 ± 2.75 mmol/l Nova StatStrip (positive bias 0.27 ± 0.16).
4. 8.12 ± 3.17 mmol/l Medisense PXP (positive bias 0.44 ± 0.15).

Haematocrit values 72.52 ± 8.35 (above manufacturer specified limits)glucose concentration:

1. 8.56 ± 3.24 mmol/l cobas b 221.
2. 8.01 ± 3.06 mmol/l Accuchek Inform II (negative bias -0.55 ± 0.25).
3. 8.12 ± 3.17 Nova StatStrip (negative bias -0.44 ± 0.15).
4. 8.00 ± 3.02 mmol/l Medisense PXP (negative bias -0.56 ± 0.34).

Conclusion. All POC systems demonstrated a negative bias with elevated haematocrit and positive bias with decreased haematocrit. Bland Altman analysis showed similar

mean and bias derivation for all POC systems. This would not preclude their use in those clinical areas where this may occur, but does require effective education and training of all personnel.

D-14

Assessment of Neonatal Hyperbilirubinemia using GEM Premier 4000 Total Bilirubin Assay

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Background: The American Academy of Pediatrics recommends measurement of total bilirubin (TBili) in newborns to assess the risk of developing severe neonatal hyperbilirubinemia and bilirubin encephalopathy. Traditionally, these measurements are performed on serum samples using automated chemistry analyzers or non-invasively using transcutaneous bilirubin meters. Recently, Point-of-care (POC) testing of TBili in whole blood has become available on the GEM Premier 4000 blood gas analyzer (GEM). Potential advantages of the GEM method include smaller sample size and elimination of transport delay and light exposure. This study aims to (1) evaluate the correlation of whole blood TBili results using the GEM to serum results obtained on an automated chemistry analyzer, and (2) determine the concordance of hyperbilirubinemia risk classification by the two methods.

Methods: Sixty-two blood samples were collected from infants of 26 to 40 weeks gestational age (GA). TBili (mg/dl) was measured on whole blood using the GEM and on simultaneously collected serum samples on the Siemens Dimension Vista chemistry analyzer (Vista). The correlation between the TBili results by the two methods was assessed by linear regression and Bland-Altman analysis of bias. Twenty-four infants, age less than 7 days, were classified into categories for risk of sequelae of severe hyperbilirubinemia using an established postnatal hour-specific nomogram (Bhutani, et.al. Pediatrics Jan. 1999). Four hyperbilirubinemia risk grades (low, low intermediate, high intermediate and high) were assigned and concordance was evaluated for risk category classification based on the GEM and Vista TBili results.

Results: TBili results ranged from 0.2 to 16.9 mg/dl (GEM) and 0.2 to 16.9 mg/dl (Vista). These results were from 5 term infants (GA ≥37 weeks) and 57 pre-term infants (GA < 37 weeks). The GEM TBili results show reasonable agreement with the Vista automated chemistry analyzer (Slope: 1.09; Intercept: 0.02; St Err Est: 0.83). GEM results showed a mean bias of +0.42 mg/dL compared to Vista. For the 24 infants whose TBili levels were measured within 7 days of birth, 16 (67%) were classified into the same risk grade by the two analytic methods, and 8 (33%) of the infants were assigned different risk grades. Compared with the Vista method, 4 of the discordant infants (50%) were assigned higher risk grades and 4 (50%) had lower risk grades by the GEM method. Only one infant's risk status changed by more than one grade.

Conclusion: Although the GEM method shows fair overall agreement with the Vista chemistry analyzer in measuring neonatal Tbili levels, assignment of clinical risk categories may differ between the two methods.

D-15

Analytical Accuracy and Efficacy of Roche Maltose Independent Chemistry ACCU-CHECK® Blood Glucose Measurements to Detect Neonatal Hypoglycemia

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Background: Accuracy in neonatal blood glucose determinations is extremely important, as falsely low blood glucose values can result in unnecessary interventions and falsely normal results might delay beneficial therapy. We evaluated the accuracy of a new maltose independent chemistry (MIC) whole blood glucose strip used with three different Roche whole blood glucose meters (BGM). Split blood samples were run on the BGM and a reference method. Laboratory hypoglycemia was defined by reference method glucose values less than 40mg/dL (2.2mmol/L). The BGMs' diagnostic sensitivities and specificities were then calculated for all BGM values.*

Methods: Each of three hospital neonatology units evaluated one of the ACCU-CHEK Inform II, ACCU-CHEK Performa or the ACCU-CHEK Aviva meters. Three test strips, all different lots, were dosed with the blood and then run on the hospital specific Roche BGM. Two whole blood samples from the same aliquot were deproteinized and run on the reference Hitachi 917 hexokinase method.**

Results: Of the 631 neonate samples collected, data from 70 were excluded from analysis due to high variation in the duplicate reference values, unknown hematocrit, or other error. The hematocrit ranged from 23-65%. The Table summarizes the average bias to reference and the rate of disagreement using modified limits that are more stringent than those currently recommended by ISO 15197:2003. Using the hypoglycemia definition of <40mg/dL, <1% of samples with reference glucose >40mg/dL were classified incorrectly (specificity ≈100%). Using a BGM cutoff of 51mg/dL (2.8mmol/L), all samples with reference glucose <40mg/dL were detected.

Conclusion: Based on our data, we believe that all three of the BGMs using this test strip can reliably detect hypoglycemia while minimizing unnecessary intervention in normoglycemic neonates.

** Neonate claims not intended for US use.*

***All systems in development. Not available in US.*

ACCU-CHEK, ACCU-CHEK INFORM, ACCU-CHEK AVIVA and ACCU-CHEK PERFORMA are trademarks of Roche.

Summary of Results

Meter Type	Glucose Range	Bias mg/dL(mmol/L) vs Reference Glucose in Samples < 50 mg/dL(2.77mmol/L)	Rates of Bias vs. Reference Glucose Exceeding 15 mg/dL(Samples <100mg/dL) or 15% (Samples >100 mg/dL)
ACCU-CHEK Aviva	28-100 mg/dL (1.6-5.6 mmol/L)	2.3 mg/dL (0.12 mmol/L)	0.0%
ACCU-CHEK Performa	11-359 mg/dL (0.6-19.9 mmol/L)	1.2 mg/dL (0.07 mmol/L)	0.0%
ACCU-CHEK Inform II	18-153 mg/dL (1.0-8.5 mmol/L)	1.8mg/dL (0.10 mmol/L)	0.5%

D-17

Critical Evaluation of Point of Care Blood Glucose Meters in the Maintenance of Appropriate Glycaemic Control in the Critically Ill Patient

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Introduction. Stress induced hyperglycaemia is common in hospitalised patients with or without diabetes. The association between stress induced hyperglycaemia and adverse outcome has been observed in numerous patient groups ranging from patients admitted to the general ward to those who have experienced myocardial infarction , stroke, surgery , burns and head trauma.

Maintenance of appropriate Glycaemic control (AGC) in diabetic and non-diabetic patients within surgical and medical intensive care units has shown a significant reduction in patient mortality and morbidity.

Rapid and precise methods for the determination of blood glucose levels are important for the clinical implementation of AGC. Point of Care (POC) blood glucose test systems have been shown to be of value in the maintenance of AGC, offering rapid and precise results at the patient's bedside. However, careful considered evaluation of POC devices compared to reference methodology is imperative before implementation.

Within this critical evaluation the new Accu-Chek Inform II test strip for use with the Inform II POC blood glucose test system (Roche Diagnostics), Nova Stat Strip (Nova Biomedical) and Medisense PXP (Abbott Diagnostics) evaluated to determine whether they demonstrated the required accuracy and precision to allow implementation of a AGC protocol in critically ill patients compared to the reference blood gas methodology currently in routine use within our critical care and intensive care areas (cobas b221, Roche Diagnostics).

Methodology. 200 paired, random heparinised arterial blood samples were obtained from critically ill patients with Blood Glucose determined with all devices and compared to Reference methodology.

Statistical Methods: Spearman Rank Correlation, Student Paired T test, Bland Altman analysis.

Results. 1. Reference cobas b221 Mean Glucose 8.61 ± 4.60 mmol/l, Accu-Chek Inform II 8.54 ± 4.58 mmol/l, Nova StatStrip 8.62 ± 4.57 mmol/l and Abbott PXP 8.74 ± 4.55 mmol/l.

2. Correlation with Reference cobas b221: Accu-Chek Inform II r^2 0.997, slope 0.994, intercept -0.02, Nova StatStrip r^2 0.997, slope 0.993, intercept 0.07, Abbott PXP r^2 0.995, slope 0.988, intercept 0.23.

3. Student Paired T Test compared to Reference b221: Accu-Chek Inform II p 0.875, Nova StatStrip p 0.983, Abbott PXP p 0.771

4. Bland Altman analysis compared to Reference cobas b221: Accu-Chek Inform II -0.07 ± 0.26 (limits of agreement -0.58 - 0.44), Nova StatStrip 0.01 ± 0.26 (limits of agreement -0.50 - 0.52), Abbott PXP 0.13 ± 0.32 (limits of agreement -0.50 - 0.76).

Conclusion. All POC blood glucose test systems within this evaluation demonstrated statistically significant correlation with reference methodology. Paired Student T test demonstrated no significant difference between all POC systems and Reference cobas b221. The Accu-Chek Inform II and Nova StatStrip showed excellent mean bias when compared to reference across the working range. Medisense PXP showed acceptable mean bias when compared to reference but increased limits of agreement. However; this did not reach levels of clinical significance and would not preclude its use in AGC protocols. This study demonstrated that all POC systems could be used within a hospitalised setting when implementing an AGC protocol.

D-18

Variability in determining patient blood glucose values with the glucose meter SureStepFlexx®. A practical example

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Background: The variability of glucose meters in determining patient blood glucose values and consequently in accurately detecting changes in patient glycemia may affect both patient care and outcomes. The precision of a glucose meter, namely SureStep Flexx, used by the Sentara Health system was evaluated at SVBGH by performing repeated assays on individual patient specimens.

Methods: One SureStep Flexx glucose meter (Serial# L9325SA00023, LifeScan, Johnson and Johnson) was employed by one of us to perform ten repeated assays with reagent strips from one lot (Lot# 3053672003) on each of twenty-nine individual patient specimens obtained by venipuncture in green-top tubes. Since previous experience with linearity and method comparison studies using patient specimens had indicated an increase in variance for increasing values of glucose, the patient specimens were selected in the interval 20-450mg/dL and they were devided in four groups (group1: 20-40mg/dL; group2:41-70mg/dL; group3: 71-180mg/dL; group4: 181-450mg/dL). The observations were stored in Minitab® (Version 16, Minitab Inc.) statistical software and they were analyzed with Bartlett's, Levene's statistical tests for homogeneity of variance, Bonferroni's inequalities for standard deviation with their 95% CI and their graphic representation.

Results: Summary statistics showed that the standard deviation increased for increasing values of blood glucose. Group1 (20-40mg/dL) s=0.6-1.6mg/dL, group2 (41-70mg/dL) s=1.1-1.8mg/dL, group3 (71-180mg/dL) s=1.4-4.4mg/dL, group4 (181-450mg/dL) s=4.1-16.3mg/dL. While for group 1 and 2 Bartlett's and Levene's tests did not detect statistically significant differences between variances(P >0.05), for group 3 and 4 they detected statistically significant differences (P <0.005). The graphic representation of the standard deviations and their Bonferrroni's 95% CI visually confirmed the results of the statistical tests. Furthermore, for group3 (71-180mg/dL) the graph showed that this group could be devided in two subgroups (71-100mg/dL and 120-180mg/dL) with homogeneous variance as estimated by Bartlett's and Levene's tests (P >0.05). For group4 (181-450mg/dL) the graph revealed an umpredictable variability pattern of the standard deviation (s varying between 4.1 and 16.3 mg/dL) for increasing values of glucose so that subgrouping was not feasible.

Conclusion: Our study indicated that the standard deviation of patient blood glucose values determined with a glucose meter increased in a predictable fashion between 20 and 180 mg/dL. Furthermore, the standard deviation was homogeneous for groups of observations between 20-40mg/dL, 41-70mg/dL, 71-100mg/dL and 120-180mg/dL. However, the standard deviation varied in an upredictable pattern for glucose values between 181-450mg/dL. The interpretration of these results is limited by the small number of patient specimens assayed with one glucose meter by a single operator using one lot of reagent strips. However, they alert both the Laboratorian and the Clinician that for blood glucose values greater than 180mg/dL the interpretration of the glucose meter results may be affected by an umpredictable variability with standard deviation varying between 4.1 and 16.3 mg/dL. Consequently, clinicians may consider to confirm blood glucose values obtained with glucose meters with those obtained with a Laboratory method prior to therapeutic interventions.

D-19

Critical Evaluation of Siemens Diagnostics DCA Vantage compared to Reference HPLC Technology in the assessment of HBA1c status in the Diabetic Patient

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Introduction. The predictive power of HbA1c variables is of importance in prognosis of diabetic complications, clinical guidelines, health-economical analyses and in the design of clinical trials of antidiabetic agents. HbA1c is used for patient education and counselling, feedback re diabetic control, improved patient motivation, and to effectively monitor management. Measurement should be accurate and precise and provided rapidly in a same-visit setting. To achieve this goal in the clinical setting POC solutions may provide this adjunct to care.

Siemens DCA Vantage was evaluated compared to Reference HPLC methodology in order to ascertain whether it provided the level of accuracy and precision for incorporation into a POC care pathway for Diabetic assessment.

Methodology. 1. Precision of DCA Vantage determined using bi-level Quality Control Media (10 replicates).

2.Correlation: 50 random whole blood samples, were analysed for HbA1c using the DCA and compared to HPLC methodology.

Results. 1. Precision: Quality Control Media Normal : NGSP Mean 5.42 % SD 0.06 CV% 1.17, IFCC Mean 36.1 mmol/mol SD 0.88 CV% 2.43. Quality Control Media Abnormal; NGSP Mean 10.44 % SD 0.13 CV% 1.29, IFCC Mean 90.4 mmol/mol, SD 1.34, CV% 1.49.

2. Correlation: HbA1c NGSP r^2=0.975, slope 0.94 intercept 0.27. Bland Altman analysis: Mean difference -0.20 ± 0.35 % with 95 % confidence interval -0.89 - 0.48%.HbA1c IFCC r^2=0.970, slope 0.93 intercept 2.37.Bland Altman analysis: Mean difference -2.42 ± 4.25 mmol/mol with 95 % confidence interval -10.76 - 5.91 mmol/mol.

Conclusion. DCA showed excellent precision across the working range with CV < 2.5 %.Correlation compared to HPLC was excellent for both NGSP and IFCC. Bland Altman demonstrated minimal scatter across the working range for both HbA1C % and mmol/mol. This pilot study suggests DCA can be used to effectively implement a same- visit setting.

D-20

Dynamic Temperature and Humidity Profiles for Assessing the suitability of Point-of-Care Testing During Emergencies and Disasters

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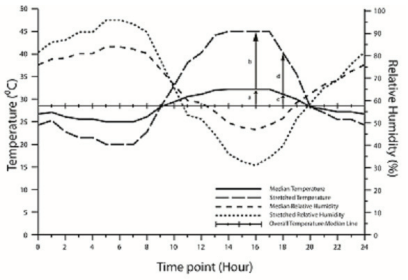
Background: Environmental standards for the performance of point-of-care testing (POCT) during emergencies and disasters do not adequately reflect dynamic environmental stresses. First responders use POCT in uncontrollable and austere conditions involving high and low temperatures and humidity extremes. The objectives are to characterize temperature and humidity conditions before, during, and following important contemporary disasters, and to use simulated conditions to conduct dynamic stress testing of POC reagents.

Methods: Diurnal profiles were developed using median hourly temperature and humidity. Data were collected from meteorological stations in a 31-day period from the National Climatic Data Center (www.ncdc.noaa.com). The hourly medians proportionally stretched to include the highest and lowest temperature and humidity recorded during actual disaster events.

Results: Figure 1 shows the stretched profile for New Orleans during hurricane Katrina, where height = (c/a)*(height max) and (height max)=a+b. Temperature range is 20 to 45 Celsius, and humidity, 30 to 96%. Other profiles were developed for, Port-au-Prince, Haiti, following the 2010 earthquake; Banda Aceh, Indonesia, after the 2004 tsunami; and Springfield, Massachusetts, during the month of January, 2009. Temperature and humidity fell outside the boundaries of what is allowed for common POC methods, when compared to ranges documented for FDA licensing claims.

Conclusion: Several profiles are now available for dynamic stress testing. Emergency and disaster medical teams should be aware of the effects of austere environments on current POC reagents, devices, and performance. We recommend that a) standards be established to include dynamic stresses encountered at field sites, b) guidelines for use of POCT be formulated, c) methods of storage and transport be altered accordingly, and d) POCT be performed in Alternate Care Facilities where environmental conditions can be moderated.



D-21

Critical Evaluation of the Effect of Hypernatraemia/ Hyponatraemia on Blood Glucose determination by Point of Care Blood Glucose Meters

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Introduction. Hyper and Hyponatraemia are not uncommon electrolyte disturbances in critical illness and across clinical disciplines and pathologies within hospitalised patients. Hypernatraemia occurs in approximately 1% of hospitalized patients. The condition usually develops after hospital admission. An incidence closer to 2% has been reported in debilitated elderly persons and in breastfed infants. Hyponatraemia is the most common electrolyte disorder, with a marked increase among hospitalized patients. Approximately 4.4% of postoperative patients developed Hyponatraemia within 1 week of surgery. Hyponatraemia has also been observed in approximately 30% of patients treated in the intensive care unit. Severe dehydration results in poor peripheral blood circulation or aggressive fluid resuscitation leading to oedema and fluid overload result in marked electrolyte disturbances and are contraindications to the use of POC blood glucose test systems.

Within this study Roche Accu-Chek Inform II, Nova Stat Strip and Abbott PXP were critically analysed compared to reference Roche cobas B221 blood gas methodology used within our institution.

Method. 52 Hyponatraemia and 50 Hypernatraemia patients (normal range 134 - 144 mmol/l) were critically analysed for whole blood glucose using random arterial blood samples. All samples were analysed on all POC systems and reference cobas b221 blood gas system. Sodium concentration analysis was performed on cobas b221.

Results. Hypernatraemia subset (151.94 + 4.70 mmol/l) in comparison to reference b221:

1. Accuchek Inform II mean bias = -0.17 + 0.29; limits of agreement = -0.74 - -0.40.
2. Nova StatStrip mean bias = -0.03 + 0.29; limits of agreement = -0.60 - 0.54
3. Medisense PXP mean bias = 0.02 + 0.39; limits of agreement = -0.74 - 0.78.

Hyponatraemic subset (128.95 + 4.14 mmol/l) in comparison to reference b221:

1. Accuchek Inform II mean bias = -0.05 + 0.22; limits of agreement = -0.48- -0.38.ly
2. Nova StatStrip mean bias = 0.04 + 0.23; limits of agreement = -0.42 - 0.49.
3. Medisense PXP mean bias = 0.18 + 0.27; limits of agreement = -0.35 - 0.71.

Conclusion. Low mean bias was exhibited by all POC blood glucose systems within this study for both the hypo and hypernatraemic subsets. Bland Altman analysis demonstrated minimal scatter within both subsets with all POC systems, which would not preclude their use in clinical practice across clinical disciplines and the healthcare sector. However due to the clinical risk associated with the patients condition and the importance associated with sampling site an effective training regime and certification procedure is required in situ.

D-22

Development of a rapid point of care test for NT-proBNP based on Magnotech

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Point-of-care (POC) *in vitro* diagnostic applications are very demanding in a number of areas: performance, time-to-result, and reliability. The Magnotech technology enables developing immunoassays that can meet these requirements. Here we present the current status for the assay development of NT-proBNP, a specific marker for the diagnosis and monitoring of heart failure. This NT-proBNP assay is rapid, sensitive and shows a good correlation when compared to immunoassays currently on the market.

One of the key requirements for point-of-care tests is a short time-to-result. The current NT-proBNP assay time is only 5 minutes, when using plasma. In the next phase a filter will be added allowing measuring whole blood as well as plasma. Experiments show that when using whole blood the filling time of the disposable cartridge is less than 30 seconds, resulting in a total time-to-result of less than 6 minutes.

To be a viable alternative for automated system analysers, for POC applications the assay sensitivity is important. Our Magnotech NT-proBNP assay has an analytical sensitivity in the same range (currently around 10 pg/mL) as for example the bioMérieux VIDAS NT-proBNP assay (20 pg/mL).

Method comparison was performed with VIDAS (bioMérieux) by Passing and Bablok fit using Lithium Heparin plasma samples. The corresponding slope was 1.19 (95% CI 1.04 to 1.34) and an intercept of 147.5 (95% CI -6.56 to 307.43) with a correlation of R=0.88 over the range 20 to 6518 pg/mL (n=65).

Our conclusion is that the NT-proBNP assay on the Magnotech platform shows promising performance for use in the point-of-care environment. The development of the assay is ongoing, and future steps will include introduction of a blood filter integrated into the cartridge, allowing finger prick tests. Another topic that will be addressed is the increase of the dynamic range by using the kinetic read-out or the multiplexing capabilities offered by Magnotech.

D-23

Determining the Limit of Detection of a Multiplex LATE-PCR Assay for Pathogen Detection in Critically Ill Patients

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Background. Sepsis represents a leading cause of death in non-coronary intensive care units. Rapid detection and identification of pathogens enables appropriate antimicrobial treatment, ultimately improving patient outcomes. Linear-after-the-exponential polymerase chain reaction (LATE-PCR), a novel form of asymmetric PCR utilizing limiting and excess primers that produce single-stranded DNA, has advantages over traditional blood culture by generating faster results, aiding definitive pathogen identification, and soon, becoming available at the point of care. The goal was to determine the limit of detection (LOD) of multiplex LATE-PCR tested with genomic DNA (gDNA).

Methods. DNA from 12 whole organisms obtained from the American Type Culture Collection (ATCC) was extracted using a sample preparation protocol developed by Smiths Detection Diagnostics. Eluted DNA was serially diluted to concentrations ranging from 10^7 to 10^{-2} genome copies/uL and tested in parallel with pure gDNA (ATCC) as a positive control. DNA also was tested in the presence of known concentrations of extracted DNA from whole blood of healthy asymptomatic volunteers using the same multiplex LATE-PCR assay.

Results. The LOD of LATE-PCR ranged from 10 to 100 copies of gDNA in molecular grade water and did not exhibit interference when challenged with human DNA extracted from whole blood. Additionally, LATE-PCR demonstrated multiplexing capabilities when in the presence of both bacteria and fungi (Figure). LATE-PCR differentiates species (e.g., *Enterococcus faecium* versus *Enterococcus faecalis*) by using the ratio of fluorescence measured at 35°C to that at 55 °C.

Conclusions. Multiplex LATE-PCR demonstrates LOD results potentially useful for rapid diagnosis, a valuable adjunct to blood culture when diagnosing bacteremia in critically ill patients. This study proves feasibility of an approach that will aid in early evidence-based treatment decisions of high value at the bedside in critical care.

D-24

Objective measurement of fatigue using salivary peptides

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Background: Fatigue is a state of mind and body that has long eluded objective definition. Fatigue is a leading cause of accidents both in and out of the workplace. In the workplace, many individuals have strong professional incentives to under-report fatigue. Others have a strong interest in mitigating fatigue through use of drugs, sleep, and diet or lifestyle changes. In the clinical setting fatigue is a common feature of

many illnesses including diabetes, cancer and cardiovascular disease. The estimated cost of fatigue related problems including accidents is greater than $100B problem in the US alone. The cost in terms of quality of life is even greater. In order to better understand how fatigue can be more effectively managed, objective methods of measuring fatigue are needed.

Methods: We hypothesized that fatigue alters the composition of the small-molecular weight proteome of saliva. In order to examine the hypothesis human saliva was collected from 8 healthy volunteers engaged in 10 hour sessions of moderate physical (70% of maximum) exertion. A crossover design was employed wherein the study was repeated two weeks later. Each hour, participants performed an exercise program that included upper and lower body exercises separated by short periods of recovery. Saliva was taken before the start of exercise and at various times after. Saliva was initially spin filtered to obtain the low molecular weight component of saliva. The low molecular weight components of saliva obtained before the start of exercise at after 10 hours were compared using LC/MS. Components that were abundant and showed a significant change compared to baseline were deemed to be putative biomarkers. High resolution MS was used to obtain a de novo sequence of fatigue biomarker peptides. Subsequent synthesis and evaluation on LC/MS for both retention time and fragmentation pattern confirmed peptide identity. Other known biomarkers and cytokines were measured by ELISA. In another study, biomarker levels were measured in US Special Forces prior to the start of indoctrination training characterized by a 50% failure rate.

Results: Average values of a putative fatigue biomarker measured at the beginning and end of the study differed substantially (24.3±28.0 [0Hr], 0.013±0.020 [8Hr]). Significant correlations were observed between FBI and various cytokines, including alpha amylase, interferon-gamma, tumor necrosis factor-alpha, monocyte chemotactic protein 1, interleukin 1-beta and immunoglobulin A ($p<0.00017$ with Bonferroni correction). Fatigue peptides are small fractions of a saliva unique protein called Proline-Rich Protein (PRP).

Conclusion: Taken together the evidence suggests that fatigue biomarkers can be used to evaluate fatigue objectively. Levels of fatigue biomarkers are associated with complex behavioral outcomes including success during US Special Forces indoctrination. The technology offers to change the manner in which disease characterized by fatigue is managed.

D-25

Lactate Biosensor for Point-of-Care Measurement

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Blood lactate concentration is indicative of certain pathological states such as shock, respiratory insufficiencies, heart disease and sepsis. Also, rapid measurements of blood lactate are a valuable tool for determining tissue hypoxia. Therefore, point-of-care analyzers that measure lactate concentrations are important for diagnosis, prognosis, and therapy for critically ill patients.

The OPTI Medical lactate biosensor contains the enzyme, lactate oxidase, to selectively catalyze the reaction between lactate and oxygen. The oxygen consumption is measured photochemically by an optical sensor. The rate of oxygen consumption is proportional to the concentration of lactate in the specimen. When the sensor is exposed to lactate it begins to deplete the oxygen in the enzyme layer as hydrogen peroxide is generated. As a result of diffusion, oxygen concentration in the base layer begins to decrease and the fluorescence intensity increases. The initial rate at which the fluorescence increases is proportional to the gradient between the sensing layer and the enzyme layer and hence to the concentration of lactate in the sample. The lactate biosensor is included in the OPTIMedical B-Lac cassette, which also measures pCO_2, pO_2, pH, tHb and SO_2.

The lactate biosensor measures blood lactate concentrations from 0.3 mM to 17.5 mM. 20 day precision studies performed on control buffers for a typical B-Lac cassette lot gave total CVs of 5.6% (1 mM), 3.1% (2.5 mM), and 4.2% (5 mM). Studies across these concentrations also show linearity with other FDA approved methods of analysis. Studies were also performed to determine sensor stability. It has been shown that the sensors are very stable over time, establishing a 12 month shelf-life. This stability was shown under standard refrigerated storage, as well as cycled temperature extremes to simulate shipping stress.

The B-Lac cassette was officially launched in January 2011. Data has shown the cassette to accurately measure blood lactate across physiological concentrations in a point-of-care setting. The OPTI Medical B-Lac cassette will be a competitive tool on the biosensor market as early sales indicate strong support.

D-26

Very early diagnosis of chest pain by point of care testing; comparison of the diagnostic efficiency of a panel of cardiac biomarkers compared to troponin measurement alone in the Randomised Assessment of Panel Assay of Cardiac markers (RATPAC) trial

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Introduction. A prospective randomised trial of triple marker testing by point of care testing (POCT); the Randomised Assessment of Panel Assay of Cardiac markers (RATPAC) was performed to assess the impact of POCT triple marker testing on patient management and the diagnostic efficiencies of different biomarker strategies examined.

Methods. The study randomised low risk patients presenting with chest pain to diagnostic assessment with a cardiac panel measured by POCT or to diagnosis when biomarker measurement was based on central laboratory testing (CLT).). Patients 18 years or older resenting with acute chest pain to the emergency department (ED) of 6 participating hospitals with suspected acute coronary syndrome (ACS) were enrolled. 1125 patients were randomised to POCT measurement of the triple marker panel of cardiac troponin I (cTnI), myoglobin and CK-MB on admission and 90 minutes from admission. In addition to POCT samples, samples were sent to the central laboratory for cardiac troponin measurement. The CLT arm utilised the local laboratory protocol for cardiac troponin measurement only.

POCT measurements were performed using the Stratus CS (Siemens Diagnostics). The analytical characteristics of the assays were as follows: cTnI detection limit 0.02 µg/L, analytical range 0.02 to 50 µg/L, interassay CV 4.3-5.1% (0.03 to 0.22 µg/L). The 99th centile of the assay is 0.07 µg/L. Myoglobin: detection limit 1 µg/L; analytical range 1-900 µg/L; interassay CV 1.9-12.7% (56 to 308 µg/L); 95% reference interval, males 21-98 µg/L, females 19-56 µg/L, combined 20-82 µg/L. CK-MB: detection limit of 0.3 µg/L; analytical range 0.3-150 µg/L; interassay CV 0.15-1.27% (3.7-39.3 µg/L); 95% reference interval 0.6-3.5 µg/L. At the individual sites cardiac troponin was measured as follows; Siemens cTnI ultra (3 sites) 99th percentile limit 0.05 µg/L, Abbott cTnI (1 site) 99th percentile limit 0.05 µg/L, Beckman Accu I (1 site) 99th percentile limit 0.06 µg/L and Roche cTnT (1 site) 99th percentile limit 0.01 µg/L.

Myocardial infarction was defined by the universal definition of myocardial infarction to categorise patients into those with or without acute myocardial infarction (AMI). Final diagnosis categorisation of all patients was performed by two clinicians by review of the patient case notes, and follow up investigations plus troponin measurement, utilising laboratory derived values wherever possible.

The following diagnostic strategies were compared: individual marker values (cTnI > 99th percentile, CK-MB > 5 µg/L and myoglobin >95th percentile) delta CK-MB >1.5 µg/L and myoglobin (defined as % change from admission measurement >25%) and the combination of presentation or 90 minute value plus delta value.

Results. In the admission sample measurement of cTnI was the most diagnostically efficient with areas under the ROC curve statistically significantly greater than all other analytes with a sensitivity of 0.85 (0.75-0.92) and specificity of 0.976 (0.96-0.98). At 90 minutes cTnI measurement had the highest AUC but was statistically significantly different only from delta myoglobin ($p = 0.0035$) and delta CK-MB ($p = 0.0064$).

Conclusion. Measurement of cTnI alone is sufficient for diagnosis. Measurement of a marker panel does not facilitate diagnosis.

D-27

Effect of Sample Transportation on the Measurement of Glycosylated HemoglobinA1c by A1CNOW POC Device in Diabetic Patients

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Background: The measurement of Glycosylated Hemoglobin A1c (HbA1c) as long-term assessment of glycemic control in patient with diabetes is highly recommended

by many societies. The aim of this study was to evaluate the effect of transportation of blood samples from physician's offices and primary health care clinics to central lab on the measurement of HbA1c performed on the (POC) device A1CNow+ and compared to central Tosoh G8 HPLC analyzer.

Methods: Blood samples were collected in EDTA tubes from 98 consecutive patients diagnosed previously with type 2 diabetic based on WHO criteria with no hemoglobin variants as was confirmed by hemoglobin electrophoresis. Among 98 patients, 54 (55%) were female and 44 (45%) were male, with an average age of 57.4±12.7 years. Venous blood samples were collected and immediately analyzed on immunoassay A1CNow+ device (Bayer, USA) in the clinics and then transported to the central lab by 4 to 6 hours later at 4° to 8° C. Upon received in the central laboratory, samples were analyzed again by A1CNow+ and results were compared to those obtained by Tosoh G8 HPLC (Tosoh, Japan) instrument. A 20 control samples from healthy nondiabetic subjects were performed on both analyzers simultaneously before and after transportation under the same conditions.

Results: The correlation of HbA1c results obtained on the A1CNow+ device before and after transportation were found to be similar with no significant difference (r^2 = 0.965, p=0.42). A positive and significant bias was observed between A1CNow+ and Tosoh HPLC (p<.00001). The mean and standard deviation of HbA1c for A1CNow+ and Tosoh HPLC were found to be 7.6±1.8 and 8.0±1.85 respectively (p<0.0001). **Conclusion:** The reliability of HbA1c results on the POCT device A1CNow+ is not affected prior or post sample transportation. However, there is a significant difference on measurement of HbA1c between the A1CNOW and Tosoh G8 HPLC.

D-28

Assessment And Evaluation Of Blood Glucose Monitoring Devices: A Comparison With Laboratory Based Plasma Glucose Measurement

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Background: Self monitoring of blood glucose is an important component of the treatment plan of diabetes mellitus. As self monitoring of blood glucose (SMBG) become more widely available, it is important to evaluate the accuracy of these devices.The present study compared the performance standard of the Accu-check Advantage, One Touch Ultra, One Touch Basic Plus glucometers with the ATAC 8000 Autoanalyser (reference method).

Methods: 86 apparently healthy workers were recruited for the study, made up of 44 female and 42 males, aged 18 to 55 years. A drop of fresh blood was placed on each of the strips already inserted in their specific glucometers. Testing were completed within 5, 15, and 45 seconds by One Touch Ultra, Accu-check Advantage, and the One Touch Basic glucometers. The devices were calibrated by setting their program numbers to match those on the strips currently in use. The colour changed, dependent on the blood glucose concentration is determined by the reflectance of light in the previously calibrated meters. The ATAC 8000 Analyzer assayed the plasma glucose by the hexokinase method. The accuracy of each blood glucose monitoring system was evaluated versus the ATAC 8000 by weighted linear regression analysis.

Results: The minimum glucose determined by Accu-check Advantage, One Touch Ultra, One Touch Basic Plus and ATAC 8000 auto analyzer were 4.3, 3.8, 3.8, and 3.0 mmol/L and the maximum glucose were also 24.3, 28.0, 22.3, and 22.8mmol/L respectively. Their means ranges from 4.0 to 6.0mmol/L with SE of 0. 476, 0.556, 0.430 and 0.462. The mean deviation (SD) for the assays were -1.11 (3.13) for Accu check Advantage, -1.11 (3.56) for One Touch Ultra and -0.65 (2.77) for One Touch Basic Plus. The y-intercepts of Accu-check advantage, One Touch ultra, One Touch Basic Plus versus the ATAC 8000 were 1.06, 0.26, 0.875 and slope (m) values of 0.995, 1.180, and 0.910. Regression line equations of the stated pairs were y= 0.955x-1.05, 1.18x +0.16 and 0.91x +0.87 respectively. The reproducibility (CV) for the assays was 2.7%, 1.1%, 2.0% and 1.3%.

Conclusion: The overall median deviation of the 3 glucometers assay from the reference values had an apparent identical maximum deviation value. The Accu-check Adavantage and the One Touch Ultra monitors show strong agreements by exhibiting equal median values in their deviation from the reference values. One Touch Basic Plus and the ATAC 8000 gave consistent pattern with one Touch Ultra giving the highest and One Touch Basic Plus giving the least in concentration values. Glucose levels determined by the Accu-check and the One Touch Ultra devices did not differ significantly from each other. 46.5% of the values measured with the Accu-check Advantage and One Touch Basic Ultra glucometers met the NCCLS criteria for SMBG. 90% of blood glucose tests performed on the One Touch Basic Plus fell within +/- 20% of the ATAC 8000 laboratory reference method. The mean deviation of-0.65(2.77) comparing meter with reference method performance indicate that the One Touch Basic Plus system provided the most accurate results of the three SMBG systems included in this study.

D-29

Analytical validation of Accu-check Inform II meters and strips before their use in the wards: practical experience of the CHU de Liège

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Background: The University Hospital of Liege is a 925-bed facility located on four different places. In 2004, all the wards were equipped with Accu-check Inform glucose meters (Roche Diagnostics, Mannheim,Germany), a near-patient device used to monitor blood glucose levels. In 2010, we moved to the Accu-check Inform II with the Accu-check Inform II strips. These strips do not present interference in the presence of maltose anymore, which makes them suitable for use in peritoneal dialysis. We have now 105 Informs II and are yearly running 350.000 strips. A two-levelled quality control (QC) needs to be tested every day by the nurses and is mandatory for the use of the meters. We also run an external proficiency test four times a year. Nevertheless, one of the major problems with these devices is the analytical validation of the meters and of the strips before use in the wards. We present here our experience in validating the Informs and strips.

Methods: First, we validated the Informs: each of the 6 vials contained in the Roche linearity kit (with increasing concentrations of glucose, from approximately 25 to 550 mg/dL) was run in simplicate. Next, the two-levelled QC were run in triplicate for 5 different days to evaluate the imprecision and the relative bias compared to the mean of the values. Then, we calculated the total error as TE=2.33*Imprecision (%)+|Bias|(%). A TE<14% was expected. Three Informs were then randomly selected and we assayed in triplicates, on five different days and on two different Accu-check Inform II strips lots, the 6 vials of the linearity kit. Finally, a comparison with the glucose electrode of the RapidLab 865 (Siemens, Deerfield, IL) on the three-levelled internal QC of the blood-gas analyzer was performed. We used the e-noval software to establish the accuracy profiles and we settled the tolerance limit at 10%, according to the American Association of Diabetes.

Results: The total error was lower than 14% for all meters (mean: 6.9 and 5.7% at respectively 0.49 and 2.91 mg/dL). With the linearity kit, the accuracy profile built with the predictive tolerance interval method shows that, on average, 95% of the future results that will be generated will be included in the computed tolerance intervals of ±10% in the 25-550 mg/dL studied range. With the internal QC of the Rapidlab, taking the expected mean obtained with the electrode as the reference, 95% of results are also included in the computed tolerance intervals of ±10% in the 50-200 mg/dL studied range.

Conclusions: we have completely validated the Accu-check Inform II meters and strips. Our results show that, on the different controls, we are in confidence for value ranging from 25 to 550 mg/dL, which is an important improvement for the method. Even if a true "patient" validation remains important, it is cumbersome and can not be proposed for the evaluation of all the meters and different strips lots. Our approach is pragmatic and allows us to certify the quality of the material before use in the wards.

D-30

Managing Costs and Impacting lives with HbA1c

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Background: Point of Care testing is becoming more popular both by demand and ease of use. However, some argue that point of care testing is less reliable and often more costly than the conventional laboratory methodologies. Careful and continuous management of blood glucose is primary for diabetic patients to prevent long term complications. However, in the event of a non-insured/low income patient this may prove difficult. Therefore, clinicians may opt for a HbA1C value to get an accurate depiction of how a diabetic patient is controlling their blood glucose while using a convenient and quantitative method. We have evaluated a point of care HbA1C instrument, which uses an inhibition of latex agglutination, against high performance liquid chromatography (HPLC) methodology; specifically, the Siemens DCA Vantage against the Tosoh G8. The purpose of this study is to evaluate the Vantage against the gold standard, knowing both methods are NGSP certified.

Methods: The Tosoh G8 HPLC method offers precise and accurate separation of the stable form of HbA1c from other hemoglobin fractions. This analysis is carried out

without off-line specimen pretreatment or interference from Schiff base.[1] A whole blood sample is diluted, treated, then a small volume is attached to the column. The hemoglobin fractions pass through the LED photometer and the changes are measured at 415 nm. In contrast, the DCA analyzer measures both the concentrations of hemoglobin A1C and the concentration of total hemoglobin. Additionally, the reagents needed for the test are self-contained in a cartridge. The measurement of total hemoglobin from the DCA analyzer is measured by a color development at 531 nm and is proportional to the total hemoglobin present in the sample. This is done by the oxidation of hemoglobin to form methemoglobin, then complexes with thiocyanate. For the measurement of HbA1c, an agglutination reaction is produced by using an agglutinator composed of a synthetic polymer with multiple immunoreactive copies of HbA1c. When agglutination occurs, this causes an increased scattering of light and again measured at 531 nm. HbA1c in whole blood specimens will compete for a limited number of binding sites causing an inhibition of agglutination. All measurements and calculations are performed automatically by the DCA analyzer and the result will be displayed on the screen in about 6 minutes.

Results: . 60 specimens were compared over a range of 4.4 - 14.0%. The average error index between the two methodologies was 0.09. However, the cost of the two methods differs remarkably. With the HPLC methodology costing $1.81 versus the agglutination assay total costs amounting to $12.00, the logistical placement of the DCA analyzer may justify this cost.

Conclusion: Specific logistical placement may consist of alternate site locations where underfunded or uninsured patients visit to obtain healthcare. Without treatment of the underlying disease, these patients could wind up in the Emergency Department. Knowing the results of the HbA1c test could potentially save more than just the $10.19 difference in the two methodologies.Therefore, I would recommend this instrument for placement at alternate sites to screen patients suspected of uncontrolled blood glucose.

D-31

Critical Evaluation of Point of Care Blood Glucose Meters in Patients Receiving Therapy with Maltose/Maltose Derivatives

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Introduction. Determination of blood glucose levels in patients who have received parenteral treatments that contain, or are metabolised to, maltose has been problematic in the past due to maltose interference with certain POC glucose test chemistries. This problem was additionally highlighted in intravenous immunoglobulin preparations, i.e. Octagam Such maltose interference can lead to overestimation of blood glucose levels and may result in the inappropriate administration of insulin. It is important, therefore, to determine the effectiveness of POC test systems in the presence of maltose or maltose derivatives.

Within this critical evaluation the new Accu-Chek Inform II test strip for use with the Inform II POC blood glucose test system (Roche Diagnostics), Nova Stat Strip (Nova Biomedical) and Medisense PXP (Abbott Diagnostics) evaluated to determine whether they demonstrated the required absence of cross reactivity to Maltose/ maltose derivatives to allow implementation as a global healthcare and hospital solution to glucose determination without increased patient clinical risk associated with treatment regime or drug therapy. All POC systems were compared to the reference blood gas methodology currently in routine use within our institution (cobas b221, Roche Diagnostics).

Methodology. Clinical evaluation of POC blood glucose test systems in patients receiving treatments that contain, or are metabolised to maltose: 50 paired random heparinised venous blood samples were obtained from renal patients, whose treatment involved maltose-containing solutions.

Statistical Methods: Spearman Rank Correlation, Student Paired T test, Bland Altman analysis.

Results. 1. Reference cobas b221 Mean Glucose 8.51 ± 4.50 mmol/l, Accu-Chek Inform II 8.52 ± 4.52 mmol/l, Nova StatStrip 8.49 ± 4.50 mmol/l and Abbott PXP 8.56 ± 4.48 mmol/l.

2. Correlation with Reference cobas b221: Accu-Chek Inform II r^2 0.997, slope 1.005, intercept -0.03, Nova StatStrip r^2 0.996, slope 0.999, intercept -0.008, Abbott PXP r^2 0.994, slope 0.994, intercept 0.10.

3. Student Paired T Test compared to Reference cobas b221: Accu-Chek Inform II p 0.989, Nova StatStrip p 0.985, Abbott PXP p 0.954

4. Bland Altman analysis compared to Reference cobas b221: Accuchek Inform II 0.012 ± 0.24 (limits of agreement -0.46 - 0.48), Nova StatStrip 0.004 ± 0.21 (limits of agreement -0.40- 0.41), Abbott PXP 0.052 ± 0.34 (limits of agreement -0.62 - 0.72).

Conclusion. All POC test systems within this study demonstrated statistically significant correlation with reference methodology across the working range in the determination of blood glucose concentrations in patients receiving treatments that contain, or are metabolised to, maltose Bland Altman analysis showed excellent mean bias and minimal scatter across the working range for Accu-Chek Inform II, with acceptable mean bias and scatter for Abbott Medisense PXP. However this does not reach levels of clinical significance and would not preclude its use. Paired Student T test demonstrated no significant difference between POC system and reference in patient receiving therapy with maltose/maltose derivatives. This study confirms that the performance of the Accu-Chek Inform II, Nova StatStrip and Abbott Medisense PXP systems are not adversely affected by the presence of maltose containing solutions.

D-32

Apparent Humidity Artefact Lessens Usefulness of Quality Control on the Abbott Precision Xceed Pro Self-Monitoring Blood Glucose Device

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Background: The province of Alberta is known for extreme seasonal temperature variations (-40C-+30C). While it has been shown that temperature and other environmental factors may alter the performance of certain self-monitoring blood glucose devices, these phenomena have not been systematically investigated. A humidity effect on the Abbott Precision Xceed Pro meter (PXP) results has been previously documented.

Methods: Quality control (QC) and patient results were collected from PXP meters between July 2010 and January 2011 operated in 2 central Alberta and 1 south Alberta regional hospitals. The external weekly temperatures were derived from meteorological reports and the internal weekly temperatures were derived from the PXP thermistor readings. Using multiple linear regressions, average weekly glucose QC and patient results were regressed against internal and external average weekly temperatures.

Results: Approximately 215 - 2091 patient data and 62 - 643 high and low control values were averaged weekly. During the study period, the weekly internal temperatures varied between 23C and 24.5C, but the weekly external temperatures varied from -20C to +20C. There was inconsistent correlation between patient averages and the internal and external temperatures. Two QC levels were measured (mean values of 2 and 15 mmol/L). Weak or no correlation was observed using the Low Control (LC). The High Control (HC) values showed strong positive correlations in all three hospitals (r2 = 88%, 72% and 44%) using multiple PXP strip and QC lots; these correlations were strongly associated with the external temperature (p= 0.02 to <0.00001). The HC excursion was 1 mmol/L over the temperature range.

Conclusions: The HC excursion of 1 mmol/L probably arises from seasonal interior humidity differences. In winter, humidity is very low and increases in the summer. The manufacturer's suggested limits for acceptability of the HC are very broad (11 - 19 mmol/L) and probably, in part, are a function of ambient humidity. Use of this presumably humidity-influenced control measurement makes error detection suboptimal. If strict statistical quality control limits are to be applied, two sets of control ranges based on seasonally dependent averages would be required.

D-33

A Comparison of the i-STAT Kaolin and Hemochron Methods for the Point-of-Care Measurement of Activated Clotting Time (ACT) in Various Clinical Venues

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Background: Various methods are available for the measurement of activated clotting time (ACT) at the point-of-care however the level of agreement between these methods using kaolin as an activator is not clear.

Methods: We compared the newer i-STAT kaolin ACT and Hemochron kaolin ACT methods for monitoring high dose heparin therapy in three different clinical venues.

Results: Significant negative bias was observed between methods with the i-STAT giving consistently lower results than the Hemochron 801 (HC801) and Hemochron Jr (HJ) models. Regression data for comparison studies in various venues revealed the

following: Extracorporeal Membrane Oxygenation (ECMO), n=27, i-STAT=0.469 x HJ + 50.9, r= 0.964; Cardiac Electrophysiology, n=69; i-STAT =0.377 x HC801 + 124, r= 0.684; Cardiac Catheterization, n=43; i-STAT =0.824 x HC801 + 32.1,r= 0.979.

Conclusion: We conclude that since i-STAT automates essentially all steps of the assay, the lower results obtain are more accurate and precise than corresponding results observed on the semiautomated Hemochron 801 method. We observed that control of the preanalytical variables greatly improves the lack of correlation between i-STAT and HC801 however significant negative bias remains. As anticipated, the comparison of i-STAT with the more automated HJ device showed excellent correlation however a significant negative bias was observed which appears to be operator independent.

D-34

Critical Evaluation of the Effect of PaO2 on Blood Glucose determination by Point of Care Blood Glucose Meters

G. M. Creed, T. J. Fox, R. J. Beale. *Guy's and St Thomas Foundation Hospital NHS Trust, London, United Kingdom,*

Introduction. Blood Glucose determination in the critically ill patient is fraught with contraindications, which may preclude the use of POCT Blood Glucose meters in this setting. Previous studies by Tang (2001), Pulzi (2009) have demonstrated the significant effect of changes in PaO2 on the accuracy of POCT meters. PaO2 in the normal patient ranges from 10 -13 kPa; however in the critically ill patient who may be respiratory and/or cardiovascularly compromised ventilatory strategies may be initiated which significantly elevate PaO2 levels. In addition due to the unstable nature of the critically ill patient hypoxic episodes with decreased PaO_2 are not uncommon. Marked changes in PaO_2 may contraindicate the use of POCT meters in this setting.

The aim of this critical evaluation was to determine whether the Roche Accu-Chek Inform II, Nova Stat Strip and Abbott PXP were significantly affected by changes in PaO2 compared to the reference methodology currently used our institution, Roche Cobas b221 blood gas methodology for the determination of Blood Glucose.

Methodology. 200 paired, random heparinised arterial blood samples were obtained from critically ill patients with Blood Glucose determined with all devices. PaO2 was additionally determined using reference blood gas methodology. Subsets were analysed where PaO_2 was: (1) 20.0 kPa to determine the clinical affect on Blood Glucose determination.

Results. In the Hypoxic patient PaO2 ranged 6.5 - 9.8 kPa difference between Reference methodology and;

(1) Roche Accu-Chek Inform II Mean -0.19, SD 0.27, Limits of Agreement -0.72 - 0.53 mmol/l.

(2) Nova Stat Strip Mean -0.03, SD 0.27, Limits of Agreement -0.56 - 0.50 mmol/l.

(3) Abbott PXP Mean 0.07, SD 0.37, Limits of Agreement -0.66 - 0.80 mmol/l.

In the Normal patient PaO2 ranged from 10.0 - 13.0 kPa difference between Reference methodology and;

(1) Roche Accu-Chek Inform II Mean -0.02, SD 0.23, Limits of Agreement -0.47 - 0.43 mmol/l.

(2) Nova Stat Strip Mean 0.02, SD 0.25, Limits of Agreement -0.47 - 0.51 mmol/l.

(3) Abbott PXP Mean 0.16, SD 0.30, Limits of Agreement -0.43 - 0.75 mmol/l.

With Raised PaO2 range 13.1 - 19.8 kPa difference between Reference methodology and;

(1) Roche Accu-Chek Inform II Mean -0.02, SD 0.23, Limits of Agreement -0.47 - 0.43 mmol/l.

(2) Nova Stat Strip Mean 0.04, SD 0.24, Limits of Agreement -0.43 - 0.51 mmol/l.

(3) Abbott PXP Mean 0.14, SD 0.29, Limits of Agreement -0.43 - 0.71 mmol/l.

With Markedly raised PaO2 Range 21.0 - 27.8 kPa difference between Reference methodology and:

(1) Roche Accu-Chek Inform II Mean -0.06, SD 0.25, Limits of Agreement -0.55 - 0.43 mmol/l.

(2) Nova Stat Strip Mean -0.06, SD 0.30, Limits of Agreement -0.65 - 0.53 mmol/l.

(3) Abbott PXP Mean 0.14, SD 0.42, Limits of Agreement -0.68 - 0.96 mmol/l.

Conclusion. All POCT meters within this study demonstrated no significant difference with reference methodology for all patient subsets investigated. The POCT meters critically evaluated within this study showed excellent limits of agreement across the PaO2 clinical spectrum investigated and therefore would not preclude their use in the critically ill patient.

D-35

A New Fully Integrated, Automatic Point-Of-Care Device, Samsung Blood Analyzer for Both Clinical Chemistry and Immunoassay Using the Lab-on-a-CD platform

B. Lee[1], K. Kim[1], S. Bae[1], H. Kim[1], Y. Lee[1], J. Kim[1], W. Lee[2], I. Park[2]. *[1]Samsung Electronics, Suwon, Korea, Republic of, [2]Ajou University School of Medicine, Suwon, Korea, Republic of,*

Background: The "Lab-on-a-CD (LabCD)" systems are the focus of intense research, where complex assays are embedded in fluidic networks on the centrifugal microfluidic systems, especially towards the development of *in vitro* diagnostics (IVD). Through the adaptation of miniaturization technology, multiple analysis steps and reagents can be fully integrated on a single LabCD with the Point-of-Care (POC) sample-to-answer system, Samsung Blood Analyzer (SBA). The SBA processes whole blood specimens without pretreatment in addition to serum and plasma specimens within 12 min for clinical chemistry panel, and within 25 min for immunoassay panel. Both clinical chemistry and immunoassay panels of the SBA were evaluated for the precision, linearity, and method comparision according to the CLSI guidelines.

Methods: The test parameters are three clinical chemistry (lipid profile) and one immunoassay (cancer profile): total cholesterol (TC), triglyceride (TG), high-density lipoprotein-cholesterol (HDL-C), and prostate-specific antigen (PSA). The precision and the linearity were evaluated according to EP 5-A2 and EP 6-A respectively. The quality control materials are Randox multi-sera (Ireland) for clinical chemistry and BioRad Liquichek Immunoassay Plus control (US) for immunoassay. Method comparison studies with central laboratory analyzer TBA-200FR (Toshiba, Japan) for clinical chemistry parameters, and Cobas Modular E170 (Roche, Swiss) for immunoassay parameter were performed using serum and plasma specimens, which were anti-coagulated with lithium heparin.

Results: In the method comparision with serum specimens (n=158), the correlation Y=1.09X+5.69, R=0.998 is for TC, Y=1.21X+12.6, R=0.993 for TG, Y=0.80X+1.42, R=0.975 for HDL-C, and Y=1.09X-0.11, R=0.995 for PSA. In the method comparision with plasma specimens (n=119), the correlation Y=1.07X-5.99, R=0.997 is for TC, Y=1.21X+38.0, R=0.992 for TG, Y=0.94X+11.2, R=0.958 for HDL-C, and Y=1.05X-0.09, R=0.994 for PSA. In the evaluation of precision, within-run and total CVs of low and high concentration QC materials are below 5.82% in all test parameters (total CV at low conccentration: 2.0% for TC, 3.9% for TG, 5.1% for HDL-C, 5.82% for PSA; total CV at high concentration: 0.3% for TC, 1.4% for TG, 3.7% for HDL-C, 5.81% for PSA). As for the linearity, all parameters show an $R^2 > 0.99$ ($P<0.0001$) with the observed ranges of 149~565 mg/dl for TC, 89~666.5 mg/dl for TG, 44.5~116 mg/dl for HDL-C, and 0.05~30.0 ng/ml for PSA.

Conclusion: The SBA is a unique multi-fuctional POC clinical chemistry and immunoassay analyzer. One LabCD platform can perform up to 19 clinical chemistry parameters at the same time within 12 min. Furthermore, it can also carry out both clinical chemistry and immunoassay parameters simultaneously within 25 min in a single run. With the clinical results comparable to the reference analyzer, the SBA can be operated under Equivalent Quality Control Option 2 and could be used for diagnosis and screening in Primary Health Care.

Wednesday PM, July 27

Poster Session: 2:00 pm - 4:30 pm
Proteins/Enzymes

D-37

Correlation Between Alpha-1-Antitrypsin Quantitation and Phenotype in Adult and Pediatric Populations

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Background: Over 100 genetic variants of alpha-1-antitrypsin (A1AT) have been described. Most are associated with normal A1AT production, most commonly the non-deficient "M" allele, although less common non-deficiency alleles, including "C", "F", and "E", also exist. Other variants, specifically "S" and "Z", are associated with decreased A1AT concentrations, which can lead to a variety of clinical symptoms, most often manifesting in the lungs and/or liver. Other deficiency alleles, including "I", have been described but their clinical significance is unclear.

Objective: Compare quantitation of different A1AT phenotypes in adults and pediatric cohorts.

Methods: A1AT phenotype and quantitation results were collected on adult (n=21445) and pediatric (n=2469) samples submitted for clinical testing between January, 2008 and December, 2009. Quantitation was performed by immunonephelometry (Siemens); phenotyping was performed by isoelectric focusing gel electrophoresis (Sebia). Mid-95%ile concentration ranges in each phenotype group were calculated for adult and pediatric cohorts using quantile regression. Distributions between adults and pediatrics were compared using the Kolmogorov-Smirnov test with sample size > 30.

Results: Statistically significant, yet likely not clinically relevant, differences in A1AT quantitation between adult and pediatric distributions were identified in most phenotype groups. In addition, decreased concentrations of A1AT correlated with phenotypes of the known deficiency alleles S and Z, with differences also noted between heterozygotes and homozygotes. The I allele also correlated with decreased concentrations of A1AT. Lastly, heterozygosity for the Z allele in the context of a non-M non-deficiency allele was associated with moderately decreased concentrations of A1AT in comparison to M/Z heterozygosity.

Conclusion: The same reference ranges for A1AT quantitation for each phenotype can be used for adult and pediatric patients. Rare deficiency alleles and rare non-deficiency alleles in the presence of the Z allele are associated with decreased concentrations of A1AT and warrant identification in the context of potential A1AT deficiency.

	Adult				Pediatrics				
		mg/dL				mg/dL			
	N	Mean	Total Range	95% Ref Range	N	Mean	Total Range	95% Ref Range	p-value
M/M	17355	158	47 - 628	100 - 273	2023	146	46 - 449	93 - 251	<0.0001
M/S	1709	134	50 - 364	84 - 225	149	122	50 - 263	83 - 236	<0.0001
M/Z	1580	94	30 - 322	61 - 156	135	89	48 - 153	64 - 142	0.005
Z/Z	139	28	12 - 80	15 - 57	48	34	19 - 140	22 - 55	0.0003
S/Z	161	67	33 - 145	42 - 108	22	58	36 - 112	N/A	N/A
S/S	60	102	45 - 183	49 - 181	10	92	81 - 114	N/A	N/A
I/Z	7	71	54 - 96	N/A	2	60	54 - 65	N/A	N/A
C/Z,F/Z,P/Z	22	74	36 - 141	N/A	0	N/A	N/A	N/A	N/A
N/A = Insufficient sample size for estimation of quantiles and/or p-values									

D-38

Validation of a Luminex-based Frataxin Immunoassay for Diagnosis, Population Screening, and Potential Treatment Monitoring of Friedreich Ataxia

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Background: Friedreich ataxia (FA) is an autosomal recessive disease affecting approximately 1:50,000 people in the USA. The disease is recognized by progressive ataxia, dysarthria, vision and hearing loss, muscle weakness and skeletal deformities. Hypertrophic cardiomyopathy is also present in almost two-thirds of patients with FA. Initial symptoms typically appear between 10 and 15 years of age. FA is caused by GAA expansions in the first intron of the *FXN* gene, which leads to a reduced expression of its product, the protein frataxin, and to disease. To date, the laboratory diagnosis of FA relies on a DNA-based molecular test. Described here is the development and validation of a new, frataxin protein-based method that could be used for diagnosis, population screening, and potentially to monitor frataxin-augmentation treatments.

Methods: Luminex xMAP microspheres are covalently coated with monoclonal anti-frataxin antibody. Diluted whole blood (WB) or the eluate from a 3 mm punch from a dried blood spot (DBS) is mixed with the coated microspheres. This is followed by the addition of anti-frataxin polyclonal antibodies. A reporter solution of streptavidin-R-phycoerythrin is finally added and the beads are read on a Luminex LX200 instrument in a 96-well plate format. A calibration curve of 0 to 76 ng/mL blood is generated on each plate using purified recombinant human frataxin. Validation of linearity, precision, recovery, stability, and interferences was performed using DBS and WB specimens from 125 adult, 126 pediatric, and 41 newborn subjects, 38 patients with FA, and 10 FA carriers.

Results: Expected vs. observed plots of serial dilutions with frataxin-spiked DBS and WB specimens resulted in linear regression slopes of 0.97-1.03 with an R^2 of 0.992-0.9998 (N=6). Dilutions were linear from 220 to 0.5 ng/mL blood. Frataxin intra assay precision CV's in DBS were 12%, 13%, and 10% at mean concentrations of 3, 15, and 34 ng/mL blood respectively (N=20), and in WB were 7%, 7%, and 5% at mean concentrations of 1, 15, and 27 ng/mL blood respectively (N=20). Frataxin inter assay precision CV's in DBS were 15%, 11%, and 15% at mean concentrations of 1, 15, and 35 ng/mL blood respectively (N=20), and in WB were 16%, 10%, and 13% at mean concentrations of 1, 15, and 28 ng/mL blood respectively (N=20). Mean recovery of frataxin spiked at 3 concentrations into 2 DBS and 2 WB samples analyzed on 3 days were 95%, 78%, and 99% respectively for each date. Addition of lipids (up to 1000 mg/dL) or bilirubin (up to 20 mg/dL) did not interfere with determination of frataxin. Results for DBS and WB were 99-120% and 97-109% of original values respectively.

Conclusion: We developed and validated a Luminex based immunoassay that can quantify frataxin in dried blood spots and whole blood and is useful for the diagnosis and monitoring of patients with FA. Ongoing work aims to demonstrate its clinical utility for pre-symptomatic population screening.

D-39

Cellulose acetate membrane electrophoresis coupled with sensitive colloidal silver staining for urinary protein analysis: A rapid and simple approach for predicting the renal injury

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Background: Cellulose acetate membrane (CAM) electrophoresis provides an overview of serum protein contents and is usually used in clinical diagnosis. We have previously reported that sensitive colloidal silver staining coupled with CAM electrophoresis is useful for urinary protein analysis without performing concentration of urine samples[1,2]. In this study, we compared the urinary protein profiles of patients with different renal diseases and the histopathological features of the corresponding renal biopsy specimens.

Methods: The study included urine samples obtained from 66 patients with different types of renal diseases, including IgA nephropathy, membranous nephropathy, antineutrophil cytoplasmic antibody (ANCA)-associated nephropathy, and tubulointerstitial nephropathy. The urine samples were analyzed using CAM electrophoresis and then stained using colloidal silver. Immunofixation (IFE) was performed to identify the major protein bands on the CAM.

Results: The urinary protein profiles were classified into 3 patterns on the basis of protein content; -glomerular proteins (albumin-, [Alb] and transferrin, [Tf]), tubular proteins (beta-2-microglobulin-, [β2-m] and retinol-binding-protein-, [RBP]), and mixed proteins (Figure)[2]. The glomerular pattern was observed for 61 patients (92.4%); -tubular pattern, for 2 patients (3.0%); and -mixed pattern, for 2 patients (3.0%). The protein profile for 1 patient could not be classified into any of these pattern. The sensitivity and specificity between the histopathological features of correlated renal biopsy specimens and urinary protein patterns identified using CAM electrophoresis were 100 and 85.7 for the glomerular pattern, 100 and 94.9 for the tubular pattern, 57.9 and 100 for the mixed pattern.

Conclusion: The urinary protein patterns correlated well with the histopathological features of the corresponding renal biopsy specimens. Thus, CAM electrophoresis is a sensitive and useful approach for the diagnosis of renal diseases.

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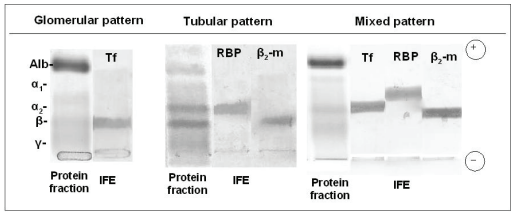

D-40

New Specific Antibodies to Immunoassay $A\beta_{1-42}$ in Alzheimer's Disease

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Background: Two of the pathological hallmarks of Alzheimer's Disease (AD) are senile plaques and neurofibrillary tangles. The main constituent of senile plaques is β-amyloid protein (Aβ), which can typically be found in the early stages of AD. Aβ, which is composed of 42 amino acids, is produced by the actions of β and γ secretases. Aβ can be deleterious to brain tissues. It has recently been demonstrated that aggregated oligomers of $A\beta_{1-42}$ are highly toxic to neurons; more so than the fibrillar $A\beta_{1-42}$ with a β-sheet structure. The purpose of this study was to detect $A\beta_{1-42}$ in minute amounts by immunoassay, using new specific monoclonal antibodies to oligomeric $A\beta_{1-42}$.

Methods: Aggregated oligomers of $A\beta_{1-42}$ were produced by the addition of KLVFF peptide ($A\beta_{16-20}$) to synthesized $A\beta_{1-42}$. $A\beta_{1-42}$ without the addition of $A\beta_{16-20}$ resulted in the fibrillar $A\beta_{1-42}$ with the β-sheet structure. For oligomeric $A\beta_{1-42}$ production, $A\beta_{1-42}$ at 2 mg/ml solution was prepared in MilliQ water and incubated for 30 min at 4 °C. Dulbecco's PBS was added to this solution, and then a 10-fold molar excess of $A\beta_{16-20}$ was added. The solution was rotated for 16 h at 37 °C at 3 rpm. $A\beta_{1-42}$ without $A\beta_{16-20}$ was incubated for 16 h in the same conditions, to prepare fibrillar $A\beta_{1-42}$. These two Aβ forms were then compared by SDS-PAGE, capillary electrophoresis, and thioflavin T (Tht) assay. Tht is a fluorescent dye which binds to amyloid fibrils, and is used to determine the concentration of fibrous structures. Oligomeric $A\beta_{1-42}$ and fibrillar $A\beta_{1-42}$ were visually observed by atomic force microscopy (AFM). The SDS-PAGE and capillary electrophoresis analyses confirmed the increase in molecular weight of $A\beta_{1-42}$. The Tht fluorescence assay revealed that fluorescence time-dependently increased for $A\beta_{1-42}$ without $A\beta_{16-20}$, and decreased for $A\beta_{1-42}$ with $A\beta_{16-20}$ addition. AFM visually confirmed the formation of oligomeric $A\beta_{1-42}$ and fibrillar $A\beta_{1-42}$. We then proceeded to make monoclonal antibodies to oligomeric $A\beta_{1-42}$, and assessed the reactivity and specificity of these antibodies by ELISA and western blot analysis.

Results: Two clones were obtained that bound $A\beta_{1-42}$ oligomers; designated 31-2 and 37-11. The subclass of both clones was IgG1 (light chain: κ). In the ELISA, the range of the calibration curve was 10 - 500 ng/ml, and the coefficients were >0.98 for both clones. Reactivity to oligomeric $A\beta_{1-42}$ was approximately 80%, and cross-reactivity to the fibrillar form was approximately 20%. We speculate that the observed cross-reactivity occurs via a reaction to a tip portion of fibrillar $A\beta_{1-42}$, projecting at the surface of oligomeric $A\beta_{1-42}$. Western blot analysis confirmed the reactivity of the antibodies to oligomeric $A\beta_{1-42}$.

Conclusion: Thus, we report here the generation of new specific monoclonal antibodies to oligomeric $A\beta_{1-42}$ that we believe may be useful in the diagnosis and treatment of AD.

D-41

Facilitating the Laboratory Diagnosis of Alpha-1-antitrypsin Deficiency

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Background: Alpha-1-antitrypsin (AAT) deficiency is an autosomal codominant disorder. The primary physiologic role of AAT is to protect the lungs from degradation by neutrophil elastase. Thus, an AAT deficiency leads to progressive deterioration of the lungs. *SERPINA1* (the gene that codes for AAT) is highly polymorphic, leading to the expression of numerous AAT variants. Many of these variants are benign, resulting in normal AAT expression and function. The most common non-deficiency allele codes for the M variant. Other AAT variants may produce deleterious effects by either decreasing the protective coating of the lungs and/or by polymerizing in hepatocytes, causing hepatocellular death and liver disease. The most common deficiency alleles code for the Z and S variants. The pulmonary damage caused by an AAT deficiency can be prevented with diagnosis and treatment. Laboratory testing is invaluable in the diagnosis of AAT deficiency and is accomplished by an amalgam of immunoassay, genetic, and electrophoresis techniques. This study facilitates the analysis of AAT phenotypes using isoelectric focusing electrophoresis (IFE) by creating a reference guide to evaluate phenotypes encountered in the laboratory. Stability of AAT in whole blood was also assessed.

Methods: Using samples collected over a two year period, a compendium of AAT phenotypes was created. These phenotypes encompass the majority of phenotypes observed in clinical testing. In addition, we evaluated the effects that various sample storage conditions had on the AAT phenotype and concentration. To assess the stability of AAT, 11 whole blood samples were collected from two individuals (n=22) and stored at 4°C or at room temperature (RT). A baseline specimen was collected to serve as a reference (n=2). One whole blood sample from each individual stored at both temperatures was centrifuged every 24 hours for 4 days (n=4) and a final set of 2 samples were processed after 7 days (n=4). At each time point, the resulting serum was stored at -20°C until all samples were processed. Total AAT concentration was quantified by immunoturbidimetric techniques (Roche Diagnostics, Indianapolis, IN). Phenotype patterns were assessed using IFE (Sebia, Inc., Norcross, GA), which produces high-resolution separation of the major and minor AAT bands associated with each phenotype.

Results: We created a compendium that displays 18 common and rare AAT phenotypes. Accompanying these visual data is detailed methodology describing how to recognize the most common AAT banding patterns and how to interpret a rare phenotype. AAT is stable for IFE phenotype analysis for at least one week in whole blood stored at 4°C or RT. The expected percent recovery of total AAT as a function of analytical precision was expected to be 96.5-103.5%. The average percent recovery of the 5 time points over a one week period met these criteria (97.5-99.7% recovery from baseline).

Conclusions: A reference compendium of most known AAT phenotypes was established that may serve as a resource for interpreting AAT phenotypes. AAT is stable in whole blood for phenotype analysis for up to 7 days. For total AAT measurements, the stability of AAT was similar whether stored at RT or 4°C.

D-42

Determining the Analytical Performance of Polyethylene Glycol (PEG)Precipitation of Macro Aspartate Aminotransferase (macroAST)

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Background: MacroAST is a high-molecular-mass complex of aspartate aminotransferase (AST) and immunoglobulin (usually IgG, rarely IgA or IgM). MacroAST complexes are benign macroenzmyes that exhibit reduced clearance from blood and result in increased serum AST activity. Confirmation of macroAST is important to reduce unnecessary and invasive diagnostic procedures. Several methods have been proposed to detect macroAST including polyethylene glycol (PEG) precipitation. However, reference intervals and cut-offs for confirming the presence of macroAST are needed.

Objective: (1) Establish reference intervals for percent AST precipitation from normal and liver disease samples, and (2) validate a macroAST assay in order to identify patients with elevated AST due to macroAST.

Methods: Residual serum samples (IRB #10-007098) with the following characteristics were obtained: (i) AST, alanine aminotransferase (ALT), and alkaline phosphatase (ALP) within reference intervals (normal, n=120); (ii) AST, ALT, and ALP results exceeding upper reference limits (elevated liver disease, n=120); (iii) AST>100 U/L, AST/ALT > 2.0, ALT and ALP activity within reference intervals (possible macroAST, n=38); (iv) clinically suspected macroAST samples based on physician order (suspected macroAST, n=14). Samples were treated with PEG6000 (12.5% final concentration) and centrifuged (1500 g, 5 mins). AST was measured on a Roche Cobas c501. Protein G (Pierce) spin columns were used to confirm immunoglobulin G (IgG) in the macro-enzyme complex. Reference intervals were determined by nonparametric analysis. Precision, recovery, and linearity were assessed using serum samples submitted for routine clinical testing and treated with PEG.

Results: Imprecision studies demonstrated intra-assay CV's of 5.8% and 0.8% at concentrations of 21 U/L and 293 U/L and inter-assay CV's of 6.6% and 4.2% at concentrations of 52 U/L and 378 U/L. The lower limit of quantitation was 10 U/L

with a CV of 13.1%. PEG-precipitated samples were linear over the measured range of 14-1584 U/L with an r^2 of 1.00 and slope of 1.00. Average recovery of AST in PEG-treated matrix was 98.4%. PEG-precipitated samples showed similar interference due to hemolysis, lipemia, and icterius as non-PEG treated samples. Reference intervals for % PEG-precipitated AST in normal and elevated liver enzyme samples were 0-51% (90% CI upper limit:45-71%) and 5-51% (90% CIs: 3 to 7%, 47 to 67 %), respectively. The mean % PEG-precipitated AST in possible macroAST samples was 26±14% (range 0-57%). Suspected macroAST samples yielded an average 94±5% PEG-precipitated AST (range 84-99%). In 7 of 8 suspected macroAST samples, protein G columns bound 69±17% of AST activity (range 43-86%). Protein G columns bound 25±8% of AST activity (range 14-41%) in ten control samples.

Conclusion: In conclusion, analytical and clinical validation suggests that PEG precipitation is a viable means of detecting macroAST using a % PEG precipitation cutoff of >80%.

D-43

Identification of Complement factor 2 deficient patient sera using a rapid, sensitive assay on the SPA PLUS turbidimeter

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Complement factor 2 (C2) is a single chain plasma serine protease which forms part of the classical pathway of complement activation. Upon triggering, C2 is bound by C4b and cleaved by C1s (or MASP-2) into C2a and C2b. C2a then forms part of the C3 convertase enzyme (known as C4bC2a). Deficiencies of the complement components result in patients having a pre-disposition to infections and autoimmune diseases such as; systemic lupus erthyematosis, glomerulornephritis and vasculitis. C2 deficiency affects approximately 1 in every 10000-20000 people. Serum levels can be measured by radial immunodiffusion techniques (RID), however C2 diagnostic testing is not in widespread use. Here we report on, the production of specific antisera and the use of these antisera to construct turbidimetric assays on the SPA PLUS analyser. C2 was purified from pooled fresh frozen plasma using ion exchange and size exclusion chromatography. Final purity was assessed by Silver Stained SDS Page and Western blot techniques. Quantification was performed using specific C2 RID (Binding Site Group Ltd) and BCA assays (Pierce) in accordance with manufacturer's instructions. Polyclonal antisera were generated using the purified C2 antigen and used to produce a 6 point calibration curve from a serum based calibration set. The standard assay curves were validated using control fluids and all dilutions were made using the instruments onboard pipetting system. Samples were initially measured at a 1/10 dilution and, if out of range the instrument automatically re-measured the samples at an alternative dilution. The assay took 10 minutes and was read at end-point. The assay range was 1 - 36mg/L using a 1/10 sample dilution, with a sensitivity of 0.1mg/L using neat sample. Intra- and inter-assay precision were assessed at three antigen levels of 2.1, 10 and 33mg/L. The coefficients of variation were 5.6% and 4.6% for the low sample, 1.1% and 2.2% for the medium sample, and 2.2% and 1.5% for the high sample respectively. The assay showed a high degree of linearity when expected values were regressed against measured values of serially diluted pooled serum:- $y=1.01x+0.01$; $r^2=1$. Sera from 24 patients were compared between RID and the SPA PLUS assay for the identification of patients with C2 deficiency. Using receiver operator curve (ROC) analysis to assess sensitivity and specificity the SPA PLUS assay identified all C2 deficient patients (area under the curve = 0.86) identified by RID. Additionally, three C2 deficient patients were identified by the SPA PLUS assay alone. We conclude that it is possible to purify C2 and use the purified protein to produce specific antisera. Furthermore, it is possible to produce a turbidimetric assay which provides a rapid alternative to RID assays, and offers similar sensitivity for the detection of C2 deficient patients.

D-44

The Significance of Subtle Abnormalities of Serum Protein Electrophoresis: Indications for Reflexing to Immunofixation Electrophoresis

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Background: In serum protein electrophoresis (SPE), subtle abnormalities such as elevated β fraction, decreased γ fraction in the cases without M-spikes may reflect the presence of a monoclonal protein below detectable level on SPE, or other causes. A wide or increased β fraction may indicate a "hidden" paraprotein co-migrating with β fraction; a decreased γ fraction may be due to the inhibition of the normal immunoglobulin in monoclonal gammopathies. Therefore, the sensitivity of SPE as a screening test for monoclonal gammopathies, is dependent on how such abnormalities are defined and interpreted. In our laboratory, β fraction above the reference interval (>1.1 g/dL) and/or γ fraction below the reference interval (<0.9 g/dL) in the absence of M-spikes, have been routinely used as criteria for reflexing to the specimens to immunofixation electrophoresis (IFE). The goal of this study is to evaluate the effectiveness and appropriateness of these criteria for reflexing to IFE.

Methods: SPE and IFE were conducted with agarose gel reagents on the Helena SPIFE 3000 (Helena Laboratories). All SPE assay were performed between January 1, 2010 and January 31. All cases with reflexed to IFE due to elevated β fraction (>1.1 g/dL) and/or decreased γ fraction (<0.9 g/dL) without M-spikes were included and analyzed in this study.

Results: Of total 565 SPE cases, 41 cases (8.1%) were with elevated β fraction (>1.1 g/dL), and 88 cases (15.5%) were with decreased γ fraction without M-spikes on SPE. Of 41 cases with elevated β fraction, 5 cases showed monoclonal proteins on IFE (IgG lambda: 2 cases, IgA kappa: 1 case, IgA lambda: 1 case, and free light chain: 1 case). The mean and SEM of the beta fractions of these 5 cases and randomly selected 12 reflexed to IFE cases with elevated β fraction without identified monoclonal proteins were 1.78 ± 0.06 g/dL and 1.27 ± 0.03 g/dL respectively ($p<0.01$). Of 88 cases with decreased γ fraction, 9 cases showed monoclonal protein on IFE (IgG kappa: 3 cases, IgG lambda: 2 cases, IgA lambda: 1 case, free light chains: 3 cases). The mean and SEM of the γ fractions of these 9 cases and randomly selected 20 reflexed to IFE cases with decreased γ fraction without identified monoclonal proteins were 0.68 ± 0.16 g/dL and 0.73 ± 0.22 g/dL respectively ($p = 0.2$). There were more IgG monoclonal immunoglobulins (56%) and free light chains (33%) than other classes of immunoglobulins identified by IFE in the cases with decreased γ fraction.

Conclusion: It is warranted that the subtle abnormalities of elevated β fraction and/or decreased γ fraction are used as criteria to reflex specimens to IFE. It is appropriate to reflex to IFE when the γ fraction level below reference interval. In order to improve the efficiency, the level of β fraction elevation as a trigger reflexing to IFE may be increased, for which further studies are needed.

D-45

Analytical evaluation of the Next Generation Lactate Dehydrogenase Assay Application for the Abbott ARCHITECT *c*Systems

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Background: Plasma and serum lactate dehydrogenase (LD) activity is used in the diagnosis of wide ranges of disorders, such as hemolytic anemia.

Abbott recently launched the ARCHITECT Next Generation Lactate Dehydrogenase Assay to ensure a better alignment with the IFCC reference method and to improve the lot-to-lot and calibration stability. This assay utilizes the conversion of lactate to pyruvate, for the determination of the enzyme activity, e.g. lactate and NAD+ are converted to pyruvate and NADH by the action of LD. The rate of NADH generation, and concomitantly the increase in absorbance at 340 nm is directly proportional to the LD activity in the sample.

We report our findings of the analytical evaluation of this assay by using the CSLI EP-10 and EP-9 protocols. In addition we report on the alignment of the ARCHITECT Next Generation Lactate Dehydrogenase Assay with the assigned value obtained with IFCC reference method.

Methodology: The precision of the assay was evaluated according to CSLI EP-10 protocol. For this pooled serum samples we prepared at 108, 578 and 1067 U/L levels. The correlation with the current on-market Abbott ARCHITECT Lactate Dehydrogenase Assay was carried out according to the CSLI EP-9 protocol. Measurements of lactate dehydrogenase were performed on the Abbott ARCHITECT *c*8000 *c*Systems.

The alignment with the IFCC reference method was determined by using so called *Calibration 2000* reference samples of the Dutch SMKL quality assessment scheme, in which the LD activity was assigned by using the IFCC reference method.

Results: Based on the EP-10 protocol, the imprecision of the Abbott ARCHITECT Next Generation Lactate Dehydrogenase Assay was below 4.0 % at all serum levels. Based on a intra-individual biological variability of 8.6 % in serum, this means at the assays meets the analytical requirements for a proper evaluation of LD activity in serum.

Based on the EP-9 protocol, the Next Generation Lactate Dehydrogenase Assay showed a correlation with the current assay of LD(next gen) = 1.05xLD - 4.01, with a 95 % confidence interval of -7.4 to -0.66 for the intercept and of 1.04 to 1.06 for the slope, respectively.

Utilizing the SKML calibration 2000 samples, the Abbott ARCHITECT results showed a good agreement with those obtained with the IFCC reference method. That is, all measurements were within 3% range of the reference method.

Conclusion: The Abbott ARCHITECT Next Generation Lactate Dehydrogenase Assay shows good analytical characteristics for the determination of LD in serum. In addition this assay shows a good alignment with the IFCC reference method.

D-46

Evaluation of common interferences on automated high resolution capillary zone electrophoresis (CZE-HR) for serum protein electrophoresis (SPE)

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Background: Serum protein electrophoresis (SPE) by automated high resolution capillary zone electrophoresis (CZE-HR) allows clear separation in 8 fractions and relative quantification of many high abundance proteins. The aim of this study is to assess interference of common clinical and analytical conditions (hemolysis, lipemia, complement degradation products (C3d), fibrinogen and C-reactive protein (CRP)) and exogenous products (radio-contrast agents) on the electrophoretic profile for patient samples analyzed by this 8 fraction high resolution serum protein capillary zone electrophoresis (CZE-HR SPE).

Methods: This study was conducted on Sebia Capillarys 2 automated system with high resolution buffer and protein detection by UV absorbance at 200 nm. Samples used to measure hemolysis, lipemia and radio-contrast agents were prepared by adding increasing amounts of hemolysate, Intralipid 10% and Omnipaque™ respectively in human biological sera with normal electrophoretic profile. C3d interference was evaluated on samples stored at 4°C or at room temperature for 0, 24h, 48h, 72h and 96h. Fibrinogen interference was observed by comparing paired plasma and serum samples. Finally, CRP interference was evaluated on serum from patients with measured CRP ranging from 7 to 567 mg/L.

Results: Hemolysis causes a decrease of haptoglobin with the emergence of a haptoglobin-hemoglobin complex on the cathodic side of the α2-macroglobulin fraction. Free hemoglobin is observed between transferrin and complement as a sharp peak. Intravascular hemolysis is characterized by decreased haptoglobin without appearance of haptoglobin-hemoglobin complex. Intralipid and Omnipaque™ interferences are observed between the α1-acid glycoprotein (AAG) and α1-antitrypsin (AAT) fractions which can affect measurement of both a1-globulin acute inflammatory protein fractions. C3d appears in early gammaglobulin region after 72h at 4°C, and after only 24h at room temperature. Fibrinogen is substantially at the same position as C3d while CRP is next to C3d, on the cathodic side.

Conclusion: Despite the fact that hemolysis is recognized as an interference, this CZE-HR SPE automated technique may allow us to detect and differentiate intravascular hemolysis from *in vitro* hemolysis. One should know the impacts of common interferences on the CZE-HR SPE profile, many of them producing a small peak similar to a small monoclonal band and contributing to a false increase of involved protein fractions (Lipemia and Omnipaque™ radiocontrast agent interferences in AAG and AAT; complement degradation (C3d), fibrinogen and CRP in gammaglobulin fraction). Adequate knowledge of interfering conditions allows optimal use of this new clinical application.

D-47

Performance Evaluation of the Helena V8 Capillary Electrophoresis System

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Background: Diagnosis and management of patients with monoclonal gammopathies requires accurate identification and characterization of monoclonal proteins. Capillary electrophoresis and immunosubstraction (also known as immunotyping or immunodisplacement) techniques have recently become popular methods to handle increasing workloads by decreasing the manual processing of methods which use agarose gels. The objective of this study was to compare a new capillary electrophoresis method, the V8 (Helena, Beaumont, TX), with the CAPILLARYS2 (Sebia, USA).

Methods: 100 consecutive samples from physician-ordered serum protein electrophoresis (SPE) tests were analyzed on the V8, CAPILLARYS2, and SIFE3000 according to the manufacturer's directions. Precision data were collected for each method using normal and hyper-gamma abnormal controls (Sebia, USA) run twice daily for 15 days. Within-run and total imprecision were calculated using EP Evaluator v9.0 consistent with CLSI guidelines (EP5-A2). For the performance comparison, each gel and electropherogram was interpreted by 3 experienced individuals to assess the ability of the SPE methods to detect abnormalities, and the ability of the immunosubtraction methods to accurately characterize monoclonal proteins. SPIFE3000 was used as the gold standard for detection of abnormalities and the isotype of monoclonal protein. Chart review was done to determine the clinical significance of discrepancies between methods.

Results: Total imprecision using the normal control for the V8 was 0.9% for albumin at 4.0 g/dL, 6.3% for alpha-1 at 0.32 g/dL, 2.9% for alpha-2 at 0.50 g/dL, 1.9% for beta-1 at 0.51 g/dL, 4.9% for beta-2 at 0.21 g/dL, and 1.9% for gamma at 0.88 g/dL. Total imprecision for the CAPILLARYS2 was 1.0% for albumin at 4.0 g/dL, 2.7% for alpha-1 at 0.26 g/dL, 4.9% for alpha-2 at 0.58 g/dL, 5.6% for beta-1 at 0.40 g/dL, 5.7% for beta-2 at 0.24 g/dL, and 1.7% for gamma at 0.93 g/dL. There were 39 abnormal samples detected by SPIFE3000. The sensitivity and specificity of the V8 SPE method to detect abnormalities was 82% and 98% respectively. In comparison, the CAPILLARYS2 SPE method had 95% sensitivity and 80% specificity. The V8 and CAPILLARYS2 were not significantly different at detecting abnormalities ($P>0.05$ using Fisher's Exact Test). Chart review revealed that the individual abnormalities misclassified by the capillary methods were not clinically significant. For accuracy of monoclonal protein characterization the V8 was significantly ($P<0.002$) more accurate at delineating the absence of abnormalities than the CAPILLARYS2. Both systems showed excellent agreement with IFE at identifying IgG monoclonal proteins. However, both capillary methods yielded mixed results with low concentration (0.3 g/dL) IgA, IgM and free light chains. Upon chart review, it was unlikely that any of these patients would have been impacted clinically.

Conclusion: Collectively, the Helena V8 system is substantially equivalent to the Sebia CAPILLARYS2. While the V8 was significantly better at characterizing normal (by IFE) samples, the methods showed no statistical or clinically significant difference in identifying abnormal samples. Both methods were less effective than agarose gel IFE at characterizing IgA, IgM, and free light chain abnormalities.

D-48

Cerebrospinal Fluid Leak Detection by High Resolution Gel Beta2-Transferrin Immunofixation

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Background and Objectives: Cerebrospinal fluid (CSF) leaks are potentially life-threatening conditions that can occur spontaneously, or as a result of laceration, blunt trauma, or surgery. CSF leaks can be diagnosed by surgical exploration, imaging or biochemical analysis of fluid samples. The most commonly used approach is measurement of β2-transferrin, which can be identified using protein electrophoresis. β2-transferrin is a desialated form of transferrin, which is post-translationally modified in the CNS and largely specific to CSF. Another less commonly available test is β-trace protein quantitation using immunoassay. β-trace protein is synthesized in the choroid plexus and is a useful marker of CSF leak. The objectives of this study were to evaluate a new immunofixation-based β2-transferrin test for detection of CSF leaks and to compare it to traditional gel electrophoresis and β-trace protein immunoassay.

Methods: For the method comparison study, 63 consecutive samples from physician-ordered β2-transferrin tests were collected and analyzed using two different electrophoresis methods, high resolution agarose gel fractionation followed by acid-violet staining (Sebia, USA), and high resolution agarose gel electrophoresis followed by β2-transferrin immunofixation (Sebia, USA). A subset of samples (n=16) with sufficient volume (>400 µL) were analyzed for β-trace protein (Siemens, USA). The results of each test were compared against patient chart data as the gold standard for the presence of a CSF leak. Additional studies were performed to assess the analytical performance of the β2-transferrin immunofixation test. To evaluate stability, a pool was generated using 5 fresh CSF specimens ordered for cell counts. The stability of the β2-transferrin isoform in the pool was tested over 7 days at room temperature (RT), 4°C, and -20°C. For analytical specificity studies, non-CSF fluids were collected from patients and volunteers. Samples included nasal drainage, serum, saliva, and whole blood. Sera from 5 patients with alcohol dependence were also collected to assess the effect of carbohydrate deficient transferrin on

the immunofixation test. The lowest concentration of visual detection was determined by serial dilution of the CSF pool using saline.

Results: The β2-transferrin immunofixation test had a sensitivity of 100% (40/40) and specificity of 71% (12/17) for detection of CSF leaks. By comparison, the agarose gel test had a sensitivity of 87% (35/40) and specificity of 94% (16/17). ROC curve analysis was used to establish a cutoff of 4.13 mg/L for the β-trace protein. At this cutoff, β-trace protein had a sensitivity of 100% (10/10) and specificity of 86% (5/6). By immunofixation, β2-transferrin in pooled CSF samples was stable over the course of 7 days at -20°C, 4°C, and RT. Body fluid samples that should not contain β2-transferrin were negative, with the exception of whole blood samples which appeared positive at a threshold of ~400 mg/dL hemoglobin. Sera from patients with alcohol dependence were negative for β2-transferrin by immunofixation. β2-transferrin was detectable in pooled CSF specimens at a dilution of 1:25.

Conclusions: Both the new immunofixation test for β2-transferrin and the β-trace protein were highly effective means of detecting CSF leaks. Users of the β2-transferrin immunofixation test should be cautioned against interpreting samples with blood contamination.

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Comparison of two Immunoturbidimetric methods for the measurement of Glycated Hemoglobin A1c

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Background: Hemoblobin A1c is the most important parameter for monitoring the metabolic control of patients with Diabetes Mellitus.

Given the need to optimize the process to measure HBA1c working with a biosafe method, an immunoturbidimetric assay in whole blood was compared with the currently used in our laboratory, performed with hemolized whole blood.**Objective:** to verify that both methods are statistically comparable without affecting the clinical interpretation of the result.

Materials and Methods: 70 samples of ambulatory diabetic patients were processed across the measurement range and medical decision levels: 5.9% and 7.0%. Two methods were compared : Tina-quant A1c Gen 2(® Roche) on a Cobas 6000 c501(whole blood samples) and Tina-quant A1c (® Roche) on Modular P (hemolized whole blood)

The performance of the comparison method (accuracy and veracity) was ascertained on a previous study through the EP15-A2 protocol CLSI. Deming linear regression (Alternate Comparison Method -EP Evaluator) was used to compare both methods. The criteria applied to verify that two methods are statistically comparable were: correlation coefficient(R) > 0.975 (indicating that the distribution of data is suitable); the confidence interval (CI) of 95% of the slope must include number one, the CI of the intercept must include number zero and there must not be any significant difference in the medical decision levels.

Results: Slope: 0.992 (CI 95% :0.981 to 1.003) ; Intercept: 0,058 (CI 95%: -0,023 to 0,138) ; R: 0,9989.Medical decision levels :

Tina quant A1c Modular P	Tina quant A1c Gen2 Cobas 6000 c501	95 % Confidence Interval
5.9	5.91	5.89 - 5.93
7.0	7.0	7.0 - 7.0

Conclusions: the results show an adequate distribution of the evaluated data. Both methods are comparable and there were no significant differences in the medical decision levels. Taking into account the optimization of the process, a decrease in the analytical error due to the pre treatment and the improvement in biosafety, the implementation of the method was accepted

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Heart Type Fatty Acid-Binding Protein levels are correlated with severity, outcome and mortality after acute stroke

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Background: Heart-Type-Fatty-Acid-Binding-Protein (hFABP) is an intracellular molecule engaged in the transport of fatty acids through myocardial cytoplasm and has been used as a rapid marker of myocardial infarction. hFABP is also expressed in the brain, although in lower concentrations than in the heart. Recent preliminary studies also investigated the usefulness of hFABP for the diagnosis and severity of acute stroke with contradictory results. Our aim was to determine whether hFABP levels are correlated with severity, outcome and mortality after acute stroke.

Methods: In a prospective study, we included 100 patients with acute stroke (75 with ischemic strokes and 25 with intracerebral hemorrhages). hFABP levels were measured at the time of admission and at 24, 48 and 72 hours thereafter. A final measurement was performed on day 7. Stroke severity was measured at the time of admission with the Scandinavian Stroke Scale (SSS). Functional outcome was measured with the modified Rankin scale (mRS) on day-7. Patients categorized into 3 severity groups according to mRS score: mild (mRS-score 0-2), moderate (mRS-score 3-4) and severe (mRS-score 5-6). hFABP was measured in EDTA plasma samples using biochip array technology on the Evidence Investigator analyzer (Randox laboratories, Crumlin, UK).

Results: hFABP median values and statistical significance are summarized in the table below. The mean age (±SD) of the patients was 75.2 (±9.4) years. Forty-two patients (42%) died during a follow-up period of 1 year. The mean time (±SD) between the onset of neurological symptoms and hospital admission was 3.22 (±1.58) hours.

Conclusions: Even from the baseline measurement levels are significantly higher in non-survivors and among the severe stroke group. Our results indicate that there is an association of low levels of hFABP with better outcome and lower mortality in acute stroke patients.

	baseline Median(IR) ng/ml	24_hours Median(IR) ng/ml	48_hours Median(IR) ng/ml	72_hours Median(IR) ng/ml	Day_7 Median(IR) ng/ml	*Difference (p*)*
Survivors	3.49 (3.40)	3.50 (2.99)	3.48 (3.82)	3.57 (4.45)	3.22 (2.79)	*Ns*
Non-survivors	4.73 (4.32)	6.54 (6.02)	7.35 (4.48)	7.49 (8.00)	6.98 (8.87)	*Ns*
Difference (p)*	*p<0.05*	*p<0.0001*	*p<0.0001*	*p<0.0001*	*p<0.0001*	
Mild	3.44 (2.92)	3.46 (2.58)	3.55 (2.72)	3.57 (4.41)	3.35 (2.46)	*Ns*
Moderate	3.89 (5.61)	5.12 (4.40)	6.00 (5.90)	4.70 (3.64)	3.78 (2.49)	*Ns*
Severe	4.79 (9.05)	6.75 (8.38)	7.56 (5.90)	8.12 (8.45)	6.95 (7.05)	*Ns*
Difference (p)*	*p<0.005*	*p<0.0001*	*p<0.0001*	*p<0.0005*	*p<0.,0005*	

* *Kruskall-Wallis-test*

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Development of a rapid, microplate-based kinetic assay for quantifying adenosine deaminase in body fluids

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Background: Adenosine deaminase (ADA) irreversibly catalyzes the deamination of adenosine into inosine and ammonia. The activity of ADA in peritoneal, pleural, or cerebrospinal fluids has clinical value in the assessment of patients with suspected tuberculous peritonitis, pleuritis, or meningitis, respectively. Our laboratory utilizes a calibrated, end-point assay to quantify ADA activity in body fluids. The lack of a certified reference material for assay calibration, a 60 minute reaction time, and the use of single cuvettes prompted us to develop a more accurate and rapid high-throughput analytical method for determining ADA activity in body fluids.

Objective: To develop and validate a rapid, microplate-based kinetic assay to quantify the ADA activity in peritoneal, pleural, and cerebrospinal fluids.

Methods: The conversion of adenosine to inosine catalyzed by ADA was monitored as a continuous decrease in absorbance at 265 nm over time. Reactions were performed in disposable 96-well ultraviolet-transparent microplates sealed with an ultraviolet-transparent film with absorbance normalized to a 1 cm path length. The molar extinction coefficients of adenosine and inosine as well as the Michaelis constant (Km) and maximum reaction velocity (Vmax) were determined using classic techniques. Accuracy, linearity, imprecision, analytical sensitivity, and enzyme stability at 4°C was established for each body fluid type using residual samples sent to ARUP Laboratories. Reference intervals were established from 120 tuberculosis-negative samples of each body fluid type.

Results: Molar extinction coefficients at 265 nm were 12,680 and 4,917 $M^{-1}cm^{-1}$ for adenosine and inosine, respectively, with their difference (7,763 $M^{-1}cm^{-1}$) used to calculate ADA activity. Km was 0.024 mM and Vmax was 0.002 ΔA/min. A reaction time of 15 minutes at room temperature was identified as providing reliable results. Accuracy was evaluated by adding ADA to a pool of each body fluid and calculating recovery. Recoveries at 12.0-24.3 U/L were between 96-110% for each fluid type. Linearity was determined for each body fluid type by adding ADA, performing serial dilutions, and testing each sample in triplicate. The assay was linear to 40 U/L for all fluid types. Within day and total precision were determined by testing two ADA pools of each body fluid type in duplicate for 20 days. For all fluid types, maximum within

day imprecision was <5 and 10% and maximum total imprecision was <9 and 19% at 20 and 7 U/L, respectively. Analytical sensitivity was 1.1 U/L as determined from the mean plus 3 standard deviations of 10 replicates of the substrate in the absence of ADA. Enzyme stability was determined from pooled samples of each body fluid type at two ADA activities. ADA activity was within 10% of baseline for at least 7 days kept at 4°C for each fluid type. Reference intervals were determined to be <1.1, <10.0, and <5.0 U/L for CSF, pleural, and peritoneal fluids, respectively.

Conclusions: The kinetic ADA assay has favorable performance characteristics. Compared to a calibrated, 60 minute end-point assay, the microplate-based kinetic ADA assay eliminates the calibration requirement, can be performed in 75% less time, and allows multiple samples to be tested simultaneously.

D-53

Liver protein profiling at an early stage in a rat model of diet induced non-alcoholic fatty liver disease.

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Background: Fatty liver is now considered to be one of the main contributing factors to cryptogenic cirrhosis of the liver. The etiology and development of fatty liver is not well understood from the early stages of disease. Animal models of fatty liver disease are valuable tools that can be used to investigate the pathogenesis of diet induced fatty liver disease or non-alcoholic fatty liver disease (NAFLD). In this study we used a diet induced obesity model in Wistar Rats.

Methods: Nulliparous Wistar rats (N=10) were mated and allowed to deliver normally. Litters were culled to 12 retaining 6 male and 6 female pups. At weaning (postnatal day 21), male and female pups from each litter were randomly assigned to a control diet or high fat diet (3 pups of each gender per diet). The high fat diet was selected to simulate the typical western high fat diet. At 7, weeks of age, two male and two female pups from each litter, one from each diet group were randomly selected for necropsy. Histology was assessed by the Brunt scoring system. A subset of the study population was randomly selected from the female pairs for proteomic evaluation of the liver samples. The animals were weighed bi-weekly and at necropsy. The intra-abdominal fat pads were weighed at necropsy, blood was collected and the liver was sampled for histology and proteomics (N=8). Proteomic analysis of the liver was accomplished using difference in gel electrophoresis, a method which uses 2D-SDS-PAGE to determine the relative abundance of over 2000 different protein spots. Protein concentration was determined after homogenization, and was used for normalizing samples. A significant change in protein abundance being defined as greater than 1.5 fold ($p < 0.05$). Western blotting for 78 kDa glucose regulated protein (GRP78; an ER-stress marker) and apolipoprotein B-100 was also performed on the liver samples.

Results: The body weights ($P = 0.006$) and abdominal fat pad weights ($p=0.003$) were significantly different between the high fat and normal fat diet. Histological changes (presence of macrovesicular fat in hepatocytes) were also identified ($p=0.08$). No fibrosis or inflammation was identified by histology. We identified 1277 individual spots with proteomics but found no significant differences between the groups. By western blotting GRP78 and apolipoprotein B-100 were found to be non-significantly increased ($p > 0.05$).

Conclusion: Although there were changes in body fat and histological changes in the liver after a 4 week exposure to a high fat diet, protein differences in the liver were not detectable. This may be due to the liver continuing to maintain homeostasis of proteins involved in fat metabolism. The changes in GRP78 and apolipoproteinB-100 suggest that there are some functional changes in the liver in the experimental group. The small sample size submitted for proteomics is a limitation of this study. Further work is required to characterize these subtle differences seen in these early stages of NAFLD so that we can identify potential tipping points that indicate progression to a metabolically detectable disease state.

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Ortho Clinical Diagnostics VITROS® Wide Range CRP (wrCRP) Assay

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Background: C-reactive protein is an acute-phase reactant that can be used in the detection and evaluation of infection, tissue injury, and inflammatory disorders. The current MicroSlide VITROS Chemistry Products CRP assay has an upper reporting range limit of 90 mg/L. We have developed the VITROS Chemistry Products wrCRP Reagent that can be used to detect C-reactive protein levels up to 300 mg/L without dilution.

Methods: The performance of the VITROS Chemistry Products wrCRP Assay was assessed on the VITROS 5600 Integrated System using CLSI EP6 for linearity and 10 day CLSI EP5 for precision. Method comparison studies followed CLSI EP9 and used both the VITROS Chemistry Products MicroSlide assay and the Siemens CardioPhase hsCRP assay run on the Siemens Behring ProSpec II as comparative methods.

Results: The VITROS Chemistry Products wrCRP assay produced acceptable linearity between 1mg/L and 300 mg/L. The assay is precise across its reportable range.

Conc. (mg/L)	1.7	10.1	17.1	73.3	230
Within run %CV	2.5	2.6	2.1	2.0	3.2
Within lab %CV	4.2	3.2	2.4	2.2	3.5

Method comparison studies demonstrate acceptable agreement with both the Behring ProSpec CRP Assay and the VITROS Chemistry Products CRP Assay (MicroSlide CRP) :

VITROS wrCRP (V5600) = 0.996 (ProSpec CRP) + 2.36 with a correlation coefficient of 0.974.

VITROS wrCRP (V5600) = 0.98 (MicroSlide CRP) - 2.76 with a correlation coefficient of 0.966

Conclusions: The VITROS Chemistry Products wrCRP Assay exhibits acceptable precision and accuracy across the reportable range of 1 mg/L to 300 mg/L.

D-55

Critical values and performance characteristics of the NMDA receptor peptide for assessment of acute ischemic stroke

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Biomarkers for acute ischemic stroke (IS) may help to diagnose and rapidly triage stroke patients for appropriate treatment, allow to assess clinical outcome and may predict the risk of complications. The NR2 peptide, a proteolytic breakdown product of the brain specific NMDA receptor, is rapidly released into the bloodstream during acute cerebral ischemia and can be used as a marker for acute IS. The study goals were (1) to assess the performance characteristics and critical values of the NR2 peptide in patients with acute IS presenting within 24h of symptom onset and (2) to correlate NR2 peptide levels with neurological status and results of advanced neuroimaging studies (brain MRI including DWI/PWI mismatch and perfusion CT), performed at initial presentation.

Methods: Cohort study of 152 study participants (n=50 acute IS, n=102 controls). A blinded investigator performed the NR2 peptide assays according to the manufacturer's manual (Gold Dot Test, CIS Biotech, Inc., Atlanta, GA). NR2 peptide concentrations in plasma were determined by plotting their absorbance values on a calibration curve constructed from the absorbance units of each calibrator and their known concentrations.

Results: Healthy controls and individuals with vascular risk factors had NR2 peptide concentrations below 0.25 and 0.4 µg/L, respectively, while those with stroke mimics had mean concentrations of 0.5 µg/L. Acute IS patients had significantly higher NR2 peptide levels compared to all control groups (ANOVA, $p<0.0001$). Test sensitivity for IS was 98% with a predictive value of 78% at a cutoff point of 0.5 µg/l. A significant correlation ($r_s=0.8$) between NR2 peptide levels and cortical lesion size (< 200 cc) was found.

Conclusion: The NR2 peptide is a sensitive and specific biomarker for the diagnosis of IS which can differentiate acute IS from stroke mimics. Increased NR2 peptide levels of > 0.5 µg/l can identify patients with acute IS within 24 h of symptom onset and correlate with findings on neuroimaging studies.

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Plasma Neutrophil Gelatinase-Associated Lipocalin (NGAL) Can Be a Biomarker for Diabetic Nephropathy

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Background: Neutrophil gelatinase-associated lipocalin (NGAL) is a recently introduced renal biomarker whose increase suggests tubular injury. Diabetic nephropathy, a leading cause of end-stage renal disease, causes typical changes in the

kidney that are characterized by glomerulosclerosis and eventual tubular damage. In the present study, we attempt to validate the usefulness of plasma NGAL (pNGAL) as a biomarker for detecting diabetic nephropathy.

Methods: The plasma NGAL level of 145 type 2 diabetes mellitus patients was measured using fluorescent immunoassay along with the Triage NGAL test (Biosite, San Diego, CA). The patients were divided into 3 groups according to urinary albumin excretion (UAE) and estimated glomerular filtration rate (eGFR), and the pNGAL differences among each group were analyzed. The serum creatinine concentration and eGFR were also compared with the pNGAL level.

Results: The subjects comprised 91 men and 54 women with the median age of 58 years (28-82 years). The mean pNGAL level was 70.2 ± 30.83 ng/mL. The pNGAL level was significantly increased in patients with severe albuminuria (67.0 ± 19.26 ng/mL for 123 normoalbuminuric, 81.3 ± 55.02 ng/mL for 19 microalbuminuric, and 130.0 ± 106.86 ng/mL for 3 macroalbuminuric patients, $P < 0.001$) and patients with decreased glomerular function (68.4 ± 27.63 ng/mL for 129 patients with eGFR above 60 mL/min/1.73 m^2, 72.9 ± 19.35 ng/mL for 15 patients with eGFR between 30 and 60 mL/min/1.73 m^2, and 253 ng/mL for a patient with eGFR less than 30 mL/min/1.73 m^2, $P < 0.001$). Further, pNGAL was found to be positively correlated with the serum creatinine concentration ($r = 0.460$; $P < 0.001$) and inversely correlated with eGFR ($r = -0.405$; $P < 0.001$).

Conclusions: The pNGAL concentration was increased in diabetic patients and was significantly correlated with the markers for renal damage. This study demonstrates that pNGAL might be a useful and practical biomarker for monitoring the extent of renal impairment in diabetic patients.

Keywords: *NGAL, diabetic nephropathy*

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Plasma Matrix Gla Protein and Gas6 levels in patients with coronary artery disease

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Background: Atherosclerosis is one of the most important factors that play a role in the pathologies of myocardial infarction, stroke, coronary and peripheral artery diseases. Although there have been many studies about atherosclerosis, the risk factors and the causes of atherosclerosis have not yet been explained clearly. Atherosclerosis is commonly characterized as a lipid accumulation, inflammation and calcification of large and/or mid-sized blood vessels. Matrix Gla Protein (MGP) is a vitamin K dependent protein which is produced in cartilage, bone, heart and blood vessels. It's shown by biochemical and genetic studies that MGP is an inhibiting factor of the calcification of cartilage and blood vessel wall. Another vitamin K dependent protein, Growth Arrest Specific 6 (Gas6), has functions such as keeping the cells alive, phagocytosis of apoptotic cells, resorption of bone, induction of the cell migration and cell to cell communication. Some Gas6 knock-out animal studies have shown that mice are protected against venous and arterial calcification. The objective of this study is to find out if there is any relation between coronary artery diseases and serum MGP, and also plasma Gas6 levels.

Methods: We collected blood samples from 42 patients (38 male, 4 female) on the same day before their coronary by-pass operation. Also, citrated blood samples of 42 healthy volunteer (38 male, 4 female) were collected as the control group. Control group patients were selected from non-smoking people who do not have a chronic or an active acute disease, and not using any drugs for 7 days. Plasma MGP levels were assayed by ELISA kit (Biomedica, Austria). Plasma Gas6 levels were assayed by ELISA kit (R&D) which were optimized and validated in our laboratory. We have used Unpaired T test for MGP levels and Mann-Whitney U test for Gas6 levels. $P<0.05$ has been taken as statistically significant.

Results: The results have shown that plasma MGP levels of coronary artery patients (18.22±5.36 nM) were not significantly different (P=0.61) with the control group (17.40±4.75 nM). Plasma Gas6 levels of the patients with coronary artery disease (4.47 ng/mL, min: 4.40, max: 18.55) were found to be significantly decreased ($P<0.001$) when we compare them with the control group (7.62 ng/mL, min: 2.13, max: 17.99).

Conclusion: The preliminary results show that there is a relation between coronary artery disease and plasma Gas6 levels where there is no significant correlation between MGP and coronary artery disease. This study gave us a brief overview on the relation between coronary artery diseases and vitamin K dependent proteins, Gas6 and MGP. Further studies will provide more trustable information from a large population based project on the relation between coronary artery diseases and vitamin K dependent proteins.

D-58

Development of a nephelometric immunoassay for the measurement of serum secretory IgA and its use for the assessment of patients with primary biliary cirrhosis

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Secretory IgA (SIgA) is the main immunoglobulin found in mucous secretions where it participates in immunological protection of these surfaces. Minute amounts of SIgA have been found in normal serum (range 1-10 mg L^{-1}). There is evidence that damage (including cancer) to the liver, pancreas and digestive tract can result in increased serum levels of SIgA. Thus, there is the possibility of a diagnostic utility for the measurement of serum SIgA in liver diseases. Primary biliary cirrhosis (PBC) is a chronic and progressive autoimmune disease resulting in destruction of the biliary duct-tree and ultimately liver cirrhosis.

Specific antibodies have been raised that recognise SIgA and secretory component but not other serum proteins. These have been used to develop automated, turbimetric immunoassays on the SPA PLUS™ analyser for the quantification of SIgA in serum. The main assay characteristics are summarised below:

Range (mg/L)	1 - 60
Sample dilution	1/10
Min. sample dilution	1/1
Sensitivity (mg/L)	1
Assay time (min)	15
Linearity	y=1.163x-1.424 R2 = 0.997
Intra assay precision % CV (n=15)	2.03%

The assay has been standardised with a polyclonal reference protein purified from human milk. The preparation was greater than 99% pure by silver-stained SDS-PAGE. The concentration was determined by absorbance at A280nm and BCA protein assay. SIgA concentrations were measured in 49 normal (blood donor) sera; median 5.77mg/L (range 0.65mg/L - 11.44mg/L). SIgA concentrations were also measured in 93 patients expressing M2 antigen (PBC biomarker, confirmed by histopathology) collected from the Department of Immunity and Infection, University of Birmingham, UK; median 14.46mg/L (range 1.25mg/L - 152.20mg/L). SIgA was shown to be significantly (Mann-Whitney $P<0.0001$) elevated in this PBC population. There was weak correlation with normal serum IgA levels in these patients (Spearman coefficient =0.347, P=0.0006). The assay data was supported by western blot staining for 5 normal and 5 M2-patient samples. We conclude that this assay provides a rapid and precise method for quantifying SIgA in serum. This may provide a sensitive method for detecting and monitoring liver disease.

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High resolution capillary zone electrophoresis (CZE-HR) for serum proteins: Clinical uses and reference values

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Background: High resolution capillary zone electrophoresis (CZE-HR) for serum protein electrophoresis (SPE) allows resolution of many proteins in 8 fractions and their relative quantification. We illustrate CZE-HR electrophoretic patterns in different clinical pathologies and their specific protein values with relevant laboratory data. We establish reference values for each of the 8 protein fractions of CZE-HR SPE electrophoregram.

Methods: This study was conducted on Sebia Capillarys 2 with high resolution buffer and protein detection by UV absorbance at 200 nm. First, temporary reference values were determined with selected normal patient samples (n=95) as defined by capillary Paragon CZE electrophoresis, run the same day (Beckman Coulter, clinical biochemist interpretation) and by specific proteins results. Alpha-1-acid glycoprotein, alpha-1-antitrypsin, haptoglobin, alpha-2-macroglobulin, CRP, complement C3 and C4 were determined by nephelometry; transferrin by turbidimetry and albumin

by BCP. Patients with CRP > 8 mg/L were excluded. Those reference values were implemented for routine SPE analysis. Second, definitive reference values were determined retrospectively 1,5 years post-implementation, selecting an 8-month interval for selection of adult (> 20y) outpatients with SPE interpretation within normal limits, normal total protein concentration (64 to 83 g/L), after exclusion of outliers (> ±3SD) for each fraction of the electrophoregram (n=942, 44% male/56% female, mean and median of 53 years). The Boothstrap nonparametric (n-1) procedure has been used for both studies.

Results: By CZE-HR SPE, acute inflammatory state is clearly characterized by increased inflammatory proteins (AAG, AAT, Haptoglobin and C3) and decreased albumin and transferrin. We illustrate clear electrophoretic resolution of haptoglobin from alpha-2-macroglobulin allowing discrimination between acute inflammatory state, nephrotic syndrome, intravascular haemolysis and in vitro haemolysis. Polyclonal hyper-IgA is clearly identified as well as transferrin increase or decrease. Definitive reference values (in %) for each of 8 fractions were established as follow: Albumin (59,4 to 71,9), AAG (0,3 to 1,3), AAT (1,1 to 2,8), haptoglobin (1,5 to 5,9), alpha-2-macroglobulin (3,0 to 5,7), transferrin (4,8 to 7,6), complement & IgA (2,9 to 6,1) and gammaglobulins (8,9 to 18,0). CZE-HR SPE fraction reference intervals were greater than those for corresponding specific proteins as established for nephelometric/turbidimetric methods because of co-migration of low abundant proteins, except for alpha-1-antitrypsin (similar) and alpha-1-acid glycoprotein (lower).

Conclusion: CZE-HR SPE reflects and differentiates more accurately some clinical conditions while allowing resolution and direct measurement of many specific proteins. Availability of this new high resolution technology on a daily basis improves the diagnostic information and clinical usefulness of first line laboratory services.

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Plasma Gas6 levels in diabetic nephropathy

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Background: Growth Arrest-Specific 6 (Gas6) is a vitamin K-dependent growth factor for mesangial cells that plays a key role in the progression of various kidney diseases. Diabetes mellitus is the most important cause of end-stage renal diseases in many countries. Diabetic nephropathy is the leading cause of end-stage renal disease. With a worldwide increase in diabetes, it is inevitable that diabetic nephropathy will also become a major issue in the future. Diabetic renal disease is underdiagnosed and undertreated even now, when detection of the early stages is simple through routinely available laboratory testing. The mechanism of diabetic nephropathy, however, remains incompletely understood. A recent series of studies in animal models has revealed the role of Gas6 and its receptor Axl in the progression of acute and chronic glomerulonephritis, diabetic nephropathy and chronic allograft rejection. Because Gas6 correlated well with kidney dysfunction, we sought to evaluate whether it could be used as a marker for diabetic nephropathy in human. The objective of this study is to investigate if plasma Gas6 levels correlates with microalbuminuria in diabetic patients.

Methods: In this study, 32 diabetic patients with nephropathy, 34 diabetic patients without nephropathy and 34 healthy control subjects were recruited as three comparing groups. Plasma Gas6 levels were determined by ELISA kit from R&D Systems. Whole blood samples for hemoglobin A1c (HbA1c), serum samples for C reactive protein (CRP), plasma samples for fibrinogen; 24 hour urine samples for microalbuminuria were analyzed by Primus PDQ, Beckman Coulter Immage 800, STA Compact, Roche Cobas Integra 800 Analyzer, respectively. Statistical difference of Gas6 among three groups and correlation between Gas6 with microalbuminuria, CRP and fibrinogen was investigated by using SPSS (Statistical Package for Social Sciences) for Windows 17.0.

Results: There was statistically significant difference among three groups in terms of Gas6 when Kruskal Wallis Test was used (P=0.035). Gas6 plasma levels were higher in diabetic patients with nephropathy (23.5±10.1 ng/mL) compared to those with diabetic patients without nephropathy (18.5±6.6 ng/mL). Also, there was a significant positive correlation between Gas6 and microalbuminuria (p=0.003); between Gas6 and HbA1c (P<0.01) by Spearman correlation analyzing. Moreover, the correlations between microalbuminuria and CRP (P<0.05), microalbuminuria and fibrinogen (P<0.001), microalbuminuria and HbA1c (P<0.05) were significant.

Conclusion: We found positive correlation between Gas6 and microalbuminuria. Microalbuminuria is a clinically useful diagnostic marker for predicting future overt nephropathy, but both sensitivity and specificity of microalbuminuria are not high enough for detecting the initial stage of nephropathy. Gas6 might be an earlier diagnostic marker in diabetic nephropathy. Plasma Gas6 levels in an early phase of diabetes can predict the future development of the complication of nephropathy in diabetic patients. It needs further investigations to elicit the pathogenesis of diabetic nephropathy.

D-61

Development and Validation of an Immunoassay for the Quantitation of Progranulin in Plasma

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Background: Progranulin, also known as PC cell-derived growth factor is an 88 kDa secreted glycoprotein. This growth factor has been implicated in a variety of physiological and pathological roles including diseases such as cancer and frontotemporal lobar degeneration (FTLD). Null mutations in the progranulin gene (*GRN)*, which result in decreased translation of the protein, have been linked with frontotemporal dementia. Because molecular testing is relatively expensive and given that recent studies have shown a strong correlation between progranulin levels and FTLD status, reduced protein expression may offer a useful screening tool for differentiating between Alzheimer's disease and FTLD.

Objective: To develop a sandwich immunoassay for the determination of progranulin levels in plasma.

Methods: A mouse monoclonal and a rabbit polyclonal antibody are used in the progranulin assay. The monoclonal antibody (0.5µg antibody per well) is immobilized to high-binding, flat-bottom 8-well strips for 14-16 hours at 4°C. The wells are then washed and blocked with Superblock® for one hour at room temperature. Patient plasma, recombinant standards and controls are added and incubated with agitation for two hours at room temperature followed by a washing step. An acridinium ester labeled polyclonal antibody is added to each well and is allowed to incubate with agitation for 2 hours at room temperature. The wells are then washed and analyzed. The resulting chemiluminescent signal is directly proportional to the concentration of progranulin in the samples.

Results: The specimen of choice was EDTA plasma. Progranulin was stable refrigerated for 7 days or frozen at -20°C with up to 3 freeze/thaw cycles. Limit of quantitation was 4 ng/mL with a CV of 9.2% (n=20). The limit of detection of the assay was 1.2 ng/mL and the critical limit was 0.43 ng/mL. The analytical measurable range of the assay was 4 to 100 ng/mL Intra-assay imprecision (CV) was found to be 5-9% (n=20) at four different levels of progranulin ranging from 17-92 ng/mL. Inter-assay imprecision was found to be 13-16% (n=20). Linearity of patient plasma samples ranged from 80.7-118.1% with a mean value of 101%.

Conclusions: We have developed and validated an immunoassay for the quantitation of plasma progranulin levels. This assay could be used as an inexpensive screening tool in patients presenting with early-onset dementia to identify FTLD patients who will benefit from progranulin genetic testing.

Wednesday PM, July 27

Poster Session: 2:00 pm - 4:30 pm
Technology/Design Development

D-62

Study for basic performance of automated glucose analyzer GA09 model

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Introduction: Other than automated chemistry analyzers, bench-top glucose analyzers with GOD electrodes are used to measure blood glucose level. Currently in Japan, there are 2 companies that provide such automated bench-top glucose analyzers which differ in features: GA09 based on GOD immobilized O2 electrode by A&T Corporation, and GA1170 based on GOD immobilized H2O2 electrode by ARKRAY Corporation.

The newly developed GA09 by A&T maintains high accuracy and stability with its measurement principal while enabling non-centrifuged whole blood samples to be tested. Glucose measurement in our laboratory is performed promptly after whole blood samples are collected by the automated glucose analyzer GA1170 (ARAKRAY Corporation). In this study, we examined the basic performance of automated glucose analyzer GA09, in comparison with GA1170.

Methods: All tests were performed with GA09 analyzer with the appropriate GA09 reagent kits. Whole blood and plasma samples were tested on the GA09 and GA1170. The correlation between GA09 and GA1170, reproducibility, stability calibration and the effect of hematocrit (Hct) were evaluated. In correlation testing, glucose concentration (mg/dL) of whole blood and plasma samples was measured by GA09 and GA1170.

Results: Reproducibility of CV 0.51% (mean 87.7 mg/dL) and CV 0.41% (mean 212.5 mg/dL) were shown by two whole blood samples, and CV 0.48% (mean 87.5 mg/dL) and CV 0.33% (mean 199.7 mg/dL) were shown by two plasma samples, each measured consecutively for 10 times. The correlation between GA09 and GA1170 was Y=1.001X+4.303 and the correlation coefficient was 0.998. Moreover, the correlation between whole blood and plasma samples which were measured by GA09 was Y=0.994X+2.959 and the correlation coefficient was 0.998. In stability calibration test, there was no additional need for calibration up to 1 hour with GA09. The percentages of Hct sample which could be measured by GA09, 0-60%, were also measured using the adjusted whole blood.

Discussion: As a result, GA09 was good in its basic performance, and also showed good correlation with the conventional method (GA1170). Because GA09 was developed based on the principle of GOD immobilized O2 electrode method, clear stability calibration and the accuracy were more excellent than those of the conventional model (GA1170). It is apparent that there is an advantage of high accuracy by introducing the GA09 which uses the electrode approach accepting whole blood. Moreover, the promptness from blood collection to result report promptly obtained in our clinical laboratory indicates the absence of centrifuge being a cost and space reduction. Since the GA09 method in emergency situations offers the convenience of obtaining rapid results at the patient bedside, it could also be said that GA09 is optimal as a point-of-care testing device.

Conclusion: In conclusion, GA09 was evaluated as a very useful analyzer for routine testing.

D-63

Novel method for ANA quantitation using IIF imaging system

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Objective A variety of antinuclear antibodies (ANA) are found in the sera of patients with rheumatic diseases. Detection of abnormal ANA titers is critical in diagnosing and evaluating the activity of systemic lupus erythematosus (SLE) and other connective tissue diseases. At present, two methods (IIF and ELISA) are applied in detecting ANA. Indirect fluorescence assay (IIF) on HEp-2 cells, the gold standard method, can determine the presence of ANA and provide information about the localization of the antigens which can be useful in diagnosis, while it has limited utility in diagnosis, prognosis and monitoring of disease activity due to the lack of standardization in performing the tests, subjectivity in interpreting the results and the fact that it is only semiquantitative. On the other hand, ELISA for detection of ANA can quantitate ANA but couldn't provide further information about the localization of the autoantigens. It would be better to integrate both of the quantitative and qualitative methods. To address this issue, this study was conducted to quantitatively detect ANAs by using IIF imaging analysis system.

Methods 1699 sera from patients with positive ANAs including various patterns such as speckled, homogeneous, nuclear-mixture and cytoplasmic-mixture patterns, were detected for ANA titers by the classical IIF. At the same time, the image of each sample was acquired by the digital Imaging System (SPOT32, American) and the green fluorescence intensity was quantified by the Image-Pro plus software (ipwin32, American). To estimate the reproducibility of the novel ANA quantitation method, 135 samples were quantitated for ANA in triplicates. For quality control of the novel ANA quantitation method, the controls supplied in ANA test kit were quantitated for ANA in parallel with patients' sera.

Results The fluorescence intensities of ANA measured by the novel ANA quantitation method were positively correlated to the ANA titers determined by the classical IIF method in all ANA patterns. The correlation coefficients (R^2) of various ANA patterns were all higher than 0.96 (speckled 0.9726, homogeneous 0.9655, nuclear-mixture 0.9698, cytoplasmic-mixture 0.9665). The fluorescence intensity measured by the novel ANA quantitation method was highly reproducible at various dilutions and has no statistic difference($P>0.05$). The quality controls were detected in parallel with patients' sera and showed good reproducibility with Mean±SD of 126.4±9.6 and CV 7.6%.

Conclusion A novel method for ANA quantitation using IIF imaging system was established in this study. The ANA intensities of patients sera measured by the novel ANA quantitation method were positively correlated to the ANA titers detected by the classical IIF method and showed good reproducibility. The novel ANA quantitation method can provide both the fluorescence intensities, which could precisely reflect the change of ANA levels in patient's sera, and the useful information on the localization of the antigens to clinicians in diagnosing and evaluating autoimmune disease.

D-64

The Optimization and Clinical Application of EGFR and K-RAS Mutation Detection in PlasmaWith the Combined Use Of Restriction Endonuclease Digestion Enrichment and DHPLC

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Background: The application of Blood-based approaches for the assessment of EGFR and KRAS mutations in non-small cell lung cancer and colorectal cancer patients play an important role in guiding the clinical use of EGFR-targeted therapy. The current study is designed to establish a sensitive and specific method for detection of EGFR and KRAS mutation in plasma based on restriction endonuclease digestion enrichment (REDE) and DHPLC.

Methods: Restriction endonucleases MseI, MscI, BstNIand BglIwere used to digest the wild type fragments of exon 19 and exon 21 of EGFR gene and code 12 and code 13 of KRAS gene respectively. The sensitivity was investigated by using plasmid standard substance. Afterwards, plasma and paraffin-embedded tissues of 120 non-small cell lung cancer patients and 120 colorectal cancer patients were detected by REDE-DHPLC. Conventional DHPLC and sequencing were used as control methods.

Results: The sensitivity of REDE-DHPLC for detecting mutations in four sites was 0.1% and that of conventional DHPLC was 1%. Plasmid standard substance that containing 0.1% mutation gene were detected by REDE-DHPLC for 2 times, results were all positive. The mutation rates of EGFR gene in plasma from 120 NSCLC patients detected by REDE-DHPLC, conventional DHPLC and sequencing methods were 27.5% ,16.7% and 12.5% respectively, and the mutation rates of KRAS in plasma from 120 colorectal cancer patients were 38.3%, 25.8% and 16.7% respectively. The mutation rates of EGFR and KRAS detected by REDE-DHPLC was significantly higher than conventional DHPLC(X^2 were 4.092 and 4.301,$P<0.05$) and sequencing method (X^2 were 8.438 and 14.127,$P<0.05$). In comparison with conventional DHPLC, the sensitivity of REMS-DHPLC for detecting EGFR exon 19 and 21 mutation were both 100% and specificity were 91.59% and 96.46%, the coincidence of the two methods were 92.50%(111/120, *Kappa*=0.702,$P<0.05$) and 96.67%(116/120, *Kappa*= 0.761,$P<0.05$).Besides, the sensitivity of REMS-DHPLC for detecting KRAS mutation was 100% and the specificity was 83.15%, the coincidence of the two methods was 87.50% (105/120, *Kappa*=0.718,$P< 0.05$). Consistency of EGFR and KRAS mutation status in plasma and tissues detected by REMS-DHPLC were 85.0%(34/40, *Kappa* =0.851,$P<0.05$)and 90.2 %(46/51, *Kappa* =0.914, $P<0.05$)respectively.

Conclusion: REMS-DHPLC is a high sensitive and specific method for somatic mutation detection in plasma, the results are easy to be identified and homozygous point mutation can also be detected, indicating its possible use in clinical laboratory

D-65

Rapid detection of Neisseria gonorrhoeae from urine with dry reagent of helicase-dependent isothermal amplification and simplified sample preparation

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Background: Neisseria gonorrhoeae (GC) is the pathogen to cause second most frequently reported sexually transmitted diseases in the United States. There is the medical need for point-of-care (POC) nucleic acid tests of Neisseria gonorrhoeae for rapid diagnostics, prompt treatments and preventions. However, there are several limitations for implement the molecular diagnostics at POC or decentralized laboratory: requirement of expensive instrument, labor extensive sample preparation procedure. In this research, we developed a molecular diagnostic assay (IsoAmp® GC) for rapid detection of Neisseria gonorrhoeae from urine. The assay is based on helicase-dependent amplification (HDA), an isothermal nucleic acid amplification technique, which does not require expensive instrument.

Methods:The urine samples were prepared by the following procedures: 0.5mL of urine was added to each 2mL urine collection tube, and spun down for 5 minutes with highest speed at benchtop centrifuge (>10k rpm). The supernatant was decanted, and 0.2mL of sample dilution buffer was added to the collection tube. The closed collection tube was heated at 95°C for 5 minutes and then spun-down again for 5 minutes with the same conditions as the first spin. And then 5 microliter of the supernatant was subjected to HDA amplification at 65°C for 30 minutes. The amplified products were detected by BEStTM cassette. If wet reagents were used for HDA amplification, the master mix was assembled by adding Enzyme mix to the Reaction mix tubes. If lyophilized reagents were used for HDA amplification, the master mix was prepared by adding lyophilization dilution buffer to the tubes with dry reagent. The dry reagents were prepared by VirTis Genesis from SP industries (Gardiner, NY).

Results:The IsoAmp® GC assay amplifies PorA gene of GC. In order to evaluate the assay specificity, inclusive (20 strains of GC from different clinical isolation) and exclusive study (34 species) were performed. It showed that the selected primers and probes are specific for GC detection, and the detection sensitivity can reach as low as 5 CFU or 50 copies per HDA reaction. The two-spin-based sample preparation method was developed and validated by pooled negative frozen urine samples and additional 58 clinical specimens (5 positives and 53 negatives) with wet reagents. In order to further simply the assay procedure and make the reagents available for ambient temperature storage and shipment, the formulation of lyophilized reagent was first developed. Accelerated stability test showed the developed formulation retained stable cake structure and intact reagent activities for up to 6 months at 40°C. The performance of the dry reagents was also evaluated by above sample preparation method and clinical samples. It indicates that HDA diagnostic reagents can be easily transported and stored at room temperature or non-frozen conditions for clinical application.

Conclusion: The IsoAmp® GC assay can detect GC from urine sample in around 1 hour. It offers clinical laboratories or POC sites with an alternative solution for rapid detection of GC from urine.

D-66

Development and Validation of a Parathyroid Hormone-Related Peptide (PTHrP) Immunoassay Using Novel Electrochemiluminescent Technology

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Background: The tumor-associated factor parathyroid hormone-related peptide (PTHrP) plays a key role in humoral hypercalcemia of malignancy and also localized osteolysis that occurs with metastatic cancer. Sharing similar N-terminal homology to parathyroid hormone (PTH), PTHrP exists as three initial translated peptide isoforms that undergo post-translational processing to generate several smaller bioactive secretory forms. We have developed a two-site immunoassay for the quantitative measurement of PTHrP in plasma using a novel plate based electrochemiluminescent detection technology (Meso Scale Discovery®, MSD).

Methods: The two-step reaction utilizes a pair of affinity-purified goat polyclonal antibodies raised against the fragments of human PTHrP. One of the pair of antibodies is immobilized on the carbon surface of the MSD 96-well plate acting as a capture antibody. The other antibody is conjugated with an electrochemiluminescent signal molecule (MSD SULFO-TAG™) to generate a signal antibody. EDTA plasma samples containing PTHrP are allowed to react in the capture antibody coated plate well. Non-PTHrP material is washed off and the PTHrP-bound plate is incubated with the signal antibody. After washing, bound PTHrP-complex emits light upon application of electrochemical stimulation initiated at the electrode surfaces of the microplate by the MSD instrument.

Results: The reportable range of the assay was from 0.7 to 70 pmol/L. The lower limit of detection was 0.07 pmol/L and the lower limit of quantitation was 0.7 pmol/L. Precision (%CV) was validated using three levels of PTHrP-spiked human plasma pools. Six replicates of each level were analyzed in three assay batches. The mean interassay CV at 1.5, 37 and 70 pmol/L was 4.9%, 6.9%, and 6.1% respectively. Dilutional linearity of three high PTHrP-spiked plasma samples diluted with the zero standard matrix produced mean recoveries of 111%-118%. Spiking high concentrations of structurally similar peptides (PTH Related Protein 1-34; 1-37; 140-173 and PTH 1-34; 1-84) did not cross react in the PTHrP assay. Method comparison with 23 plasma samples previously tested with a reference commercial PTHrP IRMA assay yielded the following regression characteristics: slope = 1.19, intercept = 1.72 pmol/L and r = 0.79. PTHrP stability was evaluated using PTHrP-spiked plasma pools. PTHrP was stable at ambient and refrigerated temperature for up to 4 hours. Stability was also confirmed after undergoing six freeze/thaw cycles.

Conclusions: The PTHrP MSD assay combines novel electrochemiluminescent technology with immunoassay performance that is comparable to a reference commercial PTHrP IRMA assay, and high assay sensitivity and precision.

D-67

Albumin Measurements by Bromcresol Green and Bromcresol Purple on the Siemens Dimension Vista

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Background: Albumin values are used to monitor renal disease morbidity. The availability of two common albumin assays employing bromcresol green (BCG) and bromcresol purple (BCP) led investigators to examine albumin recovery in dialysis patients. The NKF DOQI recommends using BCG because BCP has been shown to underestimate albumin concentration in hemodialysis patients even though BCP is known to be more specific for albumin. No previous studies have examined a BCG method on the same platforms or on Siemens Dimension instruments and not all studies have used the same patient samples for method comparisons. The Siemens Dimension Vista 500 analyzer uses BCP dye-binding reagent for albumin measurement in serum and plasma. This Vista platform allows users to configure assays using empty reagent flexes that can be filled with desired reagents in open channels and user defined parameters. The objectives of this study were to: establish BCG assay parameters for the Dimension Vista and to compare dialysis patient results using BCG and BCP on the same platform.

Methods: Siemens Dimension Vista 500 analyzer, manufacturer's defined BCP reagent (Siemens Healthcare Diagnostics) and Siemens Advia BCG reagent (XBCG) were used. The BCP method was used according to manufacturer's instructions. Assay parameters for XBCG follow. Equal dilutions of Vista Chem 4 calibrator were used for the XBCG calibration for a range of 1-11 g/dl albumin. 4 ul of serum or plasma is used with a total reaction volume of 133 ul. Total run time is 4 minutes with bichromatic detection at 600 and 700 nm. Additionally, 141 dialysis patient serum samples were analyzed to evaluate performance differences between the Vista BCP and XBCG assays on the Siemens Vista. Patient results were compared using a paired t-test and correlation coefficient.

Results: We defined and evaluated a BCG user method for the Dimension Vista using the Vista Chem 4 calibrator with Siemens Advia BCG Albumin reagent (designated XBCG). Calibration of XBCG yields a reproducible linear analytical measurement range of 1.0 - 10.0 g/dl with five calibrators and a logit data reduction. A two month calibration stability gives accurate quality control values and total precision with coefficient of variations of ~1.5 to 3.8% for each level of QC. These performance characteristics are comparable to those of the commercial BCP assay. The Siemens Advia reagent is ready to use and can be stored at room temperature before and after flex preparation. Multiple wells are filled from a single reagent bottle. The flexes are vented and filled with a syringe and sealed with tape. No significant differences were found between the two assays for dialysis patients by paired t-test (p=0) and correlation coefficient of 91.8%.

Conclusion: The XBCG method can be adopted by labs with Vista instruments who follow NKF DOQI guidelines for dialysis patient.

D-69

Comparison Of Architect I 2000 For Determination Of Scc With Imx

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Background: The SCC antigen, a tumor marker for squamous cell carcinoma, is already used for the diagnosis and follow-up of carcinoma of the cervix and the lungs. Serum concentrations of this marker correlate well with stage of disease, the presence or absence of risk factors, the effect of treatment, and the course of the disease.

Methods: The SCC concentration of 66 serum samples was determined using CMIA (chemilumineseсent microparticle immnoassay) Architect i 2000 and MEIA (microparticle enzyme immnoassay) IMx Abbott diagnostic. All patients were hospitalized at Department of Gynecologic Oncology at the University Clinics Center of Sarajevo. The normal serum range of SCC lies between 0 and 2 ng/mL. The quality control, precision and accurancy of Architect i 2000 were assessed.

Results: The quality control was done using quality control serums for low (= 2.01ng/mL), medium (= 10.1ng/mL) and high (= 49.85ng/mL). We have got good precision with CV 2.70% to 2,98 %. We established that the main difference between Architect i 2000 and IMx was statistically significant at $p < 0.05$ according to Student t-test. Correlation coefficient was r = 0.990 and regresion line had a slope 1.8131and a y axis intercept of 0.1529. The good correlation with IMx SCC allows for efficient cross-over. The some samplers have higher concentration at Architect then at IMx because sensitive of Architect assay is high (< 0.1 ng/mL).

Conclusion:The CMIA Architect technology is an applicable method significant in diagnostic of SCC tumor marker.

D-70

Rapid detection of Influenza A and B viruses by real time reverse transcriptase loop-mediated isothermal amplification and multiplex real-time reverse transcriptase polymerase chain reaction

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Background: The lateral flow assay is the main diagnostic method for influenza viruses in point-of-care testing (POCT). However, false-negative results are noted in some cases of early-stage infection. Thus, this assay should be supported by high-sensitive genetic detection. However, real-time reverse transcriptase polymerase chain reaction (RT-PCR) and other RT-PCR methods are unavailable as POCT diagnostic methods, because of faults with equipments and the time required for general genetic detection. In this study, we focused on the use of real-time loop-mediated isothermal amplification (LAMP) and multiplex real-time RT-PCR in the rapid detection of Influenza A and B and other novel viruses. To achieve this aim, we designed corresponding primers.

Methods: Initially, individual primer sets targeting the common and/or few mutation regions of the nucleo protein (NP) gene and matrix (M) gene were designed to specifically detect Influenza A and B and novel influenza A (pandemic in 2009) viruses by real-time RT-LAMP and multiplex real-time RT-PCR. In addition, virus RNA were extracted from NATtrol virus sample (ZeptoMetrix Corp., Buffalo, NY) and 12 clinical samples by using the QIAamp Viral RNA Mini kit (QIAGEN, Dusseldorf, Germany). Next, the extracted RNA were analyzed for one-step NAT by using real-time RT-LAMP (Loopamp RT-160) and multiplex real-time RT-PCR (LightCyclerII, DX400, Roche, Basel, Switzerland), and were identified as Influenza A, B, or novel type virus. Moreover, the RNA extracted from the clinical samples of novel influenza A virus were sequenced by DNA sequencer CEQ 8800 (Beckman Coulter, Fullerton, CA) and identified. Finally, the results of the genetic assay were compared with those of the lateral flow assay.

Results and Discussion: In multiplex real-time RT-PCR, clinical samples and standard samples were identified using designed primers with a TaqMan probe. As a result, 6 clinical samples were identified as influenza A virus on the basis of the fluorescence intensity of the amplified cDNA. In LAMP, the clinical samples were identified using 6 primers designed for LAMP. The results reveal that influenza A and B viruses could be identified, and novel influenza virus could be detected in 3 of the 6 samples. The time required for detection after the extraction of RNA from the samples was 1 hour for both the methods. Thus, our real-time RT-PCR and LAMP assays are potentially useful in rapidly detecting influenza viruses such as A, B, and the novel type.

D-71

Development of a Whole Proteome Native Antigen Microarray Platform for Identifying Specific Humoral Immune Responses in Breast Cancer Sera

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Background: The humoral response of a cancer patient may allow earlier detection of cancer than current methods allow. If so, the serum autoantibody repertoire from cancer patients might be exploited for autoantibody profiling and aid in the serological diagnosis of cancer. In addition, specific humoral responses to cancer cells might occur following personalized vaccination. Our specific aims are to: 1) develop a whole proteome native antigen microarray platform that can be used to monitor humoral responses, and 2) test the hypothesis that global autoantibody profiling might identify relevant disease signatures.

Methods: Whole proteome native antigen microarrays were developed to identify potential antigen targets. Briefly, tumor antigens from breast cancer cells were separated into defined antigen fractions. This was accomplished by using 2-D liquid chromatography fractionation where the 1st dimension was separation by isoelectric points and the 2nd dimension was separation by hydrophobicity. Following the 2nd dimension, the fractions were arrayed onto nitrocellulose coated microscope slides. These spotted fractions contain proteins which have relevant post-translational modifications that are specific to the natural disease state.

To demonstrate the utility of the platform and to determine whether there are specific humoral responses to cancer cells following vaccination, we tested our platform on 7 patients who underwent tumor vaccination with autologous tumor cells engineered to express GM-CSF. Pre-vaccination and 2 month post-vaccination IgGs from the same patient were isolated and fluorescently labeled, and their reactivity to specific fractions on the microarray was compared. To address whether there are relevant autoantibody signatures for early stage breast cancer, we purified IgGs from well-characterized serum samples from patients with newly diagnosed stage 1 (n=17) and 2 (n=23) invasive ductal carcinoma of the breast, and from age- and estrous cycle-matched healthy controls (n=15). The IgGs were labeled with fluorescent dyes and used as probes.

Results: For patients who underwent tumor vaccination with autologous tumor cells, 54 fractions were determined to have a p-value of ≤ 0.05 when post-vaccination IgGs were compared to the patient's pre-vaccination IgGs. In addition to unique individual responses, 4 distinct fractions were found to have increased responses in over 50% of all post-vaccination IgGs. For sera from patients with stage 1 and 2 breast cancer, we identified 17 antigen containing fractions that were differentially reactive with the cancer sera ($P \leq 0.05$). Receiver operating characteristic curves were plotted for the top 5 reactive fractions and the area under the curve (AUC) was calculated. Our findings showed that when combined, the 5 reactive fractions have an AUC of 0.898 for stage 1 breast cancers versus controls (87% sensitivity at 80% specificity), and an AUC of 0.82 for stage 2 breast cancers. Identification by mass spectrometry found multiple cancer related antigens present in the fractions including NOP16, cofillin 1, and Ki-67.

Conclusion: Our results demonstrate that we have successfully developed a whole proteome native antigen microarray platform to identify specific humoral immune responses. Our platform identified potential antigen targets to cancer vaccines as well as antigen containing fractions that appear to have significant clinical utility for separating cancers from controls.

D-72

Rapid identification of *Escherichia coli* in clinical urine samples by fluorescence in situ hybridization

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Background: *Escherichia coli* (*E. coli*) is the major pathogen in urine track infection (UTI). It can cause the asymptomatic bacterium urine and the acute nephropyelitis with noticeable symptom. Epidemiology reveals that UTI is common in female. In clinic, UTI can be diagnosed through bacteria culture of urine sample and the culture assay will take more than 48 hours. The aim of this study was to establish a rapid method for detecting and identifying *E. coli* in clinical urine samples.

Methods: Using fluorescent in situ hybridization (FISH) with 16S rRNA-targeted

FITC fluorescently labeled species-specific oligonucleotide probe, *E. coli* was rapidly identification in 120 clinical urine samples and the results were compared with the conventional culture-based assays.

Results: *E. coli* was successfully detected in 22 out of 120 clinical urine samples by both culture and FISH assay (Fig. 1). The lowest detection limit of FISH method was 10^3~10^4 CFU/ml. Compared to culture assay, the sensitivity and the specificity of FISH were 88.0% and 97.9% respectively, the positive predictive value (ppv) was 91.7% and the negative predictive value (npv) was 96.9%. The whole procedure was completed within one hour. **Conclusion:** FISH is a rapid, specific and highly sensitive technique for the detection of *E. coli* and can be used in diagnosis of UTI.

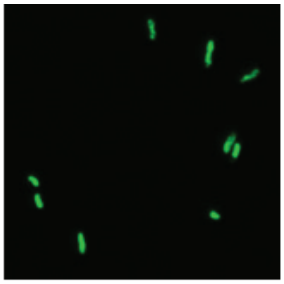

Fig. 1 Fluorescence microscopy of *E.coli*. Specimen hybridized with 16S rRNA-targeted oligonucleotide probe (magnification, ×1000)

D-73

Evaluation of the Interference of Hemoglobin, Bilirubin, and Lipids on Roche Cobas 6000 Assays

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Objective: Analytical interference can be a significant source of error in clinical laboratory measurements which can lead to wrong clinical interpretation and patient management. The effects of hemolysis, icterus, and lipemia on laboratory tests are the fundamental and key elements to be evaluated. We therefore performed such interference study on Roche Cobas 6000.

Materials and Methods: Sample preparation: Pooled lithium heparinized plasma was prepared as test sample which is not hemolyzed, icteric, or lipemic. Hemoglobin was prepared by washing, freezing, and thawing packed red cells. Various concentrations of hemoglobin were added into the plasma to simulate hemolysis. Icteric samples were obtained from pooled samples with addition of different concentrations of bilirubin. Lipemic samples were prepared by adding intralipid obtained from Pharmacy. The analytes were then measured on Roche Cobas 6000 (c501 and e601) and the change of the analyte concentrations was compared and calculated.

Results: A list of tests interfered by hymolysis, icterus, or lipemia was summarized in the Table.

Analytes	Hemolysis (H index)	Icterus (I index)	Lipemia (L index)
Acetaminophen	Increased (H 40)	Increased (I 40)	NS
ALT	Increased (H 230)	NS	Decreased (L 150)
ALP	Decreased (H 230)	NS	NS
Ammonia	Decreased (H 200)	ND	Decreased (L 50)
AST	Increased (H 40)	NS	Decreased (L 50)
Bicarbonate	Decreased (H 40)	NS	NS
Bilirubin (Total)	Increased (H 80)	NA	NS
Bilirubin (Direct)	Decreased (H 40)	NA	NS
CK	Increased (H 400)	Decreased (I 14)	Decreased(L 1200)
Creatinine	NS	Decreased (I 14)	NS
Ethanol	Decreased (H 50)	NS	NS
Haptoglobin	Decreased (H 100)	NS	NS
LDH	Increased (H 40)	NS	Decreased(L 1200)
Phosphorous	Increased (H 200)	NS	NS
Potassium	Increased (H 100)	NS	NS
Rheuma. factor	Decreased (H 300)	NS	NS
Theophylline	NS	NS	Decreased (L 50)
Troponin T	Decreased (H 300)	NS	ND
UIBC	Increased (H 50)	Decreased (I 3)	NS

The index value indicates significant interference was observed. NA: not applicable, NS: not significant, ND: not determined

Conclusions: We have found that test interferences of hemolysis, icterus, and lipemia present to some extent on Cobas 6000. Some test interferences are seen at lower concentrations of interfering substances than reported in the company's insert. More importantly we have demonstrated some test interferences which have not been reported previously. Based on the current findings, we are expanding our interference study to a larger spectrum of analytes and will incorporate these indices into middleware to be reported automatically instead of visual inspection.

D-74

Evaluation of BD vacutainer EDTA-K2 plus tubes with and without Polymer gel for plasma DNA quantification

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Background: To evaluate BD vacutainer EDTA-K2 plus tubes with and without Polymer gel for plasma DNA quantification.

Methods: A total of 20 healthy college students (10 females) were recruited with informed consent. A 6-ml blood sample was withdrawn from the antecubital vein of each individual, and equally collected into one BD vacutainer EDTA-K2 plus tube without Polymer gel (tube A) and two BD vacutainer EDTA-K2 plus tubes with Polymer gel (tube B and tube C). All blood samples were processed within 2 h of collection. After low speed (1,600 *g*) centrifugation step at room temperature for 10 min, the supernatant plasma samples were transferred into 1.5-ml polypropylene tubes, with particular care not to disturb the buffy coat layer. The plasma samples from tube A and B were then centrifuged at 16,000 *g* at 4°C for 10 min to remove any remaining blood cells. The plasma samples from tube A were added into BD vacutainer EDTA-K2 plus tubes with Polymer gel (tube D) and centrifuged at 1,600 *g* for 10 min.Fifty thousand copies of plasmid DNA were added into 200 µl cell-free plasma samples as internal controls. Thus, the plasmid DNA concentration in each plasma sample was 2.5×10^5 copies/ml. DNA was extracted from 200-µl plasma samples with an internal control using the Bilatest Viral DNA/RNA Kit (Bilatec, Viernheim, Germany), according to the manufacturer's recommendations. Duplex real-time quantitative PCR was performed for the human *β*-actin gene and internal control plasmid DNA amplification in the same volume of 25 µl with components supplied in the *TaKaRa Ex Taq*® R-PCR, version 2.1 (TaKaRa). DNA amplification was carried out in a 96-well reaction plate format in the Applied Biosystems 7500 Sequence Detector (Applied Biosystems, CA, USA). Fluorescence signals were detected in channels 1 and 2 for FAM- and JOE-labeled probes, respectively. The plasma DNA concentrations were calculated according to the threshold cycle (Ct) values and internal control's concentration. Paired t-test was used to detect differences of plasma DNA concentrations among tube A, B and C.

Results: The DNA concentrations of plasma samples from BD vacutainer EDTA-K2 plus tubes with Polymer gel by one-step or two-step centrifugation (tube B and C) were both lower than those of plasma samples from BD vacutainer EDTA-K2 plus tubes without Polymer gel by two-step centrifugation (tube A) (paired t-test, $P = 0.0064$ and 0.0068). There was no statistically significant difference of DNA concentrations between plasma samples from BD vacutainer EDTA-K2 plus tubes without Polymer gel by two-step centrifugation (tube A) and plasma samples of tube A added into BD vacutainer EDTA-K2 plus tubes with Polymer gel (tube D) (paired t-test, $P = 0.1479$).

Conclusion: The BD vacutainer EDTA-K2 plus tubes with Polymer gel was more suitable than the tubes without Polymer gel for collection of cell-free plasma samples for plasma DNA quantification.

D-76

Bias (Trueness) of Six Field Methods Estimated by Comparison to Reference Methods and Comparability of Results Within a Family of Analyzers

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Background: Assay accuracy depends on precision (reproducibility, random error) and bias (trueness, measurement error). Bias is often derived from method comparison studies of field (routine) methods, one designated the "reference method," the other the test method, but a field method is not a true "reference method," and the estimated bias is relative, not absolute. Comparison to recognized reference methods of highest metrological order that provide target values representing "scientific truth" yield the best estimate of bias. All members of a family of analyzers should produce comparable results.

Methods: Survey Validate Reference Materials (SVRMs) for six routine assays (Na, K, Cl, Glucose, BUN, and Creatinine) and creatinine standardization samples were tested with Abbott ARCHITECT cSystems (c4000, c8000, c16000) field methods. Six samples for each analyte were tested in triplicate for three days (n = 9) and mean concentrations were compared to reference method target values. Results from all analyzers were compared to each other to evaluate comparability.

Results: The table summarizes average bias for the three ARCHITECT cSystems compared to the target values assigned by reference methods. Average bias for five SVRMs was less than +/- 1.0% of reference method target values. Creatinine SVRM average bias ranged from -5.78% to +2% compared to ARM and for creatinine standardization samples from -0.35% to +7.25% compared to ID-GC/MS. Results from all three systems are comparable and Sigma metrics are typically > 6.

Conclusion: The trueness (bias) for six routine assays for a family of automated clinical chemistry analyzers compared very well to reference method target values. Proficiency testing often uses peer group grading but it is desirable to know how closely field method results match "scientific truth" determined using recognized reference methods. Results for the same sample tested by any member of the ARCHITECT cSystems are comparable.

	Average Bias Compared to the Reference Methods (%)		
Assay and reference method	c4000	c8000	c16000
Na (flame emission)	+0.09	-0.48	-0.45
K (flame emission)	+0.70	+0.06	+0.24
Cl (coulometry)	-0.64	-1.07	-0.56
Glucose (hexokinase/G-6-PDH)	-0.42	-0.47	-0.52
BUN (urease/GDH)	-0.19	-0.73	-0.30
Creatinine-alkaline picrate Jaffe [PT testing all results mean (ARM)]	-5.78	+1.34	+2.01
Creatinine-alkaline picrate Jaffe (ID-GC/MS)	-0.35	+6.8	+7.25

D-77

Detection, Detection, Detection: Considerations for Enzyme/Substrate Selection in Immunoassay Development

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Background: Detection limit, dynamic range and reproducibility are cornerstones in the development of a successful immunoassay application. During the optimization procedure, antibody/antigen systems are carefully chosen to provide the specificity and sensitivity of the desired analyte measurement. However, the choice of enzyme/substrate can also have a substantial effect on achieving the above mentioned parameters and require equal attention for optimal selection. Often the most "sensitive" substrate is chosen without consideration of necessity or tradeoff with dynamic range and reproducibility.

Methods: In order to give assay developers some guidance in the selection of detection methods, we have used HRP and AP conjugates of streptavidin to compare colorimetric (e.g. tetramethylbenzidine (TMB), 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)(ABTS), para-nitrophenol(PNPP)) and chemiluminescent (e.g. luminol and dioxetane) substrates in a simplified capture antibody assay. Using this model system, we have examined both the choice of detection enzyme as well as substrate to measure detection limit, dynamic range and reproducibility.

Results: The results indicate that the "fastest" substrate (e.g. "high sensitivity TMB") did not consistently have the best detection limit and was lacking in both dynamic range and reproducibility. Chemiluminescent substrates were shown to have less reproducibility and demonstrated plate to plate variability. Alkaline phosphatase based substrates (PNPP) were the optimal choice when a large dynamic range was desired and reduced plate to plate variations.

Conclusion: Several enzyme/substrate systems were examined and compared with respect to detection limit, dynamic range and reproducibility. Understanding the impact of these detection systems on the immunoassay application is essential and should always be a primary consideration for design.

D-78

New DNA Binding Filter Plates Compatible with Genomic DNA Whole Blood Lysis and Purification Reagents

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Objectives: Evaluate the performance of a new glass fiber material in a 96-well filter plate for the purification of genomic DNA from whole blood using commercially available lysis and purification reagents.

Background: The analysis of genomic DNA from whole blood samples for diagnostic purposes is on the rise and will continue to increase. The cost of DNA purification is expensive due to reagents and equipment. We evaluated a glass fiber DNA binding material in a new 96-well plate design for compatibility with readily available bulk cell lysis and genomic DNA purification reagents.

Materials and Methods: Whole Blood, <48 hr post collection, in citrate anticoagulant used to purify gDNA. Reagents were purchased from Qiagen, Invitrogen, Promega, and Zymo Research. Manufacturer recommended protocols were used. Genomic DNA was quantitated using SYBR green and evaluated on agarose gels.

Results: In all cases, gDNA quality is good, with fragment distribution being within a fairly narrow range. SYBR green determination of gDNA concentration showed variability among kits, but good well-to-well reproducibility from each protocol using new AcroPrep™ Advance filter plates. A qPCR method designed to detect possible inhibitors of PCR demonstrated a complete lack of inhibitory effect.

Conclusions: The new AcroPrep Advance DNA binding plate yields high quality genomic DNA when used in conjunction with a number of different reagent systems. In most cases, genomic DNA yield is similar or slightly better than plates included with the commercial kits. The high level of performance suggests that this plate could easily be used with commercially available or lab prepared lysis and gDNA binding reagents as a way to reduce costs associated with the purchase of kits. Additionally, this plate will fit into most centrifuge plate holders avoiding the cost associated with the purchase of a specially designed centrifuge.

D-79

A sensitive quantitative test strip based albuminuria assay

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Background: Chronic kidney disease (CKD) is becoming a major health problem, also in the third world countries. Global guidelines require screening of albuminuria. Therefore, a affordable and sensitive albuminuria screening test is needed. We explored the potential of urine strips, generally reported in the ordinal scale, measured on a automatic strip reader for reporting quantitative and sensitive albumin results.

Methods: We compared reflectance data from 3 different lots of Combur-Test® urine strips obtained from the commercial strip reader (Cobas U411) with micro-albumin data from a nephelometer BNII (Siemens) for 381 nonpathologic and pathologic urine samples.

Results: Imprecision of the reflectance signal of the Cobas U411 was measured with a high and low commercial control material (Liquicheck™ Urinalysis control 1 & 2, Bio-Rad). Between-run CVs for the 3 lots ranged from 4.6 to 8.3% for the high QC material and from 0.8 to 1.8 %for the low QC material. Within-run CVs were for both QC materials on the 3 lots lower than 4.7%.

Fair agreement was obtained between the albumin concentration of the BNII (AlbU_BNII) and the protein test strip reflectance data (Pro_ref) (n=381): $Y\ (10000/Pro\_ref;\ 1/\%) = 156+0.161\ X\ (AlbU\_BNII;\ mg/L)-0.0000209\ X^2\ (AlbU\_BNII;\ mg/L)$, $r^2=0.921$.

Less agreement was found between the pyrogallol red-molybdate protein assay (Modular P, Roche) and the strip reflectance ($r^2=0.831$).

Linear calibration curves were made for the 3 lots between 23.7 and 121.5 mg/L AlbU_BNII concentrations, $r^2 \geq 0.99$. The lower limit of quantification (LLOQ) for the 3 lots of teststrips were resp. 39.8, 54.3 and 42.2 mg/L.

There was no significant influence of manual handling on reflectance data and calculated concentrations of strips after 0, 10, 30 and 60 seconds prior to measurement on the teststrip reader ($p>0.05$).

Conclusion. The present study has demonstrated that the reflectance data generated by a test strip reader allows quantitative analysis of albumin. Despite the fact that the lower limit of the microalbumin range (30 mg/L albuminuria) cannot be achieved with the dye binding method, the results are satisfactory for screening purposes.

D-80

Development of Reverse Blot Hybridization Assay (REBA) HPV-ID available to REBA Autoprocessor and REBASCAN for Genotyping and Detection of Human Papilloma Virus (HPV) in Clinical Specimen

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Background: Today, infection with HPV has been detect in 95 to 100% of cervical cancer, the second most common female cancer worldwide, and is closely associated with development of cancer and cervical intraepithelial neoplasia. Worldwide, cervical cancer has been traditionally diagnosis by pap smear cytology, recently, combine of pap smear and HPV genotyping could be diagnosis of 99% in cervical cancer.

Methods: YD MolecuTech REBA HPV-ID possible to an *in vitro* molecular identification of 32 genotypes human papilloma virus (HPV) in human specimens of cervical brushes, cervical biopsies or cells in Cyto-preservation solution. This is used in conjunction with cervical cytology to aid in screening for cervical cancer. The REBA HPV-ID test is an technology for available genotyping of 32 HPV types DNA from cervical specimens 18 high-risk types (16,18,26,31,33,35,39,45,51,52,53,56,58,59,68,66,69,73), 1 probable high-risk type (34), and 13 low-risk types (6/11/32/40/42/43/44/54/70/72/81/84/87), at once. The REBA HPV-ID employs one-tube nested PCR used MY09/11/GP5-1/6 primer set and reverse blot hybridization assay (REBA) based on the membrane bounded line probe assay for identification of HPV genotypes and screening of carcinogenic HPV in cervical specimen. REBA is composed of 4 steps: The first step is DNA extraction from human specimens of cervical brushes, cervical biopsies or cells in Cyto-preservation solution. The second step is PCR amplification using by HPV specific primers. The third step is reverse blot hybridization by using the REBA autoprocessor, which is hybrid to PCR product on nylon membrane strip bound probe and color detection by chromogenic reaction. The final step is data interpretation by using the REBASCAN.

Results: The object of this study evaluates the performance of developed the REBA HPV-ID which was combined REBA autoprocessor and REBASCAN and was to confirm clinical availability in cervical cancer diagnostics. We performed REBA for the 39 clinical specimen analyzed HPV genotyping to DNA chip assay and the 80 clinical specimen used to HCII assay. Overall, the sensitivity of REBA HPV-ID, HCII, and DNA chip was 100% (119/119), 62.5% (50/80), and 89.7% (35/39), respectively. These results confirmed to the sequencing analysis. In comparison to HCII results, 30 of 80 specimens was HPV negative by the HCII assay but result of REBA HPV-ID was HPV positive and was identified to HPV genotypes of high-/low-risk group. However, cytological, these 30 specimens were diagnosed to ASCUS (11/30), L-SIL (15/30), H-SIL (1/30), RCC (1/30), adenocarcinoma (1/30), respectively. In addition, in comparison to DNA chip results for 39 specimens, the results of genotyping by the REBA HPV-ID were agreement on 76.9% (30/39), however, in comparison to sequence based genotyping results, REBA HPV-ID was agreement on 94.1% (112/119) and DNA chip was agreement on 84.6% (33/39).

Conclusion: Consequently, this study demonstrated that REBA HPV-ID is the high concordance with DNA chip assay and HCII assay and it is available to simultaneous diagnosis of cervical cancer and HPV genotyping from direct specimen, effectively. REBA HPV-ID is to 100% in clinical sensitivity and is to $10^1 \sim 10^3$ copies per reaction in technical sensitivity, therefore it was available to high-throughput test, clinically.

D-81

Increasing Speed And Security Of The Manual Sample Preparation Step For Immunesuppressant Monitoring On The Architect Analyzer Using A New 'Locked Sample Identity' Rack Principle

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Background: The newly available immunological chemoluminescent assays for the determination of tacrolimus, sirolimus and cyclosporine in whole blood on the ARCHITECT analyzer platform have, besides a number of definite advantages over other immunological tests, also one major disadvantage: Because of the multiple manual pre-treatment steps, not only the manual workload is increased but it further inherits a much higher risk of sample mix-up during sample preparation.

Methods: A new sample rack system has been designed. It is composed in a three row design based on the logical principle, that the primary whole blood (patient) samples are logically linked to the secondary precipitation tubes and the tertiary tubes for the ARCHITECT analyzer (LSI-'Locked Sample Identity'). The secondary tube racks are designed in such, that they are detachable from the rack compound and in such a format, that they can be vortexed directly and loaded into a lab-centrifuge for sedimentation together at once without the need to handle sample tubes individually. Additionally, for the sirolimus assay, secondary tube racks can directly be heated during incubation.

Results: The newly designed rack system proved, that, even with a high sample load, sample exchange by mistake is totally avoided, and that samples can be vortexed, incubated and centrifuged in large batches (up to 40) without the need to touch individual secondary sample tubes during all processes: All these steps may be performed without the need to label the secondary precipitation tubes, nor the ARCHITECT analyzer tertiary sample tubes.

The newly designed rack system has been evaluated for the ARCHITECT tacrolimus and sirolimus assays comparing them to the officially proposed manual protocol. No differences were detected, except of a (non-significant) trend to even more reproducible results with the new rack system.

Conclusion: Up to 40 patient samples can be handled in parallel in much shorter time compared to the officially proposed procedure. By integrating such a rack system into a manual sample preparation process all pipetting and transfer steps will be speeded up with an additional improvement of the precision, reducing the manual handling of sample tubes to a minimum and additionally minimizing involuntary sample exchange.

D-82

Development of a genotyping method to detect multiple polymorphisms in human serotonin transporter gene

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Background: The serotonin transporter or 5-HTT is an integral membrane protein that transports the neurotransmitter serotonin from synaptic spaces into presynaptic neurons. Selective serotonin reuptake inhibitors block the action of the serotonin transporter and are used to treat depression and other neuropsychiatric disorders. Three polymorphisms in the 5-HTT gene that have been implicated in treatment response and neuropsychiatric disorders are a 44 bp promoter ins/del polymorphism (5HTTLPR) that produces long and short alleles due to either 14 (short) or 16 (long) repeats of a highly conserved 23 bp unit, a 17-18 bp Variable Number Tandem Repeat (VNTR) found in intron2 (StIn2) is expressed as triallelic content with 9, 10 or 12 repeats (StIn2.9, StIn2.10 or StIn2.12). Finally a single nucleotide polymorphism (SNP) rs25531 in the promoter alters the function of the long promoter allele. We developed a PCR based fragment analysis assay electrophoresed on an ABI sequencer with which we are able to detect all 3 simultaneously.

Methods: The assay consisted of 4 PCR set up to amplify the Long/Short allele, the StIn2 VNTR region and allele specific PCR to amplify the A and G allele for the SNP rs25531. All 4 PCR reactions were set up at the same time under same conditions. The reverse primers of the 5HTTLPR, StIn2 VNTR and rs25531 allele specific primers were fluorescently labeled and detected on ABI 3130 sequencer and analyzed using Gene marker software v1.9 (Soft genetics). The samples analyzed by fragment analysis were verified by direct DNA sequencing.

Results: The different alleles expected for the promoter region are L (Long), S (Short), LS (Long/Short), LA, LG, SA and SG. The StIn2 VNTR alleles would be StIn2.9, StIn2.10 or StIn2.12 (9R, 10R or 12R repeats). The expected peak sizes in bp are as follows: The L peak at 450, S peak at 406, LA peak at 327, LG peak at 318 and the SA peak was at 284. The StIn2 alleles yielded fragments of 248 (StIn2.9), 265 (StIn2.10) and 298 (StIn2.12). Using this technique, we identified novel sequences which demonstrate promoter repeat regions containing 1) a 17 bp repeat with rs25531 A/G polymorphism, 2) two containing 18 repeat units 3) one with 19 repeat units, and 4) a 24 repeat sequence, with all variants confirmed by direct sequencing of gel-purified amplicons.

Summary: We developed a method to genotype 5HTTLPR, StIn2 and the rs25531 SNP simultaneously by fragment analysis and are detected in a single lane or injection on ABI Genetic Analyzer. Labeling with 2 different dyes provides a significant increase in throughput over the more traditional methods in detecting the different alleles. The Allele Specific PCR for the rs25531 is more accurate than the current procedure which utilizes a restriction enzyme digest and running gels. The analysis and interpretation is easy and is cost effective and is more appropriate for clinical setting.

D-83

Investigation of methylation patterns of APC gene in lung cancer with a novel fluorescence melting curve analysis assay

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Background: To identify methylation patterns in the promoter region of APC gene in lung cancer cell lines and two lung cancer patients by fluorescence melting curve analysis assay.

Methods: After bisulfate treatment, DNA samples of lymphocytes from cord blood without and with trans-methyl treatment were amplified. The amplicons were then cloned into plasmid vector and employed as negetive and positive controls. A pair of general primes were designed to amplify the target sequence in the p APC gene romoter region comprising 21 CpG sites. DNA melting curves were acquired by measuring the fluorescence of a double-stranded DNA-binding dye (SYBR Green I) during the dissociation stage. The methylation patterns of 4 lung cancer cell lines and 2 lung cancer patients' tumor tissue cells were determined by comparison of melting temperatures (Tm) with negetive and positive controls and sequencing.

Results: Melting curve analysis showed that three of four lung cancer cell lines (NCI-H446,SPCA1,NCI-H520) displayed a melting temperature 83°C as low as the unmethylated negetive control, while the other one (NCI-H460) displayed 2 melting peaks of 83°C and 88 °C which were corresponding to the Tm of unmethylated negetive control and fully methylated positive control, respectively. Sequencing reports were all in accordance with the melting curve analysis. The Tm values of two lung cancer patients were both between the values of negetive and positive controls.

Conclusion: The APC promoter region methylation patterns of NCI-H446 and SPCA1 and NCI-H520 are described as unmethylated alleles, while NCI-H460 cells exhibit monoallelic methylation. Two lung cancer patients' tumor tissue cells display partial methylation in APC promoter region. Integration of PCR and fluorescence melting analysis may be useful for simple and cost-effective detection of aberrant methylation patterns.

D-84

Multiplex fluorescence quantitative polymerase chain reaction for simultaneous detection of hepatitis B virus, hepatitis C virus and Human immunodeficiency virus

B. Tan, B. Ying, L. Qin. *Department of Laboratory Medicine, West China Hospital of Sichuan University, Chengdu, China,*

Objective: To construct a multiplex fluorescence quantitative polymerase chain reaction (MFQ-PCR) system for simultaneously detecting hepatitis B virus (HBV) DNA, hepatitis C virus (HCV) cDNA and human immunodeficiency virus (HIV) cDNA for reducing the false positive and false negative results, therefore increasing the efficacy and accuracy of donor blood testing. The feasibility of the MFQ-PCR system was assessed by testing 58 clincial serum samples from hospitalized patients.

Methods: TaqMan probes designed specifically for the detection of HBV, HCV and HIV viruses separately were put into the same MFQ-PCR tube, and co-amplificated with the HBV target DNA, HCV target cDNA and HIV target cDNA.

Results: In this new MFQ-PCR system, the detection limits are about 10 copies for each of standard plasmid DNA of HBV, HCV or HIV. In the test of 58 clicial serum samples, 22, 16 and 3 samples were positive with HBV, HCV and HIV viruses respectively by this new MFQ-PCR system. Compared with the traditional ELISA method, which showed that 23, 16 and 3 samples were positive for HBV, HCV and HIV respectively, the consistency rate of the new system for all positive samples is 97.6%.

Conclusion: MFQ-PCR has the advantage of simultaneous detection of HBV, HCV and HIV viruses in clinical serum samples with high efficacy and accuracy.

D-85

ColOff: a new device to collect fecal specimens

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Background: Both diagnosis and treatment of parasites from the intestinal tract depend on the recovery and identification of the etiologic agents, thus making the correct collection of fecal specimens important in terms of clinical relevance and patient care. Nevertheless the collection procedure is frequently neglected and the patients often perform the fecal collection using makeshift materials e.g. newspaper or aluminum foil. We aimed to validate ColOff® a new device to collect fecal specimens. ColOff® is a coating developed for toilet seat especially for assistance in the collection of fecal specimens.

Methods: 200 patients with a specific causative agent previously indentified by routine laboratory procedures were invited to collect a new fecal specimen using the ColOff® before starting treatment. All preparations and microscopic identifications were done by a skilled parasitologist from our laboratory following CLSI M28-A2. The results of the fecal specimen collected with ColOff® were compared to the first specimen previously indentified by routine laboratory procedures, by Pearson correlation. Statistical significance was set at R squared > 0.950.

Results: A non significance was observed for *Giardia lamblia*, *Entamoeba histolytica and Entamoeba coli* ($r < 0.950$), probably because some diarrheic feces were drained away from the device, as the ColOff® fabric was devised to facilitate elimination of contextual urine in patients with bladder stasis.

Conclusion: Considering that several laboratories are still orienting patients to inappropriate fecal specimen collection, we conclude that devices like ColOff® might represent a more suitable tool, in order to eliminate possible sources of errors. Nevertheless for a fully appropriate device for feces collection some improvements are to be made, particularly to allow collection of diarrheic feces, a frequent event in patients affected by intestinal parasites.

Wednesday PM, July 27

Poster Session: 2:00 pm - 4:30 pm
Infectious Disease

D-86

HPV genotype/species distribution in both simple and multiple infections among men and women.

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Background: HPV genotyping rather than mere detection of HPV has been recommended. Because different HPV genotypes confer distinctly different risks for development of cervical disease and persisting HPV infection is a critical step in cervical carcinogenesis. Moreover, genotyping enables the detection of multiple infections, which has been associated with an increased risk of developing cervical cancer. Furthermore, the prevalence of different genotypes varies geographically, and HPV infection in men is relatively less described and understood. Thus, the aim of this study was to describe the prevalence of HPV genotypes and species in single and multiple infections in men and women by assessing our clinical laboratory results database.

Methods: Through retrospective analysis of our HPV genotyping database, we assessed the samples results between February 2009 and September 2010. 2,134 samples were included all from HPV genotyping test of anogenital region. The genotype and species prevalences were identified and presented by gender and single or multiple infection. The patients age average was 33.0± 10.08 years, among women 32.8±9.99 years, and among men 33.7± 10.29. HPV genotyping was performed using PapilloCheck (Greiner Bio-One), which evaluates 24 different HPV genotypes (18 high-risk and 6 low risk).

Results: The database analysis demonstrates that 44% of tested samples were positive. Among these, 52.6% were single infections and 47.5% multiple infections. Men had proportionately more positive results 56.8% vs 40.4% (p> 0.0001) and also more multiple infections 57% vs 44% (p=0.0006), than women. In women, the eight most common genotypes were 16, 56, 68, 44/55, 58, 6, 42, 52 in single infections, and 56, 16, 53, 66, 51, 31, 6, 39 in multiple infections. In men, the most eight most common genotypes were 6, 56, 44/55, 53, 68, 11, 66, 39 in simple infections, and 6, 16, 42, 44/55, 56, 11, 51, 40 in multiple infections. When we grouped the HPV genotypes in species, the species A9 comprising the high-risk genotypes 16, 31, 33, 35, 52, 58, 67 was more prevalent in women and statistically different from the proportion found in men, both in single 31.8% vs 7.7% (p<0.0001) and in multiple infections in 25.7% vs 19.3% (p=0.01). On the other hand, the species A10 that comprise Low-risk genotypes 6, 11, 44 was the most prevalent among men and statistically different from the proportion found in women 31.7% vs 14.5% (p <0.0001) both in single infections and 23.4% vs 10.9% (p<0.0001) in multiple infections.

Conclusion: In this study, we observed that there is a high positivity in the HPV genotyping testing and multiple infections comprise a significant portion of these results. Moreover, there are differences in the positivity, in the prevalence of multiple infections, in the genotype distribution, of both the simple and the multiple infections, in the results between men and women. In addition, there are a higher prevalence of high risk and low risk genotypes/species in the female and male results, respectively. However, we cannot exclude that these differences are due different anatomical sites tested in men, and explained by differences in tissue tropism of specific types of HPV.

D-87

Performance Evaluation of a Prototype CHIV Assay Method on the ADVIA Centaur® System

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Objective: The prototype ADVIA Centaur HIV Ag/Ab Combo (CHIV) assay* is an in vitro diagnostic immunoassay for the simultaneous qualitative detection of human immunodeficiency virus p24 antigen and antibodies to human immunodeficiency viruses on the ADVIA Centaur system (Siemens Healthcare Diagnostics, Tarrytown, NY, US). The sensitivity and specificity of the assay were evaluated. Samples from a high-risk population were evaluated on both the CHIV and ADVIA Centaur EHIV† (Siemens) assays on the ADVIA Centaur system.

Methods: The diagnostic sensitivity of the prototype CHIV assay was evaluated with 396 HIV-positive samples obtained from ProMedDx (Norton, MA, US) and SeraCare (Mildford, MA). Specificity was determined by testing 3647 paid normal donor samples. The results were reported in index values as reactive (index ≥ 1.0) or nonreactive (index < 1.0). Forty-five commercially available HIV seroconversion panels obtained from SeraCare (formerly BBI, Milford, MA), NABI (Miami, FL, US), and Bioclinical Partners (Franklin, MA) were tested. WHO International Standard HIV-1 p24 Antigen (90/636) was serially diluted with negative plasma pool to evaluate analytical sensitivity. Precision was evaluated in a study involving 20 days and two runs per day. Six HIV-1 high positive samples and six HIV-2 high positive samples were serially diluted into the negative basepool and assayed to evaluate the high-dose hook effect. Samples (n = 2134) from high-risk population (obtained from Montefiore Medical Center, NY, US) were evaluated with the CHIV assay. Positive samples were confirmed by NAT testing using the Abbott RealTime HIV-1 assay (Abbott Park, IL, US) or the EHIV assay.

Results: All the positive samples showed reactivity by the prototype CHIV assay, resulting in 100% (396/396) sensitivity. Specificity determined by testing paid normal donor samples was 99.97% (3646/3647). The seroconversion sensitivity of CHIV assay was equivalent to the reference methods as per vendor certificate of analysis. The observed analytical sensitivity of WHO International Standard HIV-1 p24 Antigen (90/636) was 0.98 IU/mL. The CHIV assay had a total %CV of less than 6.9% over the assay range. The assay did not hook below the cutoff level with high positive samples. Additionally, 2134 high-risk samples from Montefiore Medical Center were tested on the prototype CHIV assay. The sensitivity (1428/1428) and specificity (706/706) were both 100%.

Conclusion: The results of this study show that the prototype ADVIA Centaur HIV Ag/Ab Combo assay is a reliable and accurate, fully automated qualitative method to simultaneously detect the presence of both HIV p24 antigen and HIV antibodies in human serum or plasma.

***** Under development. Not available for sale. Assay being developed by Siemens Healthcare Diagnostics Inc. for Ortho-Clinical Diagnostics, Inc.

† Assay developed, manufactured, and sold by Siemens Healthcare Diagnostics Inc., for Ortho-Clinical Diagnostics, Inc.

Keywords: HIV Ag/Ab Combo, Centaur

D-88

Development of Real-time PCR assay for the detection of five major pneumonia pathogen panels

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Background: Pneumonia, the inflammation of the lung, is a key cause of death worldwide. Pneumonia is caused by major pneumonia pathogen(*Mycoplasma pneumonia*(MP), *Chlamydia pneumonia*(CP), *Legionella pneumonia*(LP), *Streptococcus pneumonia*(SP), *Klebsiella pneumonia*(KP)). Conventional methods of detection including culture and antibody-based tests have been used to diagnose the cause of pneumonia for some time. But the inherent shortcomings of those methods, including long culture times and low accuracy of serology tests have made accurate diagnosis difficult. Molecular diagnostic methods including real-time PCR (qPCR) have recently gained favor due to high sensitivity and specificity compared to pre-existing methods. Our objective is to develop a qPCR assay for the detection of five strains of pneumonia-causing bacteria (MP, CP, LP, SP, KP).

Methods: Design of specific primer/probe sets was performed through bioinformatics analysis. Strain-specific DNA regions were targeted for PCR amplification and detection through the fluorescence of specific hydrolysis probes (5'-FAM; 3'-BHQ1) during thermal cycling. *Exicycler*™96 is a qPCR instrument with advanced optics and functionality suitable for qPCR-based pathogen detection. Accelerated storage stability tests were also performed to gauge real-world usability.

Results: *AccuPower*® MP/CP/LP/SP/KP Real-Time PCR Kits can detect five strains of pneumonia DNA with high sensitivity, accuracy, all while providing extended stability. The detection limit (sensitivity), dynamic range, and linearity were determined as 10 copies/reaction, > 7 logs, and 0.999, respectively. Specificity tests showed no signs of cross-reactivity to non-target strains. Performing the assay on clinical samples showed exact correlation with clinical laboratory test results. Accelerated stability testing showed that the kits can be considered stable for over 1 year when stored at -20 °C.

Conclusions: *AccuPower* ® MP/CP/LP/SP/KP Real-Time PCR Kits contain various features including being provided in a lyophilized premix format for user convenience and low contamination rates, outstanding reproducibility, long storage stability, improved specificity, high sensitivity, wide range of detection, equipment compatibility, the use of an internal control etc. These results suggest that our one-step RT-qPCR assay can be effective for large-scale screening and rapid detection of the major pneumonia strains MP, CP, LP, SP, KP in clinical samples.

D-89

The clinical Laboratory plays a key role in reducing Clostridium *difficile* (C. *difficile*) hospital associated infection (HAI) rates by implementing a simultaneous two test algorithm for rapid identification of C. *difficile*.

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Background: C. *difficile* is a spore-forming, gram-positive anaerobic bacillus that produces two exotoxins: toxin A and toxin B. It is a common cause of antibiotic-associated diarrhea (AAD) and it accounts for 15-25% of all episodes of AAD. C. *difficile* is the most frequent etiologic agent for healthcare associated diarrhea and may cost hospitals in the United States more than $1.1 billion annually.

Objectives: To implement a rapid, sensitive and specific test for C. *difficile* to cost effectively detect C. *difficile* infected patients using a rapid membrane enzyme immunoassay for the simultaneous detection of glutamate dehydronase antigen (GDH) and toxins A and B in a single assay device, thereby providing clinicians with key test results that will allow them to treat appropriately and isolate patients swiftly to reduce patient to patient transmission. An effective interventional surveillance program along with laboratory testing support will reduce the number of HAIs and the associated morbidity and mortality, thereby improving patient safety by reducing risks of infection and other adverse outcomes, while meeting the regulatory requirements for The Joint Commission, National Patient Safety Goals (NPSF), Goal 07.03.01.

Methods: A comprehensive, integrated, multi-disciplinary surveillance program was implemented in March 2010 using a simultaneous two test algorithm approach. The C. DIFF Quik Chek Complete™ from Alere, manufactured by TechLab Inc, uses antibodies to identify and confirm the presence of toxigenic C. *difficile* by detecting toxins A and B in a single assay device and glutamate dehydrogenase (GDH) antigen, which screens for the presence of the opportunistic C. *difficile* bacterium, delivering C. *difficile* test results in less than < 45 minutes. C. *difficile* testing is provided on demand in real time during any shift, any day, and around the clock, allowing for fast interventions by clinicians and infection control preventists when *C. difficile* is detected.

Results: Our rapid testing strategy focused on reducing the number of fecal specimens collected by nursing personnel per patient from three to one, decreasing turnaround time (TAT), improving patient bed management and reducing C. *difficile* infection rates. In 2009, before implementation of the two test algorithm in a single assay device, the infection rate was .95/1000 discharges and in 2010 after implementation of the two test algorithm for the rapid detection of C. *difficile* the infection rate was .57/1000. Comparing C. d*ifficile* infection rates between 2009 and 2010 there was a 40% reduction in C. *difficile* HAI with a corresponding 34% reduction in infection costs. Based on the average cost of infection incurred during hospitalized medical care of $35,000 dollars per infected patient, we decreased the cost of infection by $840,000 in 2010. Additionally, the total testing volume for C. *difficile* decreased by 42% in 2010 compared to 2009 and TAT decreased by 66%.

Conclusions: Rapid testing for C. *difficile* using a simultaneous two test algorithm approach reduces the time to diagnosis, treatment and cure, saving thousands of dollars in hospitalization and infection costs associated with HAIs, while enhancing patient safety and significantly reducing infection rates.

D-90

Syphilis Testing: An Automated Immunoassay Method-Comparison Study

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Introduction and Objectives: Syphilis is a multistage chronic disease that can be fatal if untreated, and infections are on the rise in the US and Europe. Transmission is primarily through sexual contact or vertical (mother-to-child) transmission. Fortunately, therapy can cure disease and prevent vertical transmission. Because the signs and symptoms associated with syphilis can be attributed to other causes, laboratory testing is an essential element in diagnosis. Serologic tests (nontreponemal and treponemal) are the most commonly used diagnostic tests. Both types of tests are essential for aiding in the diagnosis of syphilis. Using a treponemal test as the initial screening test can reduce hands-on time and provide better detection of latent and tertiary disease. This study compared the performance of three treponemal screening assays on the IMMULITE® 2000 (Siemens), BioPlex 2200 (Bio-Rad) and LIAISON (DiaSorin) systems for the detection of treponemal antibodies.

Materials and Methods: All assays were run according to the manufacturers' instructions. A total of 1360 samples were included in this study. The samples included sera from pregnant women (minimum 100 samples), sera for routine testing (minimum 1000 samples), and potentially cross-reactive sera from patients positive for other spirochete infections (minimum 20 samples). Samples were classified as negative if two of three assays were negative, and positive if two of three assays were positive. For discordant results, the final result was determined using RPR Q (RPR with titer) and TPPA tests. A single experienced operator who was familiar with all three instruments participated in the workflow comparison. Instruments were operated in batch mode.

Results: The IMMULITE 2000 and LIAISON assays demonstrated sensitivities of 100% and specificities of 98.36% and 98.04% compared to the final result. For the BioPlex assays, 4 of 136 positive samples were negative in the IgG assay (97.1% sensitivity). Two of these negative samples were positive in the IgM assay for a final sensitivity of 98.55%. In comparison to the IMMULITE 2000 and LIAISON assays, the BioPlex assays showed a lower sensitivity at comparable specificity. The time-to-first-result for the IMMULITE 2000 assay was 35 minutes, with subsequent results every 18 seconds. In comparison, the time-to-first-result for the LIAISON assay was 40 minutes, with 6 results every 125 seconds thereafter (~1 result every 21 seconds). For the BioPlex assay, the time-to-first-result was 45 minutes, with subsequent results every 36 seconds.

Conclusions: The IMMULITE 2000 and LIAISON syphilis assays showed comparable sensitivities and specificities for detection of treponemal antibodies. The BioPlex 2200 missed two specimens that were positive by the IMMULITE 2000 and LIAISON assays. The lower sensitivity is likely due to the BioPlex screening only for IgG antibodies. The workflow on all three analyzers was similar. Overall, all three systems performed well. Each has its own advantage and disadvantage. A laboratory should compare its needs against the instruments' features and functions to determine the best fit.

D-91

Identification of the Mycobacterium tuberculosis complex and its resistance to rifampin and/or isoniazid in Sourthern China

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Background: Tuberculosis (TB) is a leading cause of morbidity and mortality in the world. The WHO estimates that Mycobacterium tuberculosis infects one-third of the world's population.The emergence and spread of multidrug-resistant tuberculosis (MDR-TB) is a major medical and public problem threatening the global health. As conventional methods for mycobacteria culture and drug susceptibility testing are slow and elaborate, a simple method for the rapid identification of drug-resistant TB strains in clinical settings is urgently needed. This study uses GenoType® MTBDR*plus* (Hain Lifescience GmbH, Nehren, Germany) to establish a rapid procedure for identification of Mycobacterium tuberculosis complex (MTBC) and evaluate the local MTBC epidemiology.

Methods: A total of 334 specimens collected from West China Hospital, including 78 clinical strains and 256 clinical samples, have been determined for the MTBC and resistance to rifampin(RMP) and isoniazid(INH). Of the 78 clinical strains, 45 were sputum specimens, while the rest 33 were non-sputum specimens. Of the 256 clinical samples, 218 (209 were acid-fast bacillus (AFB) smear-positive, 9 were AFB smear-negative but PCR-positive) were sputum specimens and the rest 38 (6 were AFB smear-positive, 32 were AFB smear-negative but PCR-positive) were non-sputum specimens.

Results: The number of valid tests obtained by the GenoType® MTBDR*plus* was 82.6% (276/334). The overall drug resistance rate(RMP or INH) was 33.0%(91/276). Resistance to RMP was 25.4%(70/276) and 26.4% (73/276) was to INH, among which 82.2%(60/73)were due to the high level drug resistance and 15.1%(11/73) to the low level drug resistance. MDR-TB is defined as TB that is resistant to the two first-line anti-TB drugs RMP and INH. MDR-TB was found in 18.8% (52/276) of total valid test results. In the RMP-resistant TB, 74.3%(52/70)are resistant to INH.

Among the INH-resistant TB, 71.2% (52/73) are resistant to RMP. The identification of RMP resistance is enabled by the detection of the most significant mutations of the *rpoB* gene. For testing the high level isoniazid resistance, the *katG* gene is examined and for testing the low level isoniazid resistance, the *inhA* gene is analyzed. 65.7% (46/70)of RMP-resistant mutations were concentrated in the region of *rpoB* 530~533, in which 78.3% (36/46)were the S531L mutation. 80.8%(59/73)of resistance to INH was mediated by *kat* S315T1 mutation which confers the high-level resistance to INH. Finally, results are obtained in 1 working day compared to 1 to 2 months with conventional methods.

Conclusion: The GenoType® MTBDR*plus* assay is easy to perform and is a quick and efficient tool for the management of tuberculosis, as it allows early, appropriate treatment, which reduces transmission and spread of MDR-TB.

D-92

Developing a Highly Sensitive Immunoassay for the Screening and Diagnosis of Urogenital Mycoplasma Infection in Pregnant Women

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Background: Although urogenital Mycoplasmas are widespread commensal inhabitants of the lower genital tract in sexually active men and women, several species (such as Ureaplasma spp. and M. hominis) are proven pathogens. Subclinical urogenital infections have been implicated in up to 70% of adverse pregnancy outcome, especially preterm labor and delivery, urogenital Mycoplasmas being the most prevalent. This bacterium can spread and colonize the internal membranes and elicit an inflammatory response in the uterus initiating the cascade of events leading to precipitous delivery. Furthermore, the infant at the time of birth may be exposed to a bacterial challenge that could contribute to pathologies well known among preterm infants, including broncho-pulmonary dysplasia, chronic lung disease and respiratory distress syndrome. It is therefore crucial to diagnose infection at an early stage, but traditional diagnostic methods, such as bacterial culture or PCR, merely detect the presence of Mycoplasma, pointing to colonized carriers only. To date there is no efficient serology diagnostic test to identify those subjects that have developed an infectious disease and are at risk of developing adverse pregnancy outcome. Current serological tests reported thus far, are unsatisfactory, mainly due to the antigens used in these assays (usually ATCC lines or recombinant antigen), which do not constitute an adequate scope of antigenic variety and could result in false negative answers. In this study, we evaluated whether an antigen cocktail comprised of antigens isolated from various patients having Ureaplasma infection could be suitable for use as a sensitive serological assay. This assay is thus aimed at the screening and diagnosis of patients who developed an immune response to urogenital Mycoplasma. Relevance: We are the first to develop a highly sensitive ELISA for the detection of Ureaplasma spp. antibodies by using antigen cocktail based on serotypes isolated from symptomatic patients.

Methods: Antibodies to Ureaplasma spp. were measured by ELISA using a partially purified and sonicated antigen cocktail of serotypes representing the two Ureaplasma biovars. These mycoplasmal components were selected and isolated from symptomatic patients colonized with the bacteria. The study population included over 400 serum samples from pregnant women attending the High Risk Pregnancy Department at Soroka University Medical Center. A group of asymptomatic, healthy pregnant women with negative cervix and amniotic fluid culture were used as controls.

Results: Preliminary results using the newly developed assay demonstrated a significantly higher percentage of women with positive anti-Ureaplasma antibody titers in the high risk pregnancy group: 35/82 (42.68%) in premature rupture of membranes patients (PROM), 53/144 (36.8%) in patients with preterm labor (PTL) and 20/60 (33.3%) in pregnant woman with abortion or recurrent abortions, as compared to the control group 12/94 (12.7%).

Conclusion: The newly developed assay comprised of a mixture of mycoplasmal components (antigens) carefully selected from Mycoplasmas isolated from symptomatic patients, provides a reliable and highly specific test, which distinguishes between colonization and active infections in pregnant woman. It therefore represents a valuable tool for screening and diagnosing pregnant woman at risk.

D-95

Development of a Syphilis TPA assay* for the VITROS® ECi/ECiQ Immunodiagnostic Systems, the VITROS 3600 Immunodiagnostic System and the VITROS 5600 Integrated System

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Background. Syphilis is a sexually transmitted disease caused by the spirochete *Treponema pallidum*. The presence of antibodies to *Treponema pallidum* (TP) antigens, in conjunction with non-treponemal laboratory tests and clinical findings may aid in the diagnosis of recent, past or treated syphilis infection.

Method. The method described is a qualitative immunoassay technique, which involves the reaction of IgG, IgM or IgA antibodies present in samples with biotinylated TP antigen and horseradish peroxidase (HRP)-labeled TP antigen conjugate. The antibody-antigen complex is captured by streptavidin coated wells and unbound materials are removed by a washing step. The bound HRP conjugate, measured by a luminescent reaction, is directly proportional to the concentration of anti-TP antibody present. The performance of the VITROS Syphilis TPA assay was compared to that of other commercially available tests.

Results. Assay results are expressed as a ratio of the signal obtained at the clinical cut off. Precision was determined on all three VITROS Immunoassay Systems across a calibration interval of 28 days including evaluation of 5 recalibration events. Precision on patient sample pools measured from near the cut off to strong positives averaged 1.6% (range 0.9 - 5.0%) within run, 3.8% (range 1.9 - 5.8%) within calibration and 4.3% (range 3.0 - 6.5%) within lab. Clinical performance was assessed on a population of 1831 random donor and 233 clinical samples against a currently marketed assay. Positive percent agreement was 100% (193/193) and negative percent agreement was 99.77% (1748/1752) after resolution of 2 discordant samples by testing in two other methods. 100% agreement was obtained with commercially available performance panels from Zeptometrix and BBI-Seracare. No interference was seen with a wide range of potential interferents (Hemoglobin, Bilirubin, Tryglyceride, Intralipid, Azide, Biotin, Dipyrone) or with patient samples with other infectious agents present. No differences were observed between patient samples collected as Serum (SST, clot activator and on the clot glass tubes), as Heparin plasma (Lithium and Sodium), EDTA plasma or citrate plasma.

Conclusion. We conclude the VITROS Syphilis TPA assay meets current clinical practice needs for a fully automated assay.

* Under Development

D-96

Performance evaluation of the Access® HIV combo assay on the UniCel® DxI 800

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Objectives: A new automated HIV combo assay has been developed by Bio-Rad for the qualitative detection of HIV-1 p24 antigen and antibodies to HIV-1 (groups M-N-O) and HIV-2 using the UniCel DxI 800 Immunoassay system (Beckman Coulter). The purpose of this study was to evaluate the performance of this new assay in terms of sensitivity, specificity and precision.

Methods: All studies were performed on UniCel DxI 800 immunoassay system. The analytical sensitivity was estimated by dilution study of the AFSSAPS panel and the NIBSC Panel 90/636 (WHO standard). The clinical sensitivity was evaluated by testing 62 subtype and variant samples, 289 commercial positive samples, 199 hospital patient samples and 61 seroconversion panels (including 131 early seroconversion samples). The clinical specificity was studied with 2,552 samples from blood donors, 1,969 selected negative hospital patient samples and 617 non selected hospital patient samples. The precision study has been studied following CLSI EP5A2 guidance by the analysis of 13 samples: a negative sample, 2 low positive samples, a medium positive sample for HIV-1, HIV-2, HIV-1-O and HIV-1 antigen.

Results: The specificity was found to be 100% on blood donor samples, 99.85% on selected negative hospitalized patient samples and 100% on non selected hospitalized patient samples.

The analytical sensitivity obtained with the NIBSC 90/636 was equal to 1.1 IU/mL. It was estimated at 20.60 pg/mL with the AFSSAPS panel.

The clinical sensitivity for all positive samples including HIV-1-M, HIV-1-O, HIV-1-N, HIV-2 antibodies and HIV-1 antigen was 100%. The seroconversion sensitivity gave performance in accordance with the state of the art as 131 early seroconversion samples were all detected.

Intra-assay and inter-assay precisions were found below 10% with positive samples.

Conclusion: The evaluation of the Access HIV combo on the highest throughput UniCel DxI immunoassay system showed excellent performance in terms of global specificity, analytical sensitivity, clinical sensitivity and precision. This new Access HIV combo is fully suited for the screening of HIV, in hospitals or private laboratories.

D-97

Aberrant expression of CD8+CD28- suppressor T cells were involved in post kidney transplantation infection

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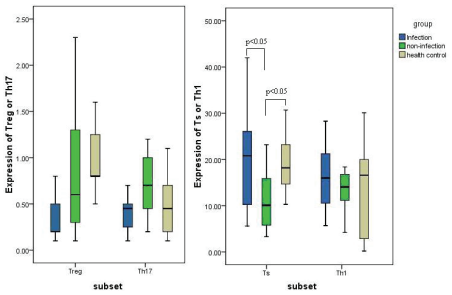
Background: The use of immunosuppressives successfully reduced transplant rejection. Due to the suppression state of immune system, infections after transplantation became an important factor to the survival of graft. Our study focused on the functions of regulatory T cells (Treg) and T helper cells (Th) in transplant recipients with infections.

Methods: 30 kidney transplant recipients were included in this study, 16 of them had infections after transplantation while 14 did not. Blood levels of cyclosporin A(CsA) and mycophenolate mofetil(MMF) were measured by an enzyme-multiplied immunoassay technique, expression of CD4+CD25+Foxp3+ Treg, CD8+CD28- suppressor T cells(Ts), Th17 and Th1 cells in the peripheral blood were analyzed by flowcytometry.

Results: Recipients with infections had higher trough levels of CsA. Compared with normal control,the expression of CD8+CD28- Ts cells decreased in non-infected group but increased in infected group. The expression of CD4+CD25+Foxp3+ Treg, Th17 and Th1 cells were similar in infected, non-infected recipients and normal controls. (Figure 1.)

Conclusions: CsA could induce a diminution in the level of CD8+CD28- Ts cells in non-infected recipients. The expansion of CD8+CD28- Ts cells in infected recipients was not directly associated with the high level of CsA, but the high level of CsA may partly induce immunosppressive state which might cause patients susceptible to infection. The increased expression of CD8+CD28- Ts cells was likely to be related to the infectious process after transplantation.



D-98

A Multicenter Evaluation of the Performance of the ADVIA Centaur® Anti-HBs2 Assay on the ADVIA Centaur Immunoassay System

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Background: The ADVIA Centaur Anti-HBs2 assay* is a sandwich immunoassay using direct chemiluminescent technology to measure the presence of antibodies to hepatitis B surface antigen in human serum and plasma. The objective of this study was to evaluate the performance of the ADVIA Centaur Anti-HBs2 assay for the qualitative and quantitative in vitro determination of total antibodies to hepatitis B surface antigen in human serum or plasma (EDTA or heparinized) using the ADVIA Centaur and ADVIA Centaur XP systems as compared to reference assays.

Methods: The ADVIA Centaur Anti-HBs2 assay was evaluated at a Siemens internal site and at two external sites. Reproducibility was evaluated with three reagent lots using an eight-member panel including two nonreactive serum samples, two low reactive serum samples, and four reactive serum samples, along with low and high calibrators and positive and negative controls. A hierarchical precision analysis of variance (replicates nested in runs, runs nested in days) was done for each site and across all three sites. A total of 2030 prospectively collected samples from subjects with signs and symptoms, at high risk for hepatitis B infection, or undergoing kidney dialysis, as well as transplant recipients, were evaluated among the three sites. In addition, unique populations were also evaluated using samples supplied from commercial vendors, including 27 matched pre- and post-HBV vaccine samples, 110 pediatric samples, and 20 neonate samples. A 95% Clopper-Pearson confidence interval was calculated for each positive and negative agreement.

Results: For reproducibility across all panel ranges ≥5.0 mIU/mL and across all lots and sites, the %CV ranged from 1.9% to 4.4% for the within-run estimates and from 5.1% to 10.2% for the total precision estimates. For the adult subject population, the overall percent agreement between the ADVIA Centaur Anti-HBs2 method and the reference assay was 97.8%, with a reactive percent agreement of 97.9% (95% CI of 96.7%-98.7%) and a nonreactive percent agreement of 97.7% (95% CI of 96.7%-98.5%). For the pediatric population, the overall percent agreement between the ADVIA Centaur Anti-HBs2 method and the reference assay was 95.5%, with a reactive percent agreement of 95.2% and a nonreactive percent agreement of 95.8%. For the neonate population, the overall percent agreement between the ADVIA Centaur Anti-HBs2 method and the reference assay was 100.0%, with both a negative and positive agreement of 100.0%. For the pre- and post-HBV vaccine samples, the overall percent agreement between the ADVIA Centaur Anti-HBs2 method and the reference assay was 100.0%, with both a negative and positive agreement of 100.0%.

Conclusion: The results of these studies demonstrate that the ADVIA Centaur Anti-HBs2 assay is a precise and accurate immunoassay for the quantitative in vitro determination of antibodies to hepatitis B surface antigen in human subjects, including pediatric and neonatal populations. Furthermore, there was excellent agreement in the testing of the vaccine samples as compared to the reference method.* Under FDA review. Not available for sale in the US. This assay is CE marked

D-99

Multiplex Cytokine Analysis for the Differentiation of SIRS and Sepsis

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Background: Sepsis is a major cause of mortality in the critically ill and is challenging to differentiate from systemic inflammatory response syndrome (SIRS). SIRS is identified by the presence of two or more of the following characteristics: abnormal body temperature, abnormal white count, tachypnea and/or tachycardia. Sepsis is SIRS in the presence of a documented infection. Rapid identification and treatment of septic patients with antibiotics and goal-directed volume resuscitation significantly reduces morbidity and mortality; SIRS patients do not require antimicrobial therapy. Thus, it is desirable to have a rapid and reliable test to rule-in sepsis in SIRS patients. Single biomarkers show low diagnostic strength to identify an infectious process among ICU patients, likely due to the complex pathobiology of the disease. Sepsis may be best detected with a biomarker panel consisting of inflammatory proteins secreted during disease progression.

Objectives: To identify a panel of biomarkers that accurately rules-in sepsis in ICU patients with SIRS.

Methods: 63 left over plasma samples collected from ICU patients on the first day they had SIRS (identified though an automated electronic medical record scan) were included. Of these, 26 had culture confirmed sepsis and 37 no bacterial infection within ±3 days of specimen collection. Concentrations of 8 cytokines IL-1β, IL-6, IL-8, IL-10, MCP-1, GM-CSF, TNFα, and INF-γ were determined by simultaneous multiplex analysis on the Luminex platform. C-reactive protein (CRP) was measured on a Roche Integra, and Procalcitonin (PCT) measured by Brahms Kryptor.

Results: ROC curves generated for each cytokine gave areas under the curve of 0.58 (p=0.26) for IL-1β; 0.74 (p=0.001) for IL-6; 0.57 (p=0.34) for IL-8; 0.64 (p=0.05) for IL-10; 0.62 (p=0.10) for GM-CSF; 0.70 (p=0.009) for MCP-1; 0.60 (p=0.18) for TNFα; 0.53 (p=0.71) for INFγ; 0.63 (p=0.086) for CRP; 0.66 (p=0.028) for PCT. Further analysis was restricted to analytes with significant (p< 0.05) curves. Cut-offs representing optimal sensitivity and specificity were 54 pg/mL, 15 pg/mL, 642 pg/mL, 97.9 ng/mL, and 0.48 ng/mL for IL-6, IL-10, MCP-1, CRP, and PCT, respectively.

At these cut-offs, the diagnostic accuracies (sensitivity, specificity, and positive likelihood ratio (LR+)) for individual analytes to predict sepsis were: 73%, 70%, 2.5 (IL-6); 69%, 68%, 2.1 (IL-10); 65%, 76%, 2.7 (MCP-1); 54%, 76%, 2.2 (CPR); and 54%, 62%, 1.4 (PCT). Combining certain analytes yielded improved diagnostic strength. Patients with positive results for both IL-6 and MCP-1, IL-10 and MCP-1, and IL-6, IL-10, and MCP-1 were more likely to have sepsis (LR+ = 3.6, 3.1, and 3.3 respectively).

Conclusions: IL-6, IL-10, MCP-1, CRP, and PCT alone show only weak ability to identify sepsis in a population of patients with SIRS. The combination of IL-6 and MCP-1 was best able to predict sepsis. A combination of biomarkers chosen based upon their role in the pathobiology of disease may accurately identify sepsis among critically ill patients with systemic inflammation.

D-100

Direct Detection of *Plasmodia* RNA in Human Blood: a Sensitive Assay Suitable for Large Scale Malaria Screening

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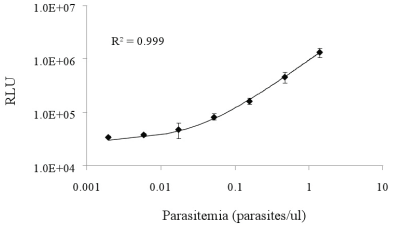
Background: Endemic malaria, coupled with increased population movements and global travel, present a diagnostic challenge to laboratories in most countries. The gold-standard microscopic examination suffers from high expertise demand and low sensitivity. Recently emerged nucleic-acid detection methods improve the sensitivity, however they lack the throughput required for large-scale screening, and their requirement for nucleic acid preparations may lead to variable diagnostic results.

Methods: We adopted a previously developed method of sandwich hybridization coupled with signal amplification to directly detect genus *Plasmodium* RNA in whole blood. A set of multiple hybridization probes were designed that hybridize to the conserved region of 18S ribosomal RNAs of the four human-infecting *Plasmodium* species, and branched DNA technology was used to amplify the hybridization signal. We evaluated the assay using cultured *P. falciparum* and tested the assay with frozen blood samples from 49 microscopically-diagnosed *P. falciparum* and *P. vivax* patients in Myanmar.

Results: The assay can detect general plasmodia directly from 20µl of blood in a 96-well plate format, without RNA purification, reverse transcription and target amplification. The detection limit of the assay for *P. falciparum* was 0.04 parasite/µl blood with signals proportional to parasite numbers (R^2=0.999). Concordance with the 49 microscopic diagnoses was 100% and the inter-assay CV was 5%.

Conclusion: The high sensitivity and the simple workflow make the assay suitable for high-throughput screening for general malaria infection.



D-101

Comparison of the Abbott Architect and the Johnson Vitros anti-HCV immunoassays results to the molecular detection of HCV RNA

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Background: Hepatitis C virus (HCV) is one of the most important causes of chronic liver disease. Acute HCV infection is usually asymptomatic. However, 85% of infected individuals develop chronic liver infection and frequently progress to cirrhosis and hepatocellular carcinoma. The screening test of HCV is anti-HCV antibody test by immunoassay and the chronic infection confirmed by nucleic acid testing of HCV. Several studies have found that specimens with low signal/cut-off (S/CO) ratios in the anti-HCV immunoassays are commonly negative when tested for HCV RNA. The objective of this study was to evaluate the possibility that positive results with low S/CO ratio in the Abbot Architect and Johnson Vitrus anti-HCV immunoassays might mean false-positive results for HCV chronic infection.

Methods: We analyzed 376 samples of the laboratory routine with medical request for anti-HCV serology that were reactive in the anti-HCV immunoassay and had the nucleic acid testing of HCV performed up to 90 days after the serologic test. The patients' ages ranged from 16 to 91 years old (mean 50.1). 243 (64.6%) patients were female and 133 (35.4%) were male. 376 samples were tested for HCV antibodies with the Abbott Architect anti-HCV immunoassay (Abbott Laboratories, Chicago, USA) and 276 were also tested in the Johnson Vitros anti-HCV immunoassay (Ortho-Clinical Diagnostics, Johson & Johson, Buckinghamshire, U.K.). The presence of HCV RNA was evaluated using the Amplicor Hepatitis C Virus (HCV) test Version 2.0 or the Cobas TaqMan HCV test V 2.0 (Roche Diagnostics GmbH, Mannheim). OuvirLer foneticamenteVer dicionário detalhado

Results: All samples evaluated in this study were positive for anti-HCV antibody. The Abbott Architect and the Johson Vitrus anti-HCV immunoassays presented a good agreement with a correlation coefficient $R^2 = 0.95$. Of the 376 anti-HCV positive patients, 202 (53.7%) were HCV RNA positive and 174 (46.3%) were HCV RNA negative. There was no significant difference related to gender ($p = 0.06$) in the HCV RNA detection. The stratification of the anti-HCV immunoassays results showed that none of the 101 patients with low S/CO ratio ranging from 1.0 to 5.0 was positive for HCV RNA, while 17 (29.3%) patients with S/CO ratio ranging from 5.1 to 10.0 and 185 (85.2%) patients with S/CO ratio above 10.0 were HCV RNA positive in the Abbott Architect anti-HCV. Similarly, none of the 64 patients with low S/CO ratio ranging from 1.0 to 9.0 was positive for HCV RNA, while eight (20%) patients with S/CO ratio ranging from 9.1 to 18.0 and 140 (85.8%) patients with S/CO ratio above 18.0 were HCV RNA positive in the Johnson Vitros anti-HCV.

Conclusions: The S/CO ratio of anti-HCV immunoassay may help to define the status of HCV infection, although it can vary among different manufacturers. In this study no patient was positive for HCV RNA in S/CO ratio of anti-HCV ranging from 1.0 to 5.0 and 1.0 to 9.0 in the Abbott Architect and Johnson Vitros anti-HCV immunoassays respectively. These results with low S/CO ratio of anti-HCV can be considered false-positive for HCV chronic infection.

D-102

Elevated troponin and Jarish-Herxheimer reaction in tick borne relapsing fever

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Background: Tick-borne relapsing fever (TBRF) is caused by many different *Borrelia* species. We report a case of *Borrelia hermsii* infection in Whatcom County, WA complicated by Jarisch-Herxheimer (J-HR) reaction and increased cardiac troponin.

Case Report: A 75-year-old woman was seen at a local hospital with febrile illness and myaglia. Past medical history was unremarkable. She had an increased heart rate and blood pressure. Urinalysis revealed numerous WBC and bacteria; culture identified *E.coli.*Chemistry showed elevated renal (BUN = 29 mg/dL; creatinine = 1.3 mg/dL) and liver (AST = 114 U/L; ALT = 163 U/L) enzymes, high glucose (154 mg/dL) and normal electrolytes. The patient was non-reactive for acute hepatitis panel and HIV. A base troponin I was normal (0.03 ng/mL). Hematology showed leukocytosis (10,000/mm^3) and thrombocytopenia (92/mm^3). A blood smear revealed numerous loosely-coiled spirochetes suggestive of borreliosis. Serological testing (acute) for *Borrelia* sp. was negative. A working diagnosis of TBRF was made. Antimicrobial therapy was started but a clinical worsening of symptoms developed in 4 hours complicated by J-HR - temperature, blood pressure and platelet count decreased rapidly. At 12 hours, spirochetes were no longer visible, and the patient became afebrile. At 36 hours, chest and low back pain developed. A troponin was 0.28 ng/mL. The patient was transferred to the cardiovascular unit. At 48 hrs, the troponin was 0.96 ng/mL. Over the next 12 hours, the thrombocytopenia improved (112/mm^3), and febrile illness and angina resolved. Fourteen days after onset of the illness, the patient's platelet count had normalized (235/mm^3). Four months after the presenting illness, the patient had seroconverted and serological testing was positive for *Borrelia hermsii* by enzyme immunoassay and Western blot.

Conclusion: The J-HR reaction is a well-known complication of antimicrobial therapy. Studies have shown that cytokines, namely tumor necrosis factor, interleukin 8 and -16 appear in the circulation transiently and correlate with symptom severity in borreliosis. Antibodies against inflammatory cytokines have been shown to decrease the J-HR. Sepsis that results from the presence of infectious organisms is frequently associated with changes in these inflammatory mediators. Elevations in cardiac

troponin in patients with sepsis are common. This is one of the first reports of transient increases in cardiac troponin in TBRF. The potential causes of troponin release during sepsis include decreased cardiac membrane integrity, bacterial endotoxins and thrombotic dysfunction. Troponin increases in borrelosis are unlikely to be caused by flow-limiting etiologies.

D-103

Natural History of Indeterminate HCV RIBA

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Background and Methods: From 4/1/06 through 9/30/10, we performed roughly 45,000 HCV antibody (HCV Ab) screening immunoassays (Siemens Centaur), of which 422 samples (0.9%) had Signal to Cut-Off Ratios (S/CO) requiring confirmatory testing (RIBA) as recommended by CDC guidelines. The RIBA results were distributed as follows: 59 POSITIVE, 216 NEGATIVE and 147 INDETERMINATE. 118 different patients accounted for the INDETERMINATE results (that is, antibody to only one specific HCV antigen was demonstrated). The CDC guidelines recommend that these patients have the HCV Ab test repeated on a new sample drawn at least 30 days following the original result.

Results: Of our 118 patients, 33 (28%) had repeat HCV Ab testing done at our institution. On these new samples, 8 turned POSITIVE, 7 turned NEGATIVE, and 18 remained INDETERMINATE.

In all 8 patients whose results converted from INDETERMINATE to POSITIVE, HCV Ab was confirmed with an additional (3rd) sample. The interval between the INDETERMINATE and initial POSITIVE result ranged from 2 to 1178 days. Just one sample was obtained at less than 30 days, but three samples were obtained more than a year later, so it is impossible to say whether the new result represented an evolution of the initial immune response or an antibody response to a new exposure.

In all 7 patients whose results converted from INDETERMINATE to NEGATIVE, the NEGATIVE result was confirmed with an additional (3rd) sample. The interval between the INDETERMINATE and initial NEGATIVE result ranged from 30 to 1342 days. All of these patients also had HCV viral loads performed, which were negative in 6. The single exception was a patient with HIV, whose HCV viral load was 800,000 and 2,400,000 when his HCV Ab results were INDETERMINATE and NEGATIVE, respectively.

Among the 18 patients whose results remained INDETERMINATE, the 30 day minimum guideline was met in 17. The interval between determinations ranged from 9 to 1253 days. In 14 of these patients, HCV viral loads were done, and all were negative. Of note, 3 of these patients had been HCV Ab POSITIVE before 2006, when we did not automatically confirm low S/CO by RIBA but simply reported them as POSITIVE. In other words, these samples may always have been INDETERMINATE.

Conclusion: We conclude that 1) Low S/CO samples are rare (1%) and should be confirmed by a more specific test; only 14% of low S/CO in our population are confirmed as POSITIVE, 2) patients with INDETERMINATE RIBA results need follow-up, and 3) among our patients, we could document follow-up in only 28% of cases; among those who did get follow-up, only 24% turned POSITIVE.

In our experience, then, INDETERMINATE confirmatory HCV tests are far more likely to represent false positive than true positive HCV Ab tests.

D-104

Coexistence of hepatitis B surface antigen and anti-HBs in Chinese chronis hepatitis B virus paitents: related to genotype C and the mutations in S gene and P gene reverse transcriptase (RT) region

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Background: We aimed to determine the prevalence of the coexistence of HBsAg and anti-HBs, analyze the clinical and virological features, including aa pattern of S gene and reverse transcriptase (RT) region in Chinese CHB patients.

Methods: 1862 CHB patients (defined by HBsAg carriage) were recruited in last 12 months. Among these, 54 subjects concomitantly carried both HBsAg and anti-HBs; HBV sequences have been obtained from 52 of them. For comparative analysis of virological patterns including HBsAg and RT regions, a control group consisting in 48 individuals with newly diagnosed HBsAg positivity and without anti-HBs was used. Measurements of HBsAg, anti-HBs antibodies, HBeAg, anti-HBe antibodies, anti-HBc antibodies, serum alanine aminotransferase level (ALT), HBV DNA quantitative, S gene and RT region sequences amplification, sequencing and analysis using in-house protocols.

Results: There was no significant difference between patients with and without anti-HBsAb regard to age, gender, alanine aminotransferase level, HBeAg positive proportion and HBcAb positive proportion (Table 1). The proportion of anti-HBeAb positive (p=0.027) and Genotype C (p=0.001) is significantly more frequent in HBsAg+/anti-HBs+ individuals. In S gene, the number of mutated residues in the HBsAg+/anti-HBs+ group was markedly increased than in control patients (p=0.022, Table 2, Figure 1). The amino acid exchange occurred mostly within the N-terminal region (p =0.023) and the "a" determinant (p =0.049, Figure 2) in two groups. For the S protein MHR antigenic loops and C-terminal region, the residue change frequencies between two groups were comparable (P>0.05). In the RT region, the mean number of substitution showed a tendency to be significantly higher in HBsAg+/anti-HBs+ patients than in controls (p=0.04,Figure 3).

Conclusion: Prevalence of anti-HBs co-existence was 2.90% and the predominance of genotype C among the HBV strains was comfirmed in this study. We observed an increased aa variability of HBV strains within both S gene and RT region in the HBsAg+/anti-HBs+ group.

Table 1. Comparison of demographic, clinical, and virologic characteristics of patients with concurrent HBsAg and anti-HBsAb and controls. (ALT, alanine aminotransferase, HBV, hepatitis B vis, HBsAg, hepatitis B surface antigen, HBsAb, hepatitis B surface antibody, HBeAg, hepatitis B early antigen, HBeAb, hepatitis B early antibody, HBcAb, hepatitis B core antibody)

	HBsAg+/anti-HBsAb+ (n=52)	Control group (n=48)	P values
Median age (years)	47(13-74)	41(24-64)	0.080
Gender(M/F)	37/16	27/21	
Male(%)	69.8%	56.3%	0.147
HBV genotype (%)			
B	6/52(11.5%)	27/48(56.3%)	0.001
C	46/52(88.5%)	21/48(43.8%)	0.001
Serum ALT level, median IU/L (range)	49(11-1165)	47(9-861)	0.928
Serum HBV DNA quantification			
<3 log10 copies/ml	18/52	12/48	0.183
>5 log10 copies/ml	28/52	30/48	0.113
HBeAg positive (%)	27/52	33/48	0.104
Anti-HBeAb positive (%)	20/52	15/38	0.027
Anti-HBcAb positive (%)	48/52	45/48	1.000

Table 2. Number of substitutions per 100 amino acid positions within HBsAg and the RT region in the HBsAg+/anti-HBsAb+ patients and control patients.

Regions of HBsAg	Number of substitutions per 100 amino acid positions		
	HBsAg+/anti-HBsAb+ Patients	Controls	P
Full length region of S protein (aa 1-226)	1.88	1.02	0.022
N-terminal region of S protein (aa 1-99)	2.15	0.87	0.023
MHR antigenic loops (aa 100-160)	2.17	0.98	0.114
"a" determinant (aa 124-147)	3.61	1.50	0.049
C-terminal region of S protein (aa 170-226)	1.01	1.34	0.874
Full length region of RT (aa 347-693)	2.34	1.46	0.378

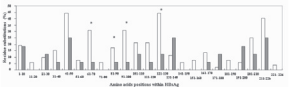

Fig 1. Frequencies of residue substitutions within the S protein from HBsAg+/anti-HBsAb+ patients (white bars, n=52) and solely HBsAg positive patients (gray bars, n=48), analyzed in intervals of 10 amino acids each. Each bar represents the percentage of patients with mutated residues for all patients in each group at each interval of 10 amino acids per group. Positions where the proportion of sequences harbouring mutations was significantly between HBsAg+/anti-HBsAb+ patients and controls are marked with an asterisk (* : P<0.05).

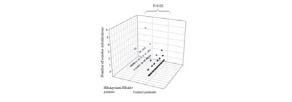

Fig 2. Numbers of residue changes from HBsAg+/anti-HBsAb+ patients and control patients within the "a" determinant. The numbers for the 52 patients (white circles) in HBsAg+/anti-HBsAb+ patients group and the 48 patients (black circles) in control group are distributed according to the numbers of residue changes in the "a" determinant. Statistical significance was calculated using a 3dmm-Whitney test (P=0.02).

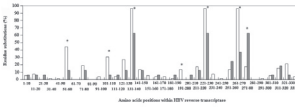

Fig 3. Frequencies of residue substitutions within the RT region from HBsAg+/anti-HBsAb+ patients (white bars, n=52) and solely HBsAg positive patients (gray bars, n=48), analyzed in intervals of 10 amino acids each. Each bar represents the percentage of patients with mutated residues for all patients in each group at each interval of 10 amino acids per group. Positions where the proportion of sequences harbouring mutations was significantly between HBsAg+/anti-HBsAb+ patients and controls are marked with an asterisk (* : P<0.05).

D-105

Relationship between IL-18 SNPs and Susceptiblity of PTB of the Tibetans from southern China

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Background: There are several reports demonstrating that host genetic factors play significant roles in susceptibility to TB . Therefore, the identification of host genes responsible for susceptibility and resistance to TB should provide a significant contribution for understanding of the pathogenesis and may lead to the development of new prophylaxis and treatment strategies. The cytokin Interleukin-18 (IL-18) , plays a vital role in both innate and acquired immunity.it stimulates IFN- production in estabilished Th1 cells and augments T cells, natural killer-cell cytotoxicity through upregulation of Fas ligand,it also enhance perforin-mediated killing and induce proinflammatory cyotokines and chemokines. Considering these multiple functions, IL-18 could have an important role in the development of TB. Recent findings show that the IL-18 gene-promoter region regulates the gene expression of this cytokine to alter the IL-18 promoter activity. contributing to the pathogenesis and pathophysiology of infectious and inflammatory diseases. The objective of this study was to determine whether the presence of IL-18 polymorphisms -607 A/C (rs1946518) and-137 G/C (rs187238) was associated with in the progression of pulmonary tuberculosis(PTB) among Tibetans populations from southwestern China.

Methods: A case control study was designed and adopted 320patients (male156, female164, mean age29.42±8.31) with PTB and 314 healthy control subjects (male184, female130, mean age26.83±7.67) were collected from Tibetan were collected from physical examinations, All subjects were unrelated ethnic Tibetan from southwestern in China, PCR amplification for A-607C and G-137C variants was carried out under the same conditions in a 96-well plate in the LightCycler® 480

Real-Time PCR System (Roche Diagnostics, Penzberg, Bavaria, Germany). and their polymorphisms were detected by using the high-resolution melting (HRM) method. The genotype and allele frequencies were analyzed ,reseparately.

Results: The studied IL-18 gene polymorphisms did not influence susceptibility to PTB in the analyzed group of patients (IL-18-607, p=0.317; IL-18-137 p=0.838) ,but may contribute to disease onset and aggressiveness.

Conclusion: Functional IL-18 gene polymorphisms do not influence the susceptibility to PTB in Tibetans but may contribute to disease onset and aggressiveness, more numbers may show a significant association between them and further examination will be needed.

D-106

Epidemical characteristics of Lamivudine-resistance mutations of HBV in Southern China

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Background: Hepatitis B virus (HBV) infection remains one of the major global public health problems and it is highly prevalent in China. Lamivudine (LMV), as the preferred oral drug of HBV, always occur resistance mutations after long time treatment. As we know, there weren't sufficient investigations reflecting whether different HBV genotypes affect the incidence of LMV-resistance mutations. In this study we investigated the chronic hepatitis B (CHB) patients in Southern China to determine the prevalence characteristics of HBV genotypes and LMV-resistance mutations, the association of HBV genotypes/LMV-resistance mutations with the age/gender features of CHB patients, as well as the relationship between HBV genotypes and LMV-resistance mutations.

Methods: 185 CHB patients living in Southern China were recruited. Enzyme-linked immunosorbent assay was tested for HBV serological markers and HBV DNA was quantified by real-time PCR; Sequencing was performed to detect HBV genotypes and mutations.

Results: There were 49.19% (91/185) CHB patients with HBV resistant to LMV. Only two genotypes were found: B and C. 62.16% (115/185) patients were infected with HBV of genotype B and 37.84% (70/185) patients were infected with HBV of genotype C. The incidence rate of LMV-resistance was not significantly different between genotype B and C (49.57% *vs.* 48.57%, $P > 0.05$). For the mean age and gender ratio, neither between patients with LMV- resistance caused by mutations and patients without any mutations, nor between patients infected with HBV of genotype B and patients infected with HBV of genotype C, no significant difference was found. The pattern of rtM204I alone was predominantly observed (36.26%, 33/91) and followed by rtM204V+rtL180M (23.08%, 21/91). The overall incidence rate of rtM204I mutation in genotype B (45.61%, 26/57) was more frequent than that in genotype C (20.59%, 7/34) (45.61% *vs.* 20.59%, $P<0.05$), but the incidence rate of other mutation patterns was not significantly different between genotype B and C.

Conclusions: This study emphasizes that a LMV-resistance test before treatment is of great importance to approach a rational and optimal CHB therapy.

D-107

Tigecycline susceptibility and distribution of efflux systems in multidrug resistant *Acinetobacter baumannii* isolates in Western China

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Background: Emergence of multidrug resistant (MDR) *Acinetobacter baumannii* has become a global challenge for the infection control. Tigecycline has been described as a new hope for the treatment of MDR *A. baumannii* infections, but there are still no uniform interpretive criteria for tigecycline against this microorganism. Recently, reduced activity of tigecycline against *A. baumannii* has emerged in many countries, and drug efflux systems were found to be involved in it. In this study, tigecycline susceptibility and distribution of efflux systems in MDR *A. baumannii* isolated from ten hospitals in Western China was determined.

Methods: A total of 26 MDR *A. baumannii* isolates recovered from the clinical specimens of hospitalized patients in ten hospitals in Western China was identified further by multiplex PCR. Minimum inhibitory concentrations (MICs) for tigecycline against MDR *A. baumannii* was determined by agar dilution method. The MIC results were interpreted using the US FDA tigecycline susceptibility breakpoints for *Enterobacteriaceae* (susceptible [S] ≤2 μg/mL; intermediate [I] 4 μg/mL; resistant [R] ≥8 μg/mL). The presence of *adeB*, *adeR*, *adeS*, *adeY*, *adeJ*, and *adeE* genes was investigated by PCR and confirmed by sequencing, respectively.

Results: The MICs of tigecycline for the 26 MDR *A. baumannii* in this study ranged from 2-8μg/mL. The percentages of susceptible, intermediate and resistant isolates were 46.2% (12/26), 42.3% (11/26), and 11.5% (3/26), respectively. The *adeB*, *adeR*, *adeS*, and *adeJ* genes were identified simultaneously in all (100%) the isolates, but neither *adeY* nor *adeE* gene was found in any isolate. It demonstrated that the AdeABC efflux system, regulated by AdeSR two-component system and found to be associated with tigecycline non-susceptibility in previous reports, was identified in all the isolates in this study. The AdeIJK efflux system, likely only contributing to intrinsic resistance, was also widely distributed in these isolates. The AdeXYZ and AdeDE efflux systems were not identified.

Conclusion: Tigecycline non-susceptible *A. baumannii* strains were widely recovered in Western China. The AdeSR regulating AdeABC efflux system could play an important role in the reduced activity of tigecycline against these isolates.

D-108

Performance Evaluation of a Prototype CMVG Assay on the ADVIA Centaur® System

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Objective: Cytomegalovirus is a member of the herpes virus family. Diagnosing CMV infection is aided by serological testing. We report the evaluation of exposure to CMV with a fully automated CMVG assay* using viral lysate in a chemiluminescence immunoassay format for the qualitative detection of CMV-specific IgG antibodies in serum or plasma on the ADVIA Centaur system (Siemens, Tarrytown, NY, US).

Methods: The diagnostic sensitivity and specificity of the assay were evaluated by testing two commercially available seroconversion panels; 2227 apparently normal samples; and samples positive for Epstein-Barr virus (EBV), herpes simplex virus (HSV), toxoplasma, rubella, varicella-zoster virus (VZV), rheumatoid factor (RF), hepatitis C virus (HCV), hepatitis A virus (HAV), and hepatitis B virus (HBV). The results were reported in index values as reactive (≥1.00), equivocal (≥0.75, <1.00), and nonreactive (<0.75). All samples were run against the VIDAS CMV IgG assay as well. Discordant samples were tested on the IMMULITE® 2000 CMV IgG assay.

Results: The ADVIA Centaur CMVG assay detected IgG reactivity on the same bleed as the VIDAS CMV IgG assay on both the Profile Diagnostic and the Boston Biomedica, Inc., panels. Among apparently normal samples, positive agreement with the comparative assay was 98.75% and negative agreement was 99.24%. After resolution of samples, the ADVIA Centaur CMVG assay's sensitivity was 99.86%, with 1 nonreactive and 1 equivocal sample (1422/1424); after resolution, the assay's specificity was 99.62%, with 1 reactive and 2 equivocal samples (787/790). All cross-reactive samples were evaluated, with no observed change in clinical interpretation. The ADVIA Centaur CMVG assay had a total %CV of <7.8% over a 20-day period.

Conclusion: The results of these studies show good performance of the fully automated ADVIA Centaur prototype CMVG assay in comparison with the VIDAS CMV IgG assay.

* For investigational use only. Not available for sale. The performance characteristics of this product have not been established.

D-109

Study of the effect of Hepatitis B virus X gene on the expression of SPG21

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Background: Hepatitis B virus (HBV) infection causes acute and chronic liver disease, which finally leads to liver cirrhosis (LC) and hepatocellular carcinoma (HCC). The SPG21 is a multifunctional protein. The aim of this study was to investigate the effect of HBV X gene on the expression of SPG21.

Methods: The expressions of SPG21 mRNA and protein in HepG2 and HepG2.2.15 cells were tested by RT-PCR and western blot. HepG2 cells were co-transfected with reporter plasmid pGL3-SPG21 and plasmids carrying individual genes of HBV, the luciferase activity was measured and the expressions of SPG21 were detected by RT-PCR and western blot.

Results: The expressions of SPG21 mRNA and protein were higher in HepG2.2.15 cells than in HepG2 cells (0.36±0.06 vs 0.21±0.05, $P < 0.05$).The activity of SPG21 in HepG2 cells transfected with pCMV-X was higher (875±27 vs 67±12, $P < 0.01$)

as compared to blank control group (transfected with pCMV-tag2B). HBV X gene enhanced SPG21 gene promoter activity, SPG21 mRNA expression and SPG21 protein production in HepG2 cells in a dose-dependent manner.

Conclusion: HBV X gene can specially activate SPG21 expression.

D-110

Two-step DNA Isolation Followed by PCR in a Fully Automated System to Detect Septicemia Agents in Whole Blood

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Objective: To develop a novel nucleic acid isolation and purification procedure on the Rheonix CARD® molecular diagnostic platform to achieve fully automated diagnosis of bloodstream infections within a three hour time frame. Relevance: The current "gold standard" method to detect bloodstream infections relies upon blood culture methods. The main disadvantages of this method are the time delays between availability of test results and the initiation of medical treatment as well as the low analytical sensitivity of the test. Since mortality rates in sepsis patients increase by approximately 8% for every hour of delay, alternatives need to be developed to overcome these shortcomings. PCR based molecular tests can potentially overcome many of these issues, however, the financial cost, personal training and the stringent lab controls required to prevent carryover and cross contamination remain serious roadblocks to widespread molecular testing in hospital labs. The Rheonix SeptiCARD is a low cost, high sensitivity and fully automated microfluidic device designed to conduct multiplexed microbial detection for sepsis diagnosis. Methodology: For high sensitivity blood sample molecular testing, the purity and quality of the nucleic acid template is one of the most critical factors. We have developed a dual-stage purification method that incorporates both magnetic bead-based and silica column based nucleic acid isolation and purification schemes into a single CARD device for fully automated molecular analysis. Once 1 ml of whole blood sample is introduced, cell lysis, dual-stage DNA purification, multiplex PCR and endpoint detection on a low density DNA array are automatically performed without any further user intervention. Validation: Whole blood was spiked with defined numbers of *Candida albicans*, E *coli* and *enterococcus* and 1.0 ml samples were processed on the bench top and on the CARD. The total recovery and purity of isolated DNA was evaluated by comparing the 260/280 nm and 260/230 nm ratios, electrophoresis gel of the isolated genomic DNA and the PCR amplicons and the microarray results. Comparison between the single and dual-stage purification methods demonstrated higher PCR detection sensitivity for the dual-stage purified sample in every test case.

Conclusions: The dual-stage nucleic acid purification scheme was developed and verified on the bench top. The concept was then incorporated into Rheonix SeptiCARD® design and the device fabricated. When the SeptiCARD is placed into the Rheonix EncompassMDx workstation, which can simultaneously run six such CARD devices, up to 12 individual samples can be automatically analyzed within a three-hour period. Carryover or cross contamination is also avoided by the closed nature of the SeptiCARD device.

D-111

Comparison of the Sensitivity and Accuracy of Nucleic Acid Based Tests for MRSA

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Background: Hospital acquired infections (HAI) are a major public health threat and methicillin resistant *Staphylococcus aureus* (MRSA) is a significant source of these infections. Infection rates are increasing (Hidron, et al., Infect Control Hosp Epidemiol. 2008; 29:996-1011). *Staphylococcus aureus* is part of the normal flora of the skin and upper respiratory tract; however, when *Staphylococcus aureus* acquires the mecA gene, it becomes resistant to methicillin. Colonization with the methicillin resistant form of *Staphylococcus aureus* greatly increases the chance that a patient will develop a hospital acquired infection and die from that infection (Davis, et al., Clin Infect Dis 2004; 39:776-82.). Therefore, many states have mandated reporting of hospital acquired MRSA infections, and hospitals have implemented MRSA surveillance programs. Although traditional culturing methods can be used for the detection of MRSA, amplified nucleic acid based tests can provide faster and more cost-effective means for detection of MRSA colonization. This study was undertaken to compare the sensitivity and accuracy of market leading nucleic acid based IVD tests for MRSA.

Methods: *Staphylococcus aureus* methicillin resistant (MRSA ATCC# 43300) and methicillin sensitive (MSSA ATCC# 29213) were grown to logarithmic phase, then heat inactivated. Just prior to heat inactivation, an aliquot of the culture was taken, serially diluted and plated on LB agar plates. The resultant colonies were counted in order to estimate the colony forming units (CFU) per milliliter in the stock undergoing inactivation. The heat inactivated stocks were used to make a panel containing a dilution series with concentrations from 1.0 x 10^5 CFU/mL down to 1.0 x 10^2 CFU/mL. The panels were tested on various assay including the Cepheid GeneXpert MRSA and Cepheid SA Nasal complete assays, the BD GeneOhm Assay, the Hain Genotype MRSA Assay and the Hain Genoquick MRSA Assay (CE marked), and the Roche MRSA Advanced Assay.

Results: All of the assays were challenged with 1.0 x 10^5 CFU/mL of methicillin sensitive *Staphylococcus aureus*, and they all correctly gave a negative result for MRSA, suggesting that the assays are all appropriately specific. The Hain assays had a lower limit of sensitivity of approximately 1.0 x 10^4 CFU/mL. The BD GeneOhm lower limit of sensitivity was 1.0 x 10^3 CFU/mL. Both assays from Cepheid as well as the Roche MRSA Advanced assays were even more sensitive, with lower limits of sensitivity of approximately 5.0 x 10^2 CFU/mL

Conclusions: All of the assays are specific and sensitive. However, the Cepheid assays and the Roche MRSA Advanced assays were the most sensitive and had the added advantage of very simplified and rapid specimen preparation steps.

D-112

Evaluation of HBs Antigen Enzyme Immunoassay for Chronic Hepatitis B Patients

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Background: The PCR based method for HBV-DNA levels is widely using in monitoring of a hepatitis treatment. However, HBV-DNA level falls immediately when treatment starting by nucleoside and nucleotide analogues such as lamivudine, adefovir, and entecavir. The HBs Antigen(HBsAg) levels are one of the important marker for hepatitis treatment monitoring. We evaluated Chemiluminescence Enzyme Immunoassay method (Sysmex HBsAg quantitative assay,Sysmex, Kobe,Japan)with chronic hepatitis B patients serum.

Methods: All serum samples were obtained at Toyama university Hospital (Toyama,Japan) after receiving informed consent for this study. The HBsAg levels in serum were analyzed using an HISCL-2000*i* automated chemiluminescence immunoassay analyzer and HISCL HBsAg reagent (Sysmex, Kobe, Japan). The comparison study for HBsAg levels were used ARCHITECT*i* 1000 (ABBOTT,USA) and for PCR based method of HBV-DNA levels were used Cobas PCR method (Roche).

Results: Basic analytical performance showed for Sysmex HBsAg method as follows: linearity 0.03 ~ 2500 IU/mL, within and day to day precision (CVs) were 2.3 ~ 3.2 %(3.2 ~ 1115 IU/mL, N=15). Correlation between Sysmex method(X) and ARCHITECT method (ABBOTT, X) were very well(Y= 1.78 X - 60, r= 0.96,N=38). The concordance study between Sysmex HBsAg levels and HBV-DNA levels with judged to be negative in HBV-DNA levels by Taqman PCR method were 100 % equality for HBV negative patients group(N=30) and 11.6 % equality for HBV positive patients group(N=30). Furthermore, Two cases of time course for treatment patients by nucleoside and nucleotide analogues within 5 months with HBsAg levels and HBV-DNA levels showed approximately similar decreasing curves. Other hand, one case with long term nucleoside and nucleotide analogues treatment patient showed HBV-DNA levels were decreased. However, HBsAg levels were increased.

Conclusion: Nucleoside and nucleotide analogues treatment decreasing HBV- DNA level in blood.However, covalently closed circular DNA(cccDNA) of HBV replicative intermediate level does not change,therefore, HBsAg is detected. Furthermore, recurrence of hepatitis is reported when HBV- DNA levels becomes less than detection limits, and stopped a treatment. Our data suggested that, both HBV -DNA levels and HBs Ag levels are useful in treatment by nucleoside and nucleotide analogues.

D-114

Comparison of rapid antigen test, ELISA, and real-time PCR assays for the detection of norovirus infection

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Background: Norovirus is a leading cause of epidemic and sporadic acute gastroenteritis worldwide. Because of the rapid transmission of the virus, early detection is important to prevent outbreak of norovirus infection. To evaluate the performance of the newly developed rapid antigen test for detecting human norovirus in stool specimens, we compared it with the established ELISA test and real-time

reverse transcription PCR.

Methods: One hundred and eighty-four stool samples were analyzed by QuickNavi-Noro (rapid antigen test), RIDASCREEN Norovirus (ELISA), and RIDAGENE Norovirus V (qRT-PCR). Overall percent agreement, percent positive agreement (PPA), and percent negative agreement (NPA) of rapid antigen test compared to ELISA and qRT-PCR were obtained.

Results: Positive rate of rapid antigen test, ELISA, and qRT-PCR from 184 stool specimens was 44.0% (81/184), 51.6% (95/184), and 42.9% (79/184), respectively. Seventy samples (38.0%) showed all positive results among three methods, and 86 samples (46.7%) showed all

negative. Overall percent agreement, PPA, and NPA of rapid antigen test compared with qRT-PCR were 89.1%, 88.6%, and 89.5%, respectively, and overall percent agreement, PPA, and NPA of rapid antigen test compared to ELISA were 90.2%, 83.2%, and 97.8%, respectively. Total procedure of rapid antigen test was finished within 20 minutes.

Conclusions: This rapid antigen test was easier and quicker to perform, and showed high agreement rate with ELISA and qRT-PCR. This test seems to be a useful tool for rapid screening of norovirus infection.

D-115

A Follow Up Study On Alteration Of Nitric Oxide, Malondialdehyde And Antioxidants In Patients With Visceral Leishmaniasis

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Background: Visceral leishmaniasis (VL), a vector borne parasitic infection, is caused by obligate intracellular parasites of Leishmania species. The parasites reside in macrophages and neutrophils of the human host. In response to the VL infection, macrophages and neutrophils generate large amount of highly toxic molecules, such as reactive oxygen species (ROS) and reactive nitrogen species (RNS) leading to oxidative stress. So, the study was undertaken to investigate and compare nitric oxide, malondialdehyde and antioxidant status in patients with VL before, after treatment with standard anti-leishmanial drugs and healthy controls.

Methods: This is a longitudinal study which included 25 confirmed cases of visceral leishmaniasis from the tropical ward of B. P Koirala Institute of Health Sciences. These cases were followed after four weeks of standard antileishmanial treatment. The age and sex matched healthy subjects from the same endemic area were enrolled as control. Nitrite, Malondialdehyde (MDA), Catalase (CAT), Superoxide dismutase (SOD) Glutathione peroxidase (GPx), Reduced Glutathione (GSH), Vitamin C and Vitamin E were assayed in blood samples of VL patients before treatment, after treatment and age-sex matched healthy controls .

Results: Plasma nitrite level was significantly lower whereas plasma MDA level was found significantly higher in VL patients before treatment compared to healthy controls ($p<0.001$). Enzymatic antioxidants (CAT, GPx and SOD) and non enzymatic antioxidants (Reduced glutathione, Vitamin C and Vitamin E) were lowered in VL patients before treatment ($P<0.001$) as compared to healthy control. Following anti-leishmanial treatment, statistically significant elevation of plasma nitrite and lowered plasma MDA level was observed, when compared to after treatment. Furthermore, antioxidant levels were also improved in VL patients after treatment compared to VL patients before treatment and it was statistically significant.

Conclusion: Finding of the study revealed an increased oxidative stress in VL patients before treatment. After treatment, increased plasma nitrite level, improved antioxidant status, and decreased malondialdehyde showed that oxidative stress was decreased and improved immune status of the patients.

D-116

Association between Polymorphisms in Interleukin-18 Gene Promoter and Hepatitis B Recurrence after Liver Transplantation in Chinese Han Population

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Background: Interleukin 18 (IL-18) is a potent proinflammatory cytokine, which can promote viral clearance. We investigated the effect of IL-18 gene G-137C and A-607C polymorphisms and IL-18 serum levels on HBV recurrence in liver transplant patients.

Methods: A total of 130 liver transplant patients were enrolled into this study. The patients' mean follow-up was 44 mo (range 16-56 mo). All liver transplant recipients were in a stable stage. We studied two single-nucleotide polymorphisms in the promoter region of IL-18 gene at the position -137 and -607 by high-resolution melting (HRM) curve analysis. The serum levels of IL-18 and IFN-γ were tested by ELISA. The patients (n=130) were divided into end-stage liver disease secondary to hepatitis B (n=81) and end-stage liver disease secondary to other diseases (n=49). Patients who became positive for HBsAg or showed elevation in HBV-DNA after transplantation were regards as HBV recurrence. There were 11 patients who had HBV recurrence after liver transplantation.

Results: IL-18 G -137C ($P=0.021$ OR, 4.47; 95% CI, 1.16-17.24) were significantly associated with HBV clearance. The allele -137G ($P=0.009$ OR, 4.00; 95% CI, 1.32-12.13) was also strongly associated with HBV clearance and avoiding HBV recurrence. The patients with the GG genotype had higher IL-18 and IFN-γ serum levels than the GC+CC genotypes, but the difference was not statistically significant ($P>0.05$). The serum levels of IFN-γ were higher in HBV no recurrence group than HBV recurrence group (figure 1.$p<0.01$). No association was found between IL-18 A-607C and IL-18 serum levels and HBV recurrence.

Conclusion: These results suggested that IL-18 gene promoter polymorphism may affect the outcome of HBV infection after transplantation. IL-18-137 G was a protective factor for HBV recurrence after liver transplantation. The non-137G HBV carriers were at higher risk of HBV recurrence and this subgroup should be reinforced with antiviral therapy.

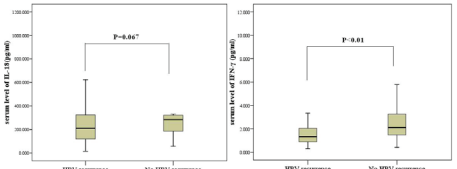

Figure 1 The IL-18 and IFN-γ serum levels between HBV recurrence group (n=11) and No HBV recurrence group (n=70). data are represented as median and data were analyzed by the Mann-Whitney U test.

D-117

Aberrant expression of soluble co-stimulatory molecules in patients with HBV infection

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Background: T cell co-stimulatory factors CD28 and CTLA-4 and their ligands CD80 and CD86 are present as receptors on T cells and antigen-presenting cells and also in soluble forms in the circulation. They are important for T lymphocyte mediated inflammatory responses. The objective of the present study is to learn whether they have a potential role in modulating anti-virus immune responses during HBV infection process.

Methods: We investigated the soluble costimulatory molecules CD80, CD86, CD28, CTLA-4 and inflammation factor IFN-γ in the sera of 20 patients with HBV infection and 20 healthy controls. Circulating levels of soluble co-stimulatory molecules were quantified by enzyme linked immunosorbent assay. We further correlated these soluble co-stimulatory molecules to other clinical parameters of importance such as HBV-DNA and quantity of HBeAg. Statistical analysis was performed by Mann-Whitney test and Spearman's correlation rank test.

Results: Serum concentrations of sCD80, sCD86, sCTLA-4 were significantly higher in HBV infected patients than those in control subjects (all $P < 0.05$). There was no significant difference for sCD28 between the two groups. The level of IFN-γ was significantly higher in patients than in the control. The levels of these factors were not related to HBV load. There was, however, a significant correlationship between the concentrations of sCD86 and HBeAg load.

Conclusion: The aberrant expression of soluble co-stimulatory molecules can be related to the activation of T cells in the progression of inflammation in HBV infection. CD86 acts as the costimulatory signal, leading to activation of T cells and production of IFN-γ, finally progressing to necrotizing inflammation. CD80 works with CTLA-4, down-regulating Th1 response and activation of CD8+T, resulting in impairment of killing immunity. Negative costimulatory molecules may have more important function in the anti HBV immunity.

D-118

Performance evaluation of the ADVIA Centaur XP HIV Ag/Ab Combo assay; ten months of routine use in a laboratory serving one million inhabitants

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Background: The ADVIA Centaur XP fourth generation HIV test (HIV1-2 antibody and p24 antigen) (Siemens Healthcare, Tarrytown, NY, USA) has been introduced as a screening test in January 2010 in the Laboratory that serves the entire Area Vasta Romagna, an area in central Italy with a population of about one million. The Laboratory carries out HIV serological tests for inpatients (admitted to clinical units in four large/middle size hospitals and in four small size hospitals). Our aim was to assess the suitability of Centaur analyzer and reagents to a mixed clinical environment requiring high throughput, sensitivity and specificity.

Methods: Between January 20th and November 20th 2010 43,515 samples have been assayed for HIV in our laboratory using Centaur analyzer and reagents. All the positive samples have been assayed using a further fourth-generation HIV test (Vidas HIV DUO Quick, Biomerieux, Marcy l'Etoile, France). The samples not known as infected that were confirmed positive underwent confirmatory RIBA (Chiron RIBA HIV-1/HIV-2 SIA, Ortho Clinical Diagnostics, Raritan, NJ, USA). In the case of negative or indeterminate RIBA, the antigen p 24 (Vidas P24II, Biomerieux) was researched. In the case of discordant results between the two screening tests a third screening test (Vironostika HIV Ag/ab A6 - Ortho Clinical Diagnostics) was carried out.

Results: ADVIA Centaur XP HIV Ag/Ab Combo assay yielded 42,696 negative results. We did not further investigated 169 samples among 819 Advia Centaur positive results obtained in known infected patients.The other 650 samples have been further assayed: 260 were confirmed to be positive and 390 had a negative result. Six seroconversions were found between January and November 2010.

Conclusion: The Centaur analyzer and reagent are suitable for the large workload of our laboratory that requires robust and highly automated analyzers. Test specificity was 99.09% while sensitivity was not calculated because HIV negative samples using Centaur were not subjected to further assay. The test positive predictive value (PPV) in our population was 40% for index value > 0.90, and 90% for index value >12.

D-119

Development of Reverse Transcriptase Polymerase Chain Reaction based Interferon Gamma Release Assay to Detect Mycobacterium tuberculosis Infection

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Background: Tuberculosis (TB) continues to be one of the most critical infectious disease and causes 3 million deaths annually. About one-third of world population is latently infected with Mycobacterium tuberculosis (Mtb). Recently, new immunodiagnostic tests for TB have been developed and called Interferon gamma releasee assay (IGRA). This assay is based on the host's immune response to Mtb specific antigens; ESAT-6, CFP-10, TB7.7. Now available commercial IGRA kit such as IFN-γ ELISA and ELISPOT have showed higher specificity and sensitivity than conventional tuberculin skin test (TST). However, IGRA test needs higher cost and has some limitations; limited number of samples tested at once and could not completely detect Mtb infection.

Methods: In this study, therefore, we have developed alternative IGRA using RT-PCR firstly. RT-PCR based IGRA quantify the expression level of IFN-γ mRNA extracted from Mtb specific antigens stimulated whole blood.

Results: Consequently, it is able to reduce turn around time, cost for test, volume of blood. Furthermore, it could have higher reproducibility and sensitivity than conventional IGRA and TST.

Conclusion: For convenience of test procedures, we need to set other quantification method such as quantitative real-time RT-PCR or real-time NASBA.

D-120

Comparison of neutrophil volume distribution width to C-reactive protein and procalcitonin as a proposed new marker of acute infection

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Background: The aim of this study was to assess the ability of Neutrophil Volume Distribution Width (NDW) and compare it to C-reactive protein (CRP) and procalcitonin (PCT) in the detection of early sepsis in Intensive Care Unit.

Methods: Coulter LH750 hematology analyzer (Beckman Coulter, Inc, Fullerton, CA) was used to obtain the total white blood cell (WBC) count, absolute neutrophils count (ANC), and NDW. NDW is the standard deviation of each cell passing through the aperture, and represents the neutrophil size variability. CRP was measured using particle-enhanced immunoturbidimetric assay on Roche Modular analytics system (Roche Diagnostics, Mannheim, Germany). This method has a measuring range from 0.5 to 258 mg/L. Imprecision was less than 5% at different levels. Reference range for healthy individuals was up to 2.5 mg/L. PCT was measured using the enzyme-linked fluorescent assay (B.R.H.A.M.S) on the VIDAS instrument (BioMérieux Sa, RCS Lyon, France). The assay had a measuring range from 0.05-200 ng/mL. Imprecision varied between 3% to 7% at concentrations of 0.22 to 155 µg/L, respectively. Reference range was up to 0.09 µg/L. For clinical evaluation 166 subjects were divided: healthy (1st), acute inflammatory (2nd), localized infection (3rd), systemic infection (4th), and hematologic disorder (5th) groups according to clinical history and cultures. NDW, CRP and PCT were compared among different groups using multivariate analysis of variance (MANOVA). Diagnostic efficacy was assessed using receiver operating characteristic and area under the curve (AUC).

Results: Using replicate sample measurements (n=78), between-run imprecision was 2.7%, 2.48%, and 3.23% for three levels of hematology controls with mean NDW of 24.5, 28.0, and 32.7, respectively. Within-run imprecision was 2.6 and 2.2% at mean NDW of 21.7 and 24.6, respectively. Lower limit of quantification was estimated at a WBC of 1000 /µL (%CV = 12.3) or ANC of 100 /µL (%CV = 0) where NDW was 56.0 (%CV = 1.0). Results for NDW were stable up to 2 hours post collection, after which they gradually increased (16% at 3 hours). NDW results were within ± 7% (result=28.9) when several dilutions (up to 10 fold) were applied using LH750 dilution buffer (sample with WBC around 50000 /µL and ANC around 40000 /µL; r^2 = 0.9771). Regarding the study groups lowest $mean_{NDW}$ was for 1st (n=41), followed by 2nd (n=20), 3rd (n=55), 4th (n=50) and 5th (n=10) group. AUC_{NDW} was higher than AUC_{CRP} and AUC_{PCT}, and equals 0.877 for infected (group 3+4) versus non-infected (group 1+2) groups, and 0.965 for systemic infection versus non-infected groups. A cutoff=21.9 resulted in 90% sensitivity, 92% specificity, 90% positive predictive value, and 92% negative predictive value (AUC_{NDW}=0.965, 95%CI:0.935-0.995). According to MANOVA, only NDW is able to differentiate acute inflammatory process from suspected early infection in postoperative patients (p=0.000).

Conclusion: NDW analytical performance is comparable to other hematology indices. It has the highest diagnostic accuracy when compared to CRP and PCT, and is available with CBC. It may be a promising parameter to aid in the diagnosis of acute infection in adults, provided the possibility of hematologic disorders is first ruled out.

D-121

Hpv 53 And 66: Can They Be Classified As Intermediate Risk?

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Background: HPV genotypes 53 and 66 (species 6, genus alpha) have been variously classified as low-risk (LR), high-risk (HR), or probable high-risk. Their pathogenicity is still debated and only a few data about histological and cytological patterns associated with them are reported in the literature. With our data we try to contribute to the evaluation of their role in HPV infection.

Methods: cervical scrape specimens were obtained (from 2005 to January 2011) from 3923 women, with ages between 16 and 80 years (mean age 36.7 years). HPV genotyping was performed by PCR amplification and hybridisation reaction, using commercial kits AlphaStrip HPV (Alphagenics) and INNO-LiPA HPV Genotyping Extra (Innogenetics). Cytological classification was reported according to the Bethesda System 2001.

Results: HPV DNA was detected in 2518 (64.2%) of patients tested, with 24.8% of samples hybridising to more than one HPV type. HPV 53 genotype was found in 267 patients (6.8% prevalence, 10.6% of the positive cases), of whom 161 were co-infected with HR-type HPV, 7 co-infected with HPV 66 (2 ASCUS, 1 LSIL, 3LSIL-

CIN1, 1 CIN3), 20 co-infected with LR-type HPV (1 negative, 10 ASCUS, 1 LSIL, 7 CIN1, 1 CIN2/3), 1 with both LR and HPV 66 types. In 78 cases HPV 53 was the only infective agent (9 negative, 25 ASCUS, 11 LSIL, 2 LSIL-CIN1, 23 CIN1, 1 CIN1/2, 1CIN2, 1 CIN3, 5 with no data). HPV genotype 66 was found in 222 patients (5.7% prevalence, 8.8% of the positive cases), of whom 130 co-infected with HR-type HPV, 7 with HPV 53, 6 with LR-type HPV (2 ASCUS, 2 LSIL, 2 CIN1), 1 with both LR and HPV 53 types. In 78 cases HPV 66 was present as the only infective agent (7 negative, 30 ASCUS, 9 LSIL, 10 LSIL-CIN1, 19 CIN1, 1 CIN2, 2 with no data).

Conclusion: as reported above, the pathogenic action of HPV genotype 53 and 66, each of them present as the only infective agent, was documented in 73 (out of 78) and 76 (out of 78) cases respectively. The ratio between cases of low-grade lesions or mild dysplasia and cases with no lesions or with cells of undetermined significance was 39/34 (1.15) for HPV genotype 53 (for which 2 cases of moderate dysplasia were documented) and 39/37 (1.05) for HPV genotype 66. In the 7 cases of co-infection with 53 and 66 the ratio was 5/2. Both genotypes appear to be often associated with LSIL/CIN1 and this suggests a greater aggressiveness than genotypes classified as LR. However, we have found neither genotype in cervical squamous cell carcinoma (SCC), in accordance with the literature, as SCC/LSIL ratios are usually reported in the range between 0.01 and 0.07. The classification of genotypes 53 and 66 as intermediate risk is therefore fully consistent with the reported data.

D-122

Prevalence of HIV and co-infection with HIV/chronic Hep b in an urban center in Eastern Nigeria

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Background: HIV and Hepatitis b are major public health problems in Nigeria .The prevalence of Hep B and HIv co-infection in Eastern Nigeria is not well known.

Objectives: We sought to determine the seroprevalence of HIV, hep B and HIV/Hep B co-infection among patients at a laboratory in eastern Nigeria.

Methods: This was a retrospective chart review of all the patients that had blood samples drawn from Jan 1 2010 to December 1 2010.A total of 720 patients had blood test drawn at the Guinness Eye center, Onitsha. The HIV kit used was the Determine Kit and Star pack kit .The Kit used for Hep B was the ACON kit.

Results: 50/720 (6.9)% of the patients were HIV positive. 7/50 of these patients (14%) were co-infected with HIV and chronic Hep B. 30/50(60%) of the HIV patients were male and 20/50(40%) were female. The average age of the patients was 18-50 years.

Conclusion: -The high prevalence rates of HIV and co-infection with both HIV and Hepatitis B in an urban center are of great concern. These patients could serve as possible means of continued diseases transmission. Hepatitis B vaccination is urgently needed in the HIV population with good CD4 counts to prevent co-infection with hepatitis b. This observation is also important in choosing treatment options that are active against both virues.

D-123

Distribution Of Hpv Genotypes In Cervical Samples From Northern Italy Women

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Background: HPV is the most common sexually transmitted infection worldwide. Two HPV vaccines are currently used to prevent the infection: Gardasil and Cervarix. While both vaccines protect against the two high-risk HPV types 16 and 18, that cause about 70% of cervical cancers, Gardasil also protects against the two low-risk types 6 and 11, responsible for 90% of genital warts. In Italy, a nationwide vaccination program with Gardasil was approved in 2007. In order to be effective, vaccines must work against high-prevalence HPV genotypes. However, high-risk HPV genotypes, with a not negligible prevalence in a population may be found, that are not present in the vaccine. With our data we evaluate the prevalence of identifiable HPV genotypes in the tested population of Northern Italy: such an evaluation may be useful in order to address toward more effective strategies of vaccinal prophylaxis.

Methods: cervical scrape specimens were obtained (from 2005 to January 2011) from 3923 women, with ages between 16 and 80 years (mean age 36.7 years). HPV genotyping was performed by PCR amplification and hybridisation reaction, using commercial kits AlphaStrip HPV (Alphagenics) and INNO-LiPA HPV Genotyping Extra (Innogenetics).

Results: HPV DNA was detected in 2518 (64.2%) of patients tested, with 24.8% of samples hybridising to more than one HPV type. A total number of 3456 infecting genotypes were identified (the prevalence in the tested population is reported in parentheses); among high or intermediate risk HPV genotypes we detected: genotype 16(16.9%); 31(9.3%); 58(7.0%); 53(6.8%); 66(5.7%); 18(5.3%); 52(4.0%); 59(3.4%); 56(3.3%); 51(3.2%); 35(2.9%); 33(2.9%); 45(2.3%); 39(2.3%); 73(1.0%); 68(0.8%); 82(0.7%); 26(0.1%). Among low-risk genotypes we detected: genotype 54(4.3%); 42(3.1%); 6(3.0%); 61(2.3%); 70(2.1%); 40(1.5%); 11(1.0%); 44(0.9%); 34(0.8%); 74(0.8%); 43(0.4%); 69/71(0.3%).

Conclusion: among high-risk HPV, genotypes 16, 31 and 58 are those more frequently found in the tested population, while genotype 18 is only the sixth one in order of prevalence. Among low-risk HPV, genotype 6 has a lower prevalence than genotypes 54 and 42, while, in order of prevalence, genotype 11 is only the seventh one. Both vaccines currently employed offer only a partial cross-protection against HPV-31 and anyway they cannot prevent infection due to various other genotypes. They are also expected to cause a positive selective pressure on the HPV genotypes that are not present in the vaccine, with consequent future higher diffusion of the same genotypes. Therefore, it must be taken into consideration the hypothesis of employing, in a not so far future, second generation wider spectrum vaccines, that in our geographic area should include at least also HPV genotypes 31 and 58.

D-124

Performance Evaluation of the Vitros Hepatitis C Virus Antibody Detection Assay in Korean Patients

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Background: Approximately 20% of patients infected with hepatitis C virus (HCV) progress to liver cirrhosis, and 3% of HCV infection can cause hepatocellular carcinoma. Effective assays for the detection of antibodies to HCV play an important role in diagnosis of HCV infection and preventing the spread of the disease. We aimed to evaluate the precision and diagnostic performances of the Vitros anti-HCV antibody detection assay in Korean population, where the prevalence of viral hepatitis is relatively high.

Methods: Precision performance was assessed based on guidelines from the Clinical and Laboratory Standards Institute (CLSI) document EP4-A2. Two levels of quality control (QC) materials were assayed in replicates of two at two separate times per day for 20 days. Additionally, total 1,011 sera from patients with suspicious HCV infections were collected and assayed for anti-HCV antibody with Vitros (Ortho Clinical Diagnostics) and Cobas (Roche Diagnostics) anti-HCV assay kits. Specimens negative for both tests were considered as negative for HCV infection, and those positive for any of the two assays were tested with another anti-HCV assay (Architect Anti-HCV, Abbott Diagnostics). Samples positive for all the three assays were regarded as positive for HCV infection. Discrepant results between the three assays were confirmed using Chiron Recombinant Immunoblot Assay (RIBA) HCV 3.0 Strip Immunoblot Assay kits (Ortho) and HCV RNA quantification (Cobas Ampliprep/Taqman HCV assay, Roche), and RIBA-positive samples were also regarded as positive for HCV infection. Assay interference was assessed through testing of additional 90 specimens from the following categories without clinical evidence of HCV infection: pregnancy (n=20), anti-nuclear antibodies-positive (n=20), hemolysed (n=10), lipemic (n=10), icteric (n=10), anti-HBc-positive (n=10) and anti-HIV antibodies-positive (n=10).

Results: Within-run and total precisions of the Vitros anti-HCV assay were respectively 6.6% and 11.6% coefficient of variation (CV) for a QC material with mean signal to cutoff ratio (S/CO) of 0.09, and 2.6% and 3.3% CV for that with mean S/CO of 6.22. Among the 1,011 serums tested for anti-HCV, 794 were negative by two assays, and 200 were positive for all three tests. Samples from 17 patients showed discrepant results between the three anti-HCV assays, and 6 and 3 of them were respectively positive and negative for anti-HCV antibodies by RIBA tests. The rest 8 cases showed indeterminate results, and HCV RNA was not detected from all of the discrepant cases. Sensitivity and specificity of the Vitros assay for detecting HCV infections were 99.5% (95% confidence interval (CI) = 97.3 to 100.0%) and 99.6% (95% CI = 98.9 to 99.9%) after the 8 RIBA-indeterminate cases were excluded. PPV and NPV of that assay were 98.6% (95% CI = 95.8 to 99.7%) and 99.9% (95% CI = 99.3 to 100.0%), when disease prevalence was 20.5%. Of the total 90 specimens from individuals without clinical evidence of HCV infection, none showed false-positive results by the assay.

Conclusion: The Vitros anti-HCV assay showed good precision performances and great diagnostic performances. This assay can be sufficiently used in routine clinical practices to screen for HCV infection from Korean population.

D-125

Detection and Characterization of Extended Spectrum β-lactamase (ESBL) and Metallo β-lactamase (MBL) in Pseudomonas aeruginosa and Acinetobacter spp

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Background: The Extended Spectrum β-lactamase (ESBL) are enzymes originally described as enterobacteria that confer bacterial resistance to β-lactamic antibiotics except for carbapenems. In recent years the presence of these enzymes has been observed in non-fermenting Gram-negative bacilli, such as Pseudomonas aeruginosa and Acinetobacter spp, which are among the main agents of hospital infections. The Metallo β-lactamase (MBL) are enzymes described mainly in non-fermenting Gram-negative bacilli, which confer resistance to all β-lactamic antibiotics except to aztreonam.The objective of this study was to determine the frequency of ESBL and MBL in isolates of P. aeruginosa and Acinetobacter spp in our environment. A descriptive observational study of transverse sections was conducted with sampling of consecutive cases of isolates of P. aeruginosa and Acinetobacter spp., from diverse biological samples (respiratory, blood, urine, surgical materials, etc.) of patients in the different participating health care facilities in Paraguay, from June 2008 to July 2009. Isolates with inhibition zones for ceftazidime and/or cefepime less than or equal 18 mm, and/or halos of imipenem and/or meropenem less than or equal 21 mm were included in this study.

Methods: Sensitivity to different antibiotics was determined by the agar diffusion method, following the rules of the Clinical and Laboratory Standards Institute for phenotypic detection of the presence of ESBL and MBL. Detection on isolates with positive phenotype was performed by PCR of CTXM, TEM SHV, GES, IMP and VIM genes.

Results:ESBL phenotypic parameters were present in 4.1% (10/242) of isolates of Acinetobacter sp and 15.1% (23/152) of P. aeruginosa, and MBL phenotypic parameters in 1.7% (4/242) of isolates of Acinetobacter sp and 7.2% (11/152) of P. aeruginosa. The presence of coding genes for ESBLs has been confirmed in 6 of 7 isolates of Acinetobacter spp, having found enzymes of the types TEM and CTX-M, and on P aeruginosa the presence of GES-type genes was confirmed in 2 of the 10 isolates analyzed so far. In terms of MBL, gene amplification was obtained in 2 of the 4 isolates of Acinetobacter spp, one VIM-type and another IMP-type. In P. aeruginosa, the IMP gene was detected in 2 isolates and VIM in 2 isolates.

Conclusion: The presence of both ESBL and MBL in non-fermentative granmnegative bacilli in Paraguay was confirmed. Routine phenotypic detection of these enzymes in P. aeruginosa and Acinetobacter spp. is recommended, given their importance. It should be mentioned that the molecular characterization of GES-type ESBL, and IMP- and VIM-type MBL has been implemented in Paraguay through this study.

D-126

In-vitro Assessment of Cell-Mediated Immunity by Demonstrating Effector-T Cells for Diagnosis of Tuberculosis and its Cross Reactivity to Leprosy in Nepalese Subjects

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Background: Tuberculosis (TB) remains a major public health problem in Nepal killing about 5000-7000 per year. Though numerous assays are available, accurate diagnosis of TB continues to be a major issue in laboratory medicine, due to poor sensitivity, time to result and cost of testing. The immune response against *mycobacterium tuberculosis* (MTB) is cell mediated. T-cells become sensitized when they encounter MTB antigens and subsequently activated effector T-cells produce a number of cytokines including interferon- γ (INF-γ) to fight the infecting organisms. Demonstration of either production of INF-γ or presence of effector T-cells sensitized to MTB specific antigens *in vitro* can be diagnostic for TB infection.

Objective of this study was to determine the efficacy of commercially available T-SPOT.*TB* kit which is used for the *in vitro* diagnosis of TB infection. Since leprosy is also endemic in Nepal and *M. leprae* is very homologous to MTB, our second objective was to determine if this test has any cross reactivity in leprosy patients.

Methods: 10 ml of heparinized blood sample was taken from 60 sputum AFB positive, 60 sputum AFB negative healthy controls and 20 cases of paucibacillary (PB) leprosy patients. The blood samples were processed to separate peripheral blood mononuclear cells. The final cell suspensions were cultured along with MTB specific antigens namely- Early Secretory Antigenic Target (ESAT-6) and Culture Filtrate Protein (CFP 10) along with negative and positive controls. The production of INF- γ was demonstrated by enzyme linked immunospot (ELISPOT) assay technique developed by T-SPOT.TB Oxford Immunotec; Oxford, UK.

Results: Six samples from smear positive patients and four from healthy controls were indeterminate due to insufficient responses to the positive control. All the remaining samples from smear positive patients produced INf-γ after exposure to MTB specific antigens. 8 (13.3%) of healthy controls were also demonstrated presence of the effector T-cells. The sensitivity and "specificity" for active disease of the ELISPOT (T-SPOT.*TB*) in respect to AFB microscopy was 100% and 85.7% respectively.

On the other hand, we found all PB leprosy patients to be negative by T-SPOT.*TB* suggesting that this test has no cross-reaction with leprosy even though L-ESAT, a *M. leprae* antigen is very homologous to the T-ESAT-6 used in this test.

Conclusion: T-SPOT.*TB* has an excellent sensitivity of 100%. Based on the high sensitivity in active disease, a negative test makes active TB infection unlikely. Therefore, this test can be very valuable in screening for tuberculosis. The lower specificity value in this study is probably due to the high prevalence of TB in our country so latent TB infection cannot be excluded in the control group. This test does not appear to have cross reactivity with leprosy.

D-127

A Multicenter Evaluation of the Analytical Performance of the ADVIA Centaur® EHIV Assay on the ADVIA Centaur CP Immunoassay System

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Background: The Siemens ADVIA Centaur CP Immunoassay System is a benchtop system that uses the same reagents, calibrators, controls, and ancillaries as the larger ADVIA Centaur Immunoassay System. The ADVIA Centaur HIV 1/O/2 Enhanced assay* (EHIV) is an antigen-bridging microparticle chemiluminometric immunoassay used for the detection of antibodies to human immunodeficiency virus type 1, including group O, and/or type 2 in serum or plasma. The objective of the study was to show concordance between the ADVIA Centaur CP and ADVIA Centaur systems and perform reproducibility studies on the ADVIA Centaur CP system.

Methods: The EHIV assay was evaluated at the Siemens Tarrytown laboratory and at two external sites. Reproducibility was evaluated with three reagent lots using a 12-member panel including two nonreactive, four HIV-1-positive, four HIV-2-positive, and two HIV-1 group O serum samples, along with low and high calibrators and positive and negative controls. A hierarchical precision analysis of variance (replicates nested in runs, runs nested in days) was done for each site and reagent lot and pooled across all three sites and all three lots. To ensure adequate volume and distribution, samples were contrived (i.e, samples were pooled) and supplied. A total of 430 nominally positive samples (target > 1.0 and including 395 HIV-1, 30 HIV-2, and 5 HIV type O) and 400 nominally negative samples (target < 1.0 index) were evaluated among three sites. Positive and negative agreements between the ADVIA Centaur CP and ADVIA Centaur systems were calculated for each site and pooled across sites. Bootstrap confidence intervals were calculated. Numerical patient sample correlations were calculated for three replicates of all samples on each platform, using Deming regression.

Results: Excluding the low index negative control, negative pools, and low calibrator, within-run and total precision pooled across sites and across lots ranged from 2.7% to 4.4% and from 9.1% to 15.2%, respectively. For the contrived positive and negative samples, the positive agreement between the ADVIA Centaur CP EHIV assay and the ADVIA Centaur EHIV reference assay across sites was 99.61%, (95% CI of 98.6% to 99.91%) and the negative agreement was 99.75% (95% CI of 99.26% to 99.90%). The nominally positive samples were all positive on the ADVIA Centaur system and each ADVIA Centaur CP system. Two nominally negative samples very near the cutoff were positive on the ADVIA Centaur system but not on each ADVIA Centaur CP system.

Conclusion: The compact ADVIA Centaur CP Immunoassay System uses the same reagents and ancillaries as the ADVIA Centaur Immunoassay System. It is generally easy and efficient to operate, generating results equivalent to those on the ADVIA Centaur system. The ADVIA Centaur EHIV assay performs well and efficiently on the ADVIA Centaur CP system, yielding equivalent results on both platforms. The ADVIA Centaur EHIV assay showed 100% positive agreement between the ADVIA Centaur® CP and the ADVIA Centaur instruments with nominally positive samples.

* Assay developed, manufactured, and sold by Siemens Healthcare Diagnostics Inc. for Ortho-Clinical Diagnostics, Inc.

D-128

Lookback of seropositivity for infectious diseases among 26.354 blood donors tested in a Brazilian reference laboratory during the year 2010

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Background: Blood transfusion saves lives and improves health, but millions of patients needing transfusion do not have timely access to safe blood. The WHO programme on Blood Transfusion Safety monitors key quantitative blood safety indicators to assess the global situation on blood safety, monitor trends and progress and identify priority countries for support. Blood transfusion was an important potential source of infectious diseases transmission. Remarkable progress has been made in transfusion safety from infection over the past three decades. Selection of healthy blood donors is essential to ensure blood safety. A laboratory guideline was designed by the Brazilian Regulatory Healthy Agency since 1992 and was recently reviewed and updated in December 2010. The risk donors are excluded by screening serological tests including screening for HIV(two screening methods), Hepatitis C, Hepatitis B, HTLV, Syphilis and Chagas Disease and their blood are excluded from use in transfusions if any sign of seropositivity is encountered. The introduction of more-sensitive viral-screening assays and the recent introduction of nucleic-acid amplification technology (NAT) have nearly eliminated transmission of HIV and hepatitis C virus (HCV) by blood transfusion in North America. In Brazil we have public and private blood banks regulated and inspected by the national Agency of Health surveillance that have recently recommended and will in the near future require the introduction of more sensitive screening tests to improve the blood transfusion safety.

Methods: During 2010 a Brazilian reference laboratory in Belo Horizonte, state of Minas Gerais, has received 26.354 blood samples from three private blood banks and screening serological tests for HIV, Hepatitis C, Hepatitis B, HTLV, Syphilis and Chagas Disease were performed, using two manufacturers Murex and Roche for all these tests.

Results: Positive serology was encountered in 2.5% of 26.354 total blood donations that were performed. Seropositive individual's tests varied and Hepatitis B was the serological test that showed the highest prevalence of 1.8% among the sampling. The other tests Chagas disease, HIV, Hepatitis C and Syphilis showed seropositivity between 0.05% to 0.3%, respectively.

Conclusion: The low rate of seropositive donors may reduce the risk of transfusion transmission of most diseases tests but the seropositivity and prevalence for Hepatitis B has indicated the importance of introducing nucleic-acid amplification (NAT) technology in Brazilian laboratories reducing occurrence of inconclusive results and improving blood transfusion safety.

D-129

Comparison of the Abbott Architect and the Johnson Vitros anti-HCV immunoassays results to the molecular detection of HCV RNA

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Background: Hepatitis C virus (HCV) is one of the most important causes of chronic liver disease. Acute HCV infection is usually asymptomatic. However, 85% of infected individuals develop chronic liver infection and frequently progress to cirrhosis and hepatocellular carcinoma. The screening test of HCV is anti-HCV antibody test by immunoassay and the chronic infection confirmed by nucleic acid testing of HCV. Several studies have found that specimens with low signal/cut-off (S/CO) ratios in the anti-HCV immunoassays are commonly negative when tested for HCV RNA. The objective of this study was to evaluate the possibility that positive results with low S/CO ratio in the Abbot Architect and Johnson Vitrus anti-HCV immunoassays might mean false-positive results for HCV chronic infection.

Methods: We analyzed 376 samples of the laboratory routine with medical request for anti-HCV serology that were reactive in the anti-HCV immunoassay and had the nucleic acid testing of HCV performed up to 90 days after the serologic test. The patients' ages ranged from 16 to 91 years old (mean 50.1). 243 (64.6%) patients were female and 133 (35.4%) were male. 376 samples were tested for HCV antibodies with the Abbott Architect anti-HCV immunoassay (Abbott Laboratories, Chicago, USA) and 276 were also tested in the Johnson Vitros anti-HCV immunoassay (Ortho-Clinical Diagnostics, Johson & Johson, Buckinghamshire, U.K.). The presence of HCV RNA was evaluated using the Amplicor Hepatitis C Virus (HCV) test Version 2.0 or the Cobas TaqMan HCV test V 2.0 (Roche Diagnostics GmbH, Mannheim).

Results: All samples evaluated in this study were positive for anti-HCV antibody. The Abbott Architect and the Johson Vitrus anti-HCV immunoassays presented a good agreement with a correlation coefficient $R^2 = 0.95$. Of the 376 anti-HCV positive patients, 202 (53.7%) were HCV RNA positive and 174 (46.3%) were HCV RNA negative. There was no significant difference related to gender ($p = 0.06$) in the HCV RNA detection. The stratification of the anti-HCV immunoassays results showed that none of the 101 patients with low S/CO ratio ranging from 1.0 to 5.0 was positive for HCV RNA, while 17 (29.3%) patients with S/CO ratio ranging from 5.1 to 10.0 and 185 (85.2%) patients with S/CO ratio above 10.0 were HCV RNA positive in the Abbott Architect anti-HCV. Similarly, none of the 64 patients with low S/CO ratio ranging from 1.0 to 9.0 was positive for HCV RNA, while eight (20%) patients with S/CO ratio ranging from 9.1 to 18.0 and 140 (85.8%) patients with S/CO ratio above 18.0 were HCV RNA positive in the Johnson Vitros anti-HCV.

Conclusion: The S/CO ratio of anti-HCV immunoassay may help to define the status of HCV infection, although it can vary among different manufacturers. In this study no patient was positive for HCV RNA in S/CO ratio of anti-HCV ranging from 1.0 to 5.0 and 1.0 to 9.0 in the Abbott Architect and Johnson Vitros anti-HCV immunoassays respectively. These results with low S/CO ratio of anti-HCV can be considered false-positive for HCV chronic infection.

D-130

Development of Molecular Identification Method for Detecting Common Dermatophytes in Korea

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Background: Dermatophytosis is caused by dermatophytes such as genera *Trichophyton*, *Microsporum* and *Epidermophyton* which are a group of keratinophilic fungi. It has a higher morbidity and cause a public health problem. For Proper anti-dermatophytosis therapy, early and accurate diagnosis of dermatophytes is very important. Current diagnosis of dermatophytosis relies on microscopic and cultural methods. However, this combination of techniques is time-consuming and has lower specificity and sensitivity. Recently, molecular-based techniques have been developed to identify the dermatophytes and to overcome limitations of conventional methods.

Methods: In this study, therefore, we have developed a PCR-reverse hybridization blot assay (REBA) which provides some advantages such as a higher simplicity, specificity and sensitivity, and lower cost. Furthermore, REBA is able to detect multiple infections at once.

Results: Therefore, we have designed species-specific oligonucleotide probes within the region of internal transcribed spacer 1 (ITS1) to detect 6 common dermatophytes in Korea and have performed with clinical isolates.

Conclusion: For more prompt or simpler detecting of dermatophytes, further tests would performed with direct clinical specimens.

D-131

Adenosine Deaminase Isoenzyme Activities in Serum and Plasma of Visceral Leishmaniasis Patients

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Background: Visceral leishmaniasis is a disease caused by *Leishmania donovani* (LD), an obligate intracellular parasite of macrophages and monocytes. Adenosine deaminase (ADA) is an enzyme which deaminates adenosine to inosine, has been implicated in the disease process by its involvement through the cell mediated immune response. Of the two isoenzymes of ADA viz. ADA_1 and ADA_2, the later is released by monocyte and macrophage on stimulation by LD and may be of significance in diagnosis of LD as a surrogate marker. This study is undertaken to compare ADA isoenzymes activities in serum and plasma of visceral leishmaniasis patients.

Materials and Methods: After approval from the institute ethical review board, fifty five diagnosed VL patients 50 age sex matched healthy individuals as control belonging to similar endemic area were enrolled. Total ADA (ADAT) and ADA_2 activities were measured spectrophotometrically by the method of Guisti and Gallanti in the presence of specific ADA_1 inhibitor, erythro-9-(2-hydroxy-3-nonly) adenine (EHNA).

Results: Our study showed that the serum level of ADAT (59.44 ± 10.06 U/L), ADA_1

(11.61 ± 2.62 U/L) and ADA_2 (47.86 ± 8.20 U/L) to be similar to the plasma level of ADAT (58.17 ± 9.34 U/L), ADA_1 (10.65 ± 2.56 U/L) and ADA_2 (46.78 ± 7.99 U/L) in VL patients and this was statistically not significant ($p > 0.05$). In the healthy controls ADAT, ADA_1 and ADA_2 activities were 11.51 ± 3.18U/L, 3.57 ± 0.93 U/L and 7.96 ± 2.45 U/L in serum and 11.22 ± 3.2 U/L, 3.39 ± 0.90 U/L and 7.61 ± 2.43 U/L in plasma respectively and were not significant ($p > 0.05$).

Conclusion: There was significantly raised activity of ADAT, ADA_1 and ADA_2 in VL patients as compared to the control. However, no significant difference was found in the ADA activities either in the serum or in the plasma of VL patients. Hence, both serum and plasma can safely be used as clinical sample for estimation of ADA isoenzymes activities.

D-133

Use of clinical chemistry parameters in P. falciparum malaria: its significance in determining the severity and outcome of patients

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Background: Malaria and its complications pose a major health issue in Pakistan. This study was undertaken to examine the relationship between Tumor Necrosis Factor α (TNF α) and other biochemical parameters in P. falciparum malaria and to investigate their usefulness in determining the severity and prognosis of malaria in patients from endemic areas of the Khyber Pukhtunkhwa province of Pakistan.

Methods: 1575 patients suspected to be suffering from P. falciparum malaria were examined between the summer months of May to October. Patients were divided into severe malaria (SM), mild malaria (MM), cerebral malaria (CM) and chronic malaria (CHM) groups. Parasite density was determined by counting the number of P-falciparum a-sexual forms/200 White Blood Cells on thick smears or 1000 Red blood Cells on thin smears. The numbers of parasites per micro liter were calculated on the basis of measured white or red blood cell count. TNF α was determined by Enzyme Linked Immunosorbant Assay. Blood glucose, bilirubin, creatinine, albumin, ALT and LDH were determined using micro lab-300(Vital Scientific). All the materials/ reagents for measuring the TNF α were provided by AmerSham/Biotrak Life Science in a kit form. Normally distributed data was analyzed using student t-test. The Kruskal-Wallis Test and Wilcoxon signed-Rank Test were used for non-normally distributed data.

Results: The TNF α levels were significaly elevated in patients with chronic P. falciparum malaria. The differences in blood glucose, bilirubin and ALT levels between severe malaria and mild malaria in adult patients was statistically significant ($P<.001$, $P<.001$, $P<.05$) each respectively. The blood glucose was decreased in severe malaria adult group (72.0 ± 08.4) and increased in mild malaria adult (MMA) group (96.8 ± 04.7, bilirubin and ALT were increased. The serum ALT, bilirubin and creatinine levels were higher in SMA group (81.5 ± 18.5, 04.4 ± 01.5 and 01.21 ± 0.27) and lower in MMA group (36.2 ± 04.3, 01.2 ± 0.19 and 0.9 ± 0.04) respectively. The difference in serum bilirubin levels between severe malaria adults and children was also significant ($P<0.02$), being increased in SMA group(04.4 ± 01.5) and decreased in SMC group(03.6 ± 01.4).Thirty percent patients with P. falciparum malaria had elevated ALT levels and 8% patients had more than three fold increase in ALT levels.

Conclusions: This study demonstrated that TNF α levels and other biochemical parameters were associated with several manifestations of severe malaria and that TNF α levels were not specific to cerebral malaria and hyperparasitemia. Furthermore, blood glucose, bilirubin and ALT levels could be used to differentiate between severe and mild malaria. The TNF α levels were not co-related to young age and fatal outcome. The TNF α levels were associated with hepatic and kidney dysfunction. Finally it is concluded and proposed that malarial hepatitis due to severe P. falciparum malaria should be kept in mind in the differential diagnosis of hepatitis particularly in malaria endemic areas.

D-134

Increased Prevalence of Vancomycin Resistant Bacteria in Long-Term Care Facilities

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Background: The emergence of vancomycin resistant bacteria is becoming a major problem, it is more common in enterococcus than any other pathogenic bacteria, it has been reported that about one quarter of all enterococcal isolate are vancomycin resistant. There are five types of Vancomycin resistance (VanA, VanB, VanC, VanD, and VanE) all externally acquired ; several cases has been described with acquisition of *vanA* by methicillin-resistant *Staphylococcus aureus* (MRSA) which is worrisome. Nonsusceptibility to glycopeptides also occurs independently from *van* genes and is a growing therapeutic challenge, especially in MRSA.

Design: we analyzed 193,014 positive cultures collected from residents in Long-Term Care Facilities from 2007 to 2010. Cultures were performed utilizing MicroScan Walkaway 96 conventional panels. The positive cultures were segregated further by the organisms isolated, and all VRE were included in this study. In addition, we calculated total vancomycin trough ordered for the same period of time.

Results: 994, 995, 1603 and 1534 cultures were positive in 2007, 2008, 2009 and 2010 respectively. Vancomycin trough samples ordered for the period are listed in table 1.

	2007	2008	2009	2010
Total VRE	994	995	1,603	1,534
Total Enterococcus	7,821	6,818	9,441	8,906
% VRE/total Enterococcus	12.7%	14.6%	17.0%	17.2%
Vancomycin trough ordered	4,072	4,510	6,486	6,662

Conclusions: increasing the use of vancomycin has big impact on the emergence of VRE especially in nursing home. Since treating VRE is very difficult and requires polypharmacological approach, prevention is the key and it should be done by educating the healthcare worker about the proper isolation techniques, environmental cleaning and hand hygiene are very effective in the prevention too. Physician should limit the use of vancomycin to the situation where it is needed and follow the recommended guidelines.

D-135

Association between 3 SNPs in miRNA-machinery genes and tuberculosis in Chinese Tibetan population

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Background: Recent studies forecasted that MicroRNA (miRNA) may be implicated in anti-tuberculosis protection immunological mechanism and single nucleotide polymorphisms (SNPs) in miRNA processing genes may promote infectious inflammation through affecting miRNA biogenesis. Our study pays attention to Chinese Tibetan population, which has a distinctive series of physiological traits differentiate from plainsmen for the high altitude environment, and investigate whether 3 SNP loci (rs636832 A/G, rs7813 C/T and rs3757 G/A loci) in miRNA-machinery genes were associated with tuberculosis in order to provide data for molecular diagnosis and clinical practices.

Methods: 320 tuberculosis patients and 314 healthy controls from Tibet native were involved. Using high-resolution melting (HRM) method on LightCycler 480 machine and GeneScan software, we respectively analyzed the genotype and allele distributions of these three SNP loci in the miRNA processing genes.

Results: The genotype distributions in both patients and controls were within Hardy-Weinberg equilibrium. Genotypic frequencies of these 3 SNPs did not show significant difference between tuberculosis patients and normal subjects (rs636832 A/G: p=0.158; rs7813 C/T: p=0.180; rs3757 G/A: p=0.422, respectively). Meanwhile, Allelic frequencies of these 3 SNPs analogously distribute between case and control group (rs636832 A/G: p=0.300, OR=0.875, 95% CI=0.679-1.127; rs7813 C/T: p=0.101, OR=0.816, 95% CI=0.640-1.041; rs3757 G/A: p=0.590, OR=0.927, 95%CI=0.703-1.222).

Conclusion: Although the existing data didn't support that the polymorphisms of these 3 loci (rs636832 A/G, rs7813 C/T and rs3757 G/A loci) in miRNA-machinery genes contribute to the development of tuberculosis, it would be inadequate to draw solid conclusions. We intended to expand the sample volume to improve this part of data in the future research.

Table 1. Comparisons gene polymorphisms between patient and control groups

Polymorphism	Patient n (%)	Control n (%)	χ2	*p**	OR	95% CI
Rs3757	320	314				
Genotype						
GG	205 (64.06)	212 (67.52)				
GA	100 (31.25)	84 (26.75)	1.725	0.422	N/A	N/A
AA	15 (4.69)	18 (5.73)				
Allele						
G	510 (79.69)	508 (80.89)	0.290	0.590	0.927	0.703-1.222
A	130 (20.31)	120 (19.11)				
Rs636832	320	314				
Genotype						
AA	191 (59.69)	168 (53.50)				
GA	104 (32.50)	125 (39.81)	3.691	0.158	N/A	N/A
GG	25 (7.81)	21 (6.69)				
Allele						
A	486 (75.94)	461 (73.41)	1.073	0.300	0.875	0.679-1.127
G	154 (24.06)	167 (26.59)				
Rs7813	320	314				
Genotype						
CC	38 (11.87)	24 (7.64)				
CT	123 (38.44)	121 (38.54)	3.426	0.180	N/A	N/A
TT	159 (49.69)	169 (53.82)				
Allele						
C	199 (31.09)	169 (26.91)	2.692	0.101	0.816	0.640-1.041
T	441 (68.91)	459 (73.09)				

OR=odd ratio; 95% CI=95% confidence interval. N/A=not applicable; * $p<0.05$ is in bold

D-136

Investigation of isolated aspergillus in intensive care unit of west china hospital from 2009 to 2010

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Background: To investigate and analyze the Aspergillus isolated situation from January 2009 to February 2010 in patients in intensive care unit(ICU) of the West China hospital, Sichuan University ,to discuss the ICU aspergillosis factors and summarize the characteristics of ICU nosocomial infection in our hospital, and to propose effective prevention and control measures.

Methods: We used LIS and HIS system to collect hospital medical unit ICU 54 cases of patients with positive aspergillus isolated laboratory and clinical data for statistical analysis .

Results: 43 cases were with basic diseases or high-risk factors, including diabetes and organ transplants, COPD, long-term use of immunosuppressant,broad-spectrum antibiotics. 9 patients were without underlying disease or high risk factors, but with trauma or surgery. 2 cases missed the medical records. 48 cases(88.89%)were with mechanical assisted entilation.The time between mechanical ventilation and the report of Aspergillus isolated from laboratory was 2d in the shortest interval , 59d in the longest interval. Doctors usually use itraconazole and voriconazole . The average treatment time was 1 week or so, but the mortality rate was still about 50% -78%.

Conclusion: Aspergillus infection in ICU is a special type of fungal infection recently, with increasing trend. Long-term high-dose broad-spectrum antibiotics, high-dose glucocorticoids and immunosuppressive application were closely related. Invasive mechanical ventilation created an opportunity for the Aspergillus infection.Clinical anti-fungal treatment was often poor and high mortality.

D-137

Development of DR Polymorphism based PCR to Classify the Sublineages of Mycobacterium tuberculosis Beijing family Isolated in the Republic of Korea

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Background: The Beijing family of Mycobacterium tuberculosis (Mtb) is one of the most successful clades in the present worldwide tuberculosis (TB). 'K strain' , a sublineage of the Beijing family, is commonly isolated from TB patients in the Republic of Korea. 'M strain' , a sublineage of the K strain , is isolated from Masan National TB Hospital. However, it has been showed different IS6110 band patterns in Mtb genome when performed with restriction fragment length polymorphism (RFLP) method. These Beijing family strains have been reported as a serious causative agent of TB in the Republic of Korea. Therefore, DNA fingerprinting of Beijing family of Mtb is very important to control the TB. Because it might provide informations about transmission route or source of internal laboratory contaminations, and able to distinguish the reactivation or exogenous reinfection.

Methods: However, RFLP is time consuming, expensive and requires skilled experts, therefore, limited laboratories can perform the RFLP technique. For clinical application, we have developed PCR-based method for genotyping of Mtb Beijing family using DR polymorphism and performed with 72 Mtb clinical isolates from Masan National TB Hospital and the results have compared with RFLP.

Results: Due to DR polymorphism based PCR generates a unique band, it may provide more useful information of K and M strain than RFLP method, therefore, we suggest DR polymorphism based PCR genotyping for correct identification of the Mtb Beijing sublineages.

Conclusion: To establish the usefulness of this newly developed method, more tests need to performed with more clinical samples.

D-139

Serum procalcitonin level and bacteremia

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Background: When patients present with fever, the risk of sepsis and the preceding bacteremia has to be evaluated. However, a diagnosis of bacteremia can be confirmed by blood culture only after 24 to 48 h of incubation. In contrast, the serum concentration of procalcitonin (PCT), a biomarker for bacteremia or sepsis, can be determined within an hour. We thus compared the serum concentration of PCT and blood culture results and analyzed their implications.

Methods: The study population comprised 628 patients (mean [SD] age, 63 [17] years) with fever and related symptoms. Blood samples were collected from all patients (208 patients were not treated, and 420, treated with antibiotics) for the measurements of serum PCT and C-reactive protein (CRP) levels and the blood culture. PCT levels were measured using BRAHMS PCT, a modular assay system (Roche Diagnostics). A bacteremia episode was defined as positive blood culture result associated with clinical symptoms of infection. If a blood culture yielded coagulase-negative staphylococci or Bacillus species, then that culture was considered contaminated.

Results: PCT and CRP levels of all patients were plotted on receiver operating curves (ROCs). The areas of PCT and CRP levels under ROC in untreated patients were 0.727 and 0.659, respectively, with the former being significantly greater ($p < 0.05$). The areas of PCT and CRP levels under ROC in antibiotic-treated patients were 0.578 and 0.420, respectively. When the cut-off value of PCT level was set at 0.5 ng/ml, the sensitivity and specificity for the diagnosis of bacteremia were 94% and 60%, respectively, for untreated patients and 69% and 50%, respectively, for antibiotic-treated patients. However, when the cut¬-off value of PCT level was set at 2.0 ng/ml, the sensitivity and specificity were 38% and 81%, respectively, for untreated patients and 41% and 71%, respectively, for antibiotic-treated patients.

Conclusion: Therefore, serum PCT levels can predict bacteremia in both untreated and antibiotic-treated patients. However, our preliminary results by serial sampling tests indicate that in some patients, even under bacteremic conditions, PCT levels may be elevated only after several hours.

D-142

Interference of oxidative stress in iron protein depletion in Pacients Co-infected HIV/TB before and after drug intervention and nutritional guidance

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Background: Tuberculosis (TB) is one of the most common opportunistic diseases associated with HIV worldwide. There is great interest in the study of oxidative stress in patients co-infected by the possibility of interference in the prognosis of the disease and on nutritional conditions, especially protein malnutrition and iron deficiency anemia. The objective is study the association of oxidative stress status with body iron, and protein in HIV / TB before and after drug intervention and nutrition.

Methods: A longitudinal study of dietary intervention conducted in HIV / TB drug-naïve. Anthropometric data were collected for biochemical and 6 months of drug therapy for TB. We measured serum albumin, transferrin, serum iron, complete blood count and malonic Dialdehyde (DMA), in addition to checking for arm muscle circumference (AMC). The initial data were analyzed with multivariate confidence

interval 95% stipulated by using statistical tests ANOVA and T studant.

Results: We studied 14 adult patients 10 (71.4%) males and four (28.6%) women, all HIV / TB, using as criterion cutoff of hemoglobin (HGB) proposed by WHO for classification of anemia, we found that all patients in spite of nutritional counseling, and independent of gender remained anemic after the end of treatment, with high involvement of iron status (n = 4), transferrin (n = 14), albumin (n = 4), hemoglobin (n = 5) and AMC (n = 5) and found statistical significance between the degree of stress, according to figures from the DMA, and nutritional variables, independent of markers (or visceral somatic) before and after treatment. It is worth noting the improvement of nutritional value during treatment with a statistical trend (Fe p = 0.08) (p = 0.45 TFR) (HGB p = 0.05) (CMB p = 0.37).

Conclusion: Co-infection HIV / TB promotes organic stress and can impose risks to the patient's nutritional status and dietary guidance was only able to promote an improvement in the values of HGB without recovery of body condition of iron and protein occurs, patients were accompanied by nutrition for 2 to 3 months up to the high nutritional value. Indicating the need for more effective nutrition intervention that prioritizes the provision of micronutrients and protein.

D-143

Vulva and perianal infection by *Pseudozyma aphidis* in a Woman with Acute Myelocytic Leukemia (AML)

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Background: We present an unusual case of fungal infection caused by *Pseudozyma aphidis* which are rarely responsible for human diseases, to provide more information for clinical diagnosis.

Methods: Sabouraud's dextrose agar was used to culture the yeast, microbiology examination was used to identify the colony, and commercial yeast identification kit failed to identification. Then sequence of ribosomal DNA ITS domains was used for A BLAST search.

Results: A BLAST search using the sequence of the 762-bp product yielded 100% homology with *P. aphidis* ITS sequences in GenBank (Accession number AB 204896.1)

Conclusion: To our knowledge, this is the first report of infection caused by *P. aphidis* in leukemic patients. Accurate diagnosis combined with effective anti-fungal therapy may prevent the development into invasive fungal infections.

D-144

Antibody screening, provirus detection and genetic characterization of Human T-lymphotropic virus in Rio de Janeiro/Brazil

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Background: It is estimated that 15-20 millions of people are infected with human T-cell lymphotropic virus (HTLV) worldwide. This virus infection is endemic in Brazil, where around 2-3 million people are infected. HTLV-1 was introduced in the country by the intense slave trade in the XVI-XVIII centuries while HTLV-2 is an ancestral virus that followed the human migrations to the Americas 10.000-40.000 years ago. Blood donor screening is mandatory in Brazil since the 1990s with enzyme immunoassays (EIAs) for antibodies to HTLV, and the positive samples later confirmed with HTLV-I/II Western blot (WB). Laboratory testing for HTLV-1 and -2 infections has become also routine in transplantation and clinical diagnoses in many countries. However the high proportion of indeterminate results of the HTLV screening test (WB) is still a challenge all over the world.

Methods: 6.548 samples of the laboratory routine with medical request for HTLV serology were screened by enzyme-linked immunosorbent assay (ELISA) Murex HTLV I+II (Murex Biotehch Limited, Dartford, UK) or Abbott Architect rHTLV-I/II immunoassay (Abbott Laboratories, Wiesbaden, Germany) and/or HTLV Blot 2.4 Western blot test (MP Biomedicals Asia Pacifics, Singapore). Eighteen reactive samples to HTLV antibody, with cell availability, were submitted to PCR analysis using generic primers to HTLV tax gene and further specific PCR to LTR region from HTLV-1 and HTLV-2. All amplicons were sequenced. The sequence analysis was performed using MEGA V.4 software and Neighbor-joining/Kimura-2-parameter.

Results: Considering the 6.548 samples submitted to HTLV antibody screening, ELISA or Abbott Architect Immunoassay and/or WB, 49 presented reactivity (0.75%). 18 samples, with cell availability, were then submitted to PCR testing. 15 (83,3%) of 18 ELISA or Abbott Architect immunoassay reactive samples were confirmed as HTLV-1 by the WB profile (reactivity to rgp46-1) and tax and LTR sequence. One sample presenting reactivity to rgp46-2 was demonstrated to be HTLV-2 by tax and LTR sequence. One sample with indeterminate WB pattern (reactivity to p19 and GD21 bands) and other one, with low reactivity in the Abbott Architect immunoassay, were negative to PCR and therefore false-positives. The HTLV-1 genetic characterization, based on the LTR sequences analyze revealed that all but one strain belong to the HTLV-1aA, Cosmopolitan, Transcontinental group and one to HTLV-1aB Cosmopolitan, Japanese group. The HTLV-2 virus was characterized as type B.

Conclusion: Since PCR analysis revealed 11.1% of the samples presenting reactivity to HTLV screening tests as false-positives we can infer that the HTLV infection prevalence in this sample was 0.67% contrasting with the prevalence among blood donors in Rio de Janeiro that is around 1.5%.

D-145

Comparison Of The Hiv Ab And The Hivag/Ab Combo Immunoassays

A. Ejilemele, M. Loeffelholz, J. R. Petersen, A. O. Okorodudu. *University of Texas Medical Branch, Galveston, TX,*

Introduction: The acquired immunodeficiency syndrome caused by the human immunodeficiency virus (HIV) is a worldwide pandemic with more than 33 million people living with the virus. Current third-generation serologic tests are based on the detection of IgG and IgM antibodies against HIV types 1 and 2. Most third generation assays incorporate several recombinant antigens derived from HIV -1 and -2 core and envelope genes with most persons having detectable antibodies after 2-8 weeks and 95% are positive by 6 months. Positive tests are usually repeated and confirmed by western blot. Fourth generation assays have been developed which combine detection of human antibodies against the HIV (antibodies) and the HIV (antigens) on the same platform. Our observations using the HIV Ab assay showed that specimens with a signal to cutoff ratio (s/c) <10 (≈75% of positive results in a low risk pregnant population) are unlikely to test positive by HIV-1 Western blot. We therefore conducted an evaluation of the new fourth generation combo Ag/Ab HIV1/2 assay to determine the proportion of weakly reactive false positive results relative to the third generation assay.

Objectives: To compare screening results obtained from the HIV Ab and HIV Ag/Ab test kits with western blotting results and evaluate the sensitivity and specificity of the two HIV kits in our laboratory.

Methods: With IRB approval 219 sera with S/C ratio <40 were retrieved over a six month period from the serology unit. All samples had been tested using an HIV Ab Instrument. Information on the western blot status was also retrieved. These samples were retested using the HIV combo assay and the results evaluated.

Results: The mean age was 32.13 ± 12.9 years and the male:female ratio was 1:1.3. One hundred and forty specimens (78.2%) which were positive using the HIV Ab kit screened negative using the HIV combo kit. Twenty-five specimens (14.3%) that were positive on the HIV Ab and twenty-five (69.4%) of positive samples using the HIV combo test kit gave a positive western blot reaction respectively. Sixty-six patients with discordant results between the HIV Ab assay and western blot were called back for repeat testing within 3-4 months. Of these samples collected for retesting, 13(19.7%) screened negative by HIV Ab and only 53 (80.3%) samples that repeated as positive on the HIV Ab assay were submitted for western blot with 2 (3.8%) confirmed as positive. Two (15.4%) of the 13 negative samples by HIV Ab assay were positive by the HIV combo assay. Relative to the western blot, the HIV combo assay had 98.1% agreement for these repeat testing. The sensitivity and specificity of the HIV Ab kit were 100% and 30% while those for the HIV combo kit was 100% and 96.9% respectively.

Conclusion: Given the population studied, the HIV combo assay is more specific for the detection of HIV. Results obtained using the HIV combo assay are more likely to correlate with western blotting results.

Thursday AM, July 28

Poster Session: 9:30 am - 12:00 pm
Endocrinology/Hormones

E-01

IFCC International Conventional Reference Procedure for the Measurement of Free Thyroxine in Serum

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Background: The IFCC Working Group for Standardization of Thyroid Function Tests proposes to establish traceability of serum free thyroxine (FT4) measurements to an international conventional reference measurement procedure (RMP) using equilibrium dialysis (ED) isotope dilution-liquid chromatography/tandem mass spectrometry (ID-LC/tandem MS). This proposal defines the measurand operationally as "thyroxine in the dialysate from ED of serum prepared under defined conditions". The rationale for proposing a conventional RMP is that, because of the physical separation step, it is unknown whether the measured FT4 truly reflects the concentration in serum. Here we describe optimization and validation of a previously developed candidate ED ID-LC/tandem MS procedure (1). The objective was to show that the procedure was ready to ask endorsement by the IFCC Scientific Division and to be nominated for listing by the Joint Committee for Traceability in Laboratory Medicine (JCTLM).

Methods: The convention is that ED has to adhere to the following requirements: use of a dialysis buffer with a composition closely resembling the ionic environment of serum/plasma; buffering of samples to a pH of 7.4 (at 37°C) before dialysis, however, without additional dilution; use of a dialysis device with a dialysand/dialysate compartment of identical volume and separated by a regenerated cellulose membrane with adequate cut-off; thermostatic control during dialysis at 37°C ± 0.5°C. The ID-LC/tandem MS procedure should be based on a JCTLM listed total T4 reference method or variant. In addition to the basic validation (1), we focused here on robustness and compliance of the ED process with the aforementioned requirements. This comprised verification of the pH and temperature during ED; dialysis time necessary to reach equilibrium; impact of changes in membrane cut-off and brand; generation of non-esterified fatty acids (NEFAs) during ED; verification of the trueness of calibration solutions and of the procedure's overall precision and trueness; estimation of the expanded uncertainty of measurement (U).

Results: Fulfillment of the pH and temperature requirements was confirmed; results after 4 hours dialysis differed not significantly (NS) from those after respectively 5, 6 and 7 hours, nor was there a significant impact of the membrane cut-off and brand; NEFAs concentrations in paired serum samples (dialyzed/not -) were NS different; for the RMP, inclusive ED, the within- and between-run CVs were 2.8% and 2.4%, respectively, the total CV 3.7% (target: 10 pmol/L, n = 61 duplicates); the CVs for MS measurement were respectively: 1.7%; 1.0% and 2.0% (target: 1.29 pmol/g, n = 66 duplicates); monitoring of the trueness of the measurement procedure, with and without ED, showed a mean bias of -0.2% (n = 61 duplicates) and +0.03% (n = 66 duplicates); U (k = 2) was estimated at 7.6%. A transferability study showed reasonable agreement between the results of the laboratories of Ghent University and Reference Material Institute for Clinical Chemistry Standards (Japan) (difference max. 4.2%).

Conclusion: The validation data documented that the optimized candidate conventional RMP is qualified to obtain IFCC endorsement and to be considered for listing in the JCTLM database.

1. Van Uytfanghe K et al. Clin Chem 2006;52:1817-21.

E-02

Vitamin D Standardization Program

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Objective: The objectives of the program are to: (1) standardize the measurement results of serum 25-hydroxyvitamin D [25(OH)D] in national health surveys around the world, (2) to study differences in 25(OH)D values in national health surveys, and (3) to standardize 25(OH)D measurement results from clinical, commercial, and research laboratories to enable transfer of survey findings to patient care and pubic health activities. The measurands addressed in the program are total 25(OH)D, $25(OH)D_2$, $25(OH)D_3$ and the 3-epi-$25(OH)D_3$.

Relevance: One of the key recommendations of the Institute of Medicine's recent released Dietary Reference Intakes for Calcium and Vitamin D is the need to standardize the measurement of serum 25(OH)D. The wide spread variation in measurement results of 25(OH)D confounds international efforts to develop evidence-based clinical guidelines.

Methodology: To assess measurement variability in currently used assays, an interlaboratory comparison study is conducted that includes laboratories from national surveys as well as research laboratories and assay manufacturers. A formal laboratory standardization program will be implemented using procedures similar to those used in the CDC Hormone Standardization Program (HoSt Program). These standardization activities will use single-donor, fresh-frozen serum collected using the CLSI C37 protocol. Values will be assigned to these sera by the reference laboratories at the National Institute for Standards and Technology (NIST) and the University of Gent (Prof. Dr. Thienpont). The initial assay performance criteria used in this standardization program are <=10% imprecision and <= 5% bias to the reference values. These performance criteria are based on biological variability data. To increase the comparability of existing data from different national surveys, studies are performed to create master equations that facilitate the conversion of already existing national survey data.

Results: The NIH Office of Dietary Supplements (ODS) and CDC National Center for Environmental Health (NCEH) established a vitamin D standardization program with a Standardization Coordinating Center at CDC. Survey laboratories from national surveys in Canada, Germany, Ireland, UK and USA are participating in this program. Additional national survey laboratories are enrolling. An initial study to establish a master equation between data obtained in the U.S. National Nutrition and Health Examination Survey (NHANES), National Center for Health Statistics, CDC and data obtained in the German National Health Surveys is conducted or is planned.

Conclusions: A Vitamin D Standardization Program has been established by NIH ODS, CDC and NIST. The program will standardize measurement results from national survey laboratories as well as clinical laboratories and assay manufacturers. This program will resolve current problems that limit the use of research data in patient care and public health.

E-03

Measurement of Serum Free Testosterone by Liquid Chromatography-Tandem Mass Spectrometry--Direct Measurement vs. Calculation

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Background: Free testosterone (FT) accounts for about1-2% of the total testosterone that circulates in blood. According to the "free hormone theory", only FT is able to penetrate the cell membrane to interact with the androgen receptor to regulate the expression of androgen-responsive target genes. Studies suggest that FT is more direct and informative than total testosterone in investigating the androgen status of the patient. Analog-based FT immunoassays are the most widely used FT methods. Recent studies conclude that analog-based FT assays provide essentially the same information as a total testosterone assay when applied to healthy adult males, and should not be used as FT assays. We successively developed two liquid chromatography-tandem mass spectrometry (LC-MS/MS) based methods to detect and quantify serum FT.

Methods: Calculation method involves three separate assays that measure serum total testosterone by LC-MS/MS, serum sex hormone binding globulin (SHBG) by chemiluminescent immunoassay, and serum albumin by colorimetry, respectively. By employing the Vermeulen algorithm, free and bioavailable testosterones are calculated using the albumin, SHBG, and total testosterone results, in conjunction with the association constants of testosterone binding to both SHBG and albumin (k_{at} = 3.6×10^4 L/mol, k_{st} = 10^9 L/mol). The direct method applies ultrafiltration (UF) at 37 °C to separate the free fraction from serum, and use of LC-MS/MS to directly measure FT in the ultrafiltrate. Ion transitions of *m/z* 289.2/109.1 and 291.2/111.1 are used to monitor testosterone and its internal standard (IS) testosterone-d_2, respectively. Both methods were validated. The results of FT from 120 samples were compared, and gender specific FT reference intervals were determined.

Results: Within-in and between-run imprecision of the direct method gave a coefficient of variations (CV) less than 4.5% at a concentration of 30 pg/mL, and 3.0% at 120 pg/mL (n=15 each). Recovery ranged from 86% to 104%. The direct method

demonstrated a linear response from 1 to 200 pg/mL, and a functional sensitivity was determined to be 1 pg/mL (CV 11.3%). The direct method for FT correlated very well with the calculation methods $FT_{direct} = 0.6986FT_{calc} - 1.3001$, r = 0.9807, n=120. Average bias of direct method compared with calculation method was -0.66 pg/mL for female subjects (0.5 - 14.0 pg/mL), and -28.7 pg/mL for male subjects (43 - 186 pg/mL). Reference intervals for males were established as 41 - 179 pg/mL using calculation method and 43 - 186 pg/mL using the direct method; for females, 0.5 - 9.9 pg/mL using calculation method and 0.5 - 14.0 pg/mL using the direct method (serum samples from 150 healthy males and 150 healthy females were used, and all subjects were 18 years and above).

Conclusion: We developed and validated two LC-MS/MS based methods to detect and quantify serum free testosterone. The direct and calculation methods for free testosterone correlate well, and the direct method has average bias of -0.66 pg/mL for females, and -28.7 pg/mL for males. This bias implies a problem with the verity of the calculation method. The direct method is more cost-effective, requires less sample volume, and offers faster turn-around-time than the calculation method.

E-04

Nonlinear Analytical Recovery in the Liaison 25 Hydroxy Vitamin D Chemiluminescent Immunoassay

E. W. Holmes, J. Garbincius, K. Mc Kenna. *Loyola University Medical Center, Maywood, IL,*

Background: The purpose of this study was to investigate the frequency and cause of falsely increased 25 hydroxy-vitamin D (25OHD) results obtained with the Liaison immunoassay (DiaSorin Inc., Stillwater, MN 55082) among specimens with results in the range of 58 - 150 ng/mL. In November of 2010, we discovered a marked under-recovery of 25OHD in a patient sample that was randomly selected and serially diluted for the purpose of verifying linearity/recovery. In view of this initial finding, we began to routinely analyze two-fold dilutions (manufacturer's specified diluent) for all patient specimens that had initial results that were > 58 ng/mL. When the recovery after dilution was < 85% of the result for the undiluted specimen, we suspected that the specimen contained an interfering substance that caused the initial result to be falsely elevated.

Methods: Subsets of the samples with nonlinear dilution profiles (NLD samples) were retested by the Liaison assay to see if the positive interference could be eliminated by: extraction/deproteinization with acetonitrile, treatment with a heterophile blocking reagent (HBT, Scantibodies Laboratory, Inc., Santee, CA 92071), or supplementation with goat IgG or rabbit gamma globulins prior to analysis. A subset of the NLD specimens was also tested for total 25OHD by LC-MS (ARUP Laboratories, Salt Lake City, UT 84108).

Results: Over an 86 day study period (11/3/10 to 1/28/2011), we tested a total of 5305 specimens for 25OHD. 152 specimens had initial results that were greater than 58ng/mL, and 31 of these (20%) had fractional recoveries of < 0.85 after dilution.The mean fractional recovery (SD) of 25 OHD following acetonitrile extraction of five samples with linear dilution profiles was 0.94(0.08) as compared to the original specimen. In contrast, the mean recovery for 6 NLD specimens was 0.36 (0.13). This difference was highly significant (p<0.001,unpaired t), and suggested that the interfering substance was a protein. Only 1 of 6 NLD specimens showed a significant decrease (>15 %) in the 25 OHD result after two successive treatments with the HBT reagent. Three of these 6 NLD specimens showed a significant decrease in the 25 OHD result after the addition of goat IgG. The total 25 OHD results for a group of 15 undiluted NLD specimens were, on average, 2.6-fold elevated (95% CI:2.0to 3.2; range:1.6 to 5.0) by the Liaison method as compared to the total 25OHD results determined by LC-MS.

Conclusion: Our studies demonstrate the presence of protein (possibly heterophile antibody) interference in the Liaison assay. The magnitude of the interference and its high prevalence among samples in the upper half of the method's AMR indicate that it is an important source of clinically significant analytical error in 25 OHD assays performed by the Liaison method.

E-06

High fraction (>70%) of hemoglobin A1c patients with only one A1c measurement during a 1-year interval: frequency data support use of diabetes risk-based A1c reference range

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Background: American Diabetes Association (ADA) recommendations for monitoring of A1c in diabetes are that measurement should occur at a frequency of not less than twice per year. ADA 2010 practice guidelines also introduced new criteria for diagnosis of diabetes to include use of hemoglobin A1c measurement as an option. Given the sanction of A1c measurement for diabetes screening, clinicians at our institution requested a change of the A1c reference range from the established non-diabetes population reference range (A1c < 6.1%) to a reference range demarcating an increased risk for diabetes (A1c > 5.6%). The extent of utilization of A1c for screening vs. monitoring at our institution was unknown, however. We therefore examined laboratory data to infer the maximum possible extent to which A1c measurement was being used as a diabetes screening test at our institution. Our premise was that the rate of patient A1c samples processed without a second A1c sample within a one-year interval (rate of solo samples) represents an upper limit on the rate of use of A1c for screening.

Methods: Primary data were all A1c measurements performed by our central hospital laboratory over a 1-year interval (Sept 2009 through Aug 2010). Patients were sorted with respect to the number of A1c measurements made during this interval.

Results: 17086 A1c measurements in the 1-year interval were from 12279 patients, among which 8952 patients had only solo measurements (72.9% of patients; 52.4% of A1c measurements). The remainder 3327 patients (27.1% of patients) were serial A1c measurement patients (having two or more A1c measurements within the 1-year interval) comprising 8134 A1c measurements (47.6% of total). The number (n) of serial measurements performed for individual patients ranged from n=2 to 14, according to the distribution of 68.5% (n=2), 22.4% (n=3), 6.7% (n=4), 2.4% (n=5 or more). There was no correlation between an individual's maximum A1c and the number of measurements performed (r = 0.117). The majority of serial patients (90.4%) had 2 or 3 A1c measurements, in accordance with ADA guidelines for diabetes. The median A1c for solo measurements was 5.9% compared to 6.7% for serial patient measurements. Solo samples as here defined would include not only screening but also other circumstances such as: 1. non-compliance of diabetes patients with respect to A1c monitoring; 2. data interval "end effects" of the rates of attrition or accretion of old and new diabetes patients; and 3. single encounters of both diabetic and non-diabetic patients with clinician specialists who routinely elect to order A1c.

Conclusions: There was a high fraction (72.9%) of solo A1c measurement patients in a 1-year interval. Although solo measurements do not exclusively represent diabetes screening, the high solo ordering rate at our institution indicated that reporting of a risk-based reference range for A1c (A1c > 5.6%: increased risk of diabetes) was reasonable and appropriate.

E-07

Comparative Analysis Of The Usefulness Of Fasting Glucose, Hba1c And Estimated Average Glucose As Screening Tests For Diagnosis Of Diabetes Mellitus And Other Categories Of Glucose Intolerance In A High Risk Population

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Background: Recently, glycated hemoglobin (HbA1c) was included in the criteria for diagnosis of diabetes in non-pregnant individuals - HbA1c of ≥ 6.5% is diagnostic for diabetes, HbA1c of 5.7% to 6.4% identifies patients at risk for diabetes (prediabetes). Converting HbA1c result to estimated average glucose values (eAG) might help understanding and interpretation of HbA1c. The aim of the current study is to evaluate and compare the utility of fasting Glucose, HbA1c and eAG as screening tests for undiagnosed diabetes and metabolic disorders in apparently healthy first degree relatives of patients with Type 2 diabetes.

Methods: We measured fasting glucose, HbA1c and calculated eAG in 575 (232M, 343F) first degree relatives of T2DM patients. Subjects were classified by the IDF criteria for the metabolic syndrome (MS). Receiver operating characteristic curve (ROC) analysis was used to examine the diagnostic performance characteristics for DM and MS.

Results: Using standard cut off values for glucose, HbA1c and eAG, the relative prevalence of undiagnosed diabetes in our cohort were 16/575 (2.8 %) for glucose, 66/575 (11.5%) for HbA1c and 62/575 (10.8%) for eAG respectively. At standard cut-off values, the prevalence of impaired fasting glucose (IFG) was 55/575 (9.6%), 195/575 (33.7%) and 437/575 (76%) for glucose, HbA1c and eAG respectively. Using the ADA glucose diagnostic criteria as reference, the areas under the ROC (0.989; 95% CI (0.874 - 0.982)) for diagnosis of DM were the same for HbA1c and eAG. HbA1c and eAG also had the same ROC areas for the diagnosis of IFG (0.719; 95% CI = 0.647 - 0.791, respectively) and the metabolic syndrome (0.678 (95% CI = 0.600 - 0.756). The ideal cut-off points with the highest sensitivity (100%) and specificity (65%) for diagnosis of DM in our cohort were HbA1c = 5.9% and eAG = 6.7 mmol/L. These values are different from recommendations in international guidelines.

Conclusion: We conclude that there are significant differences in the number of

undiagnosed diabetic subjects identified by screening with glucose, HbA1c and eAG. The differences between HbA1c and eAG could be due to the wide confidence intervals around eAG estimates. HbA1c and eAG are potentially useful tools for diagnosing subjects who are unaware of their glucose tolerance status but consensus is needed on the ideal management for normoglycemic subjects with HbA1c and eAG in the diagnostic range for diabetes mellitus.

E-08

The Role of RNase L in Type I Diabetes

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Background: The cause of type I diabetes continues to be a focus of investigation. It has been believed that type I diabetes is caused by autoimmune responses triggered by viral infection or other toxic agents, resulting in destroy of insulin producing pancreatic beta-cells, leading to an absolute deficiency of insulin. Studies have revealed that an antiviral protein known as interferon (IFN)-alpha, in pancreatic islets after viral infection and treatment with double-stranded nucleic acids (dsRNA), a mimic of viral infection, is associated with the onset of type I diabetes. Much effort has been devoted toward the understanding of the role of IFN-alpha in the development of type I diabetes over past years. However, how IFN-alpha contributes to the onset of type I diabetes remains to be obscure.

Methods: We created an RNase L deficient RIP-B7.1 mouse which is more vulnerable to environmental harmful factors such as viral infection, resulting in destruction and leading to disease. Then we used this model to determine the role of RNase L in poly I:C and STZ-induced diabetes.

Results: In this study, we found that 2-5A dependent RNase L (RNase L), an IFN-alpha-inducible enzyme that functions in IFN action against viruses and cell proliferation, played an important role in poly I:C and STZ-induced onset of type I diabetes. RNase L deficient mice showed a significant delay of diabetes onset after treatment with dsRNA and STZ. Further investigation revealed that RNase L was reponsible for beta-cell apoptosis and immune responses in the islets.

Conclusion: Our finding provides new insight into the molecular basis leading to beta-cells destruction, the cause of absolute deficiency of insulin in type I diabetes and may suggest novel therapies for treatment and prevention of the disease based on the selective regulation and inhibition of RNase L.

E-10

Validation of an immunoassay for hemoglobin A1C using a commercial HPLC method as reference

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Background: Hemoglobin A is found in red blood cells and composed of four subunits, 2 α and 2 β globins each containing a heme group. In the presence of glucose, hemoglobin A is glycosylated non-enzymatically in proportion to the glucose concentration. As a result, hemoglobin A1c (HbA1c) is an indicator of glucose levels over 2-3 months. HbA1c has been used for monitoring blood glucose control in diabetes management. In 2010 the American Diabetes Association endorsed HbA1c as one of the diagnostic biomarkers for diabetes. Both chromatography-based methods and immunoassays are available for measuring HbA1c. **Objective:** To compare an immunoassay with a high-performance liquid chromatography (HPLC) method using specimens with either normal or abnormal hemoglobin variants. **Methods:** Whole blood (EDTA) specimens from leftover clinical samples on which HbA1c was ordered were used for the method comparison. The turbidimetric inhibition immunoassay was run on the Integra 800 (Roche Diagnostics, Indianapolis, IN) according to manufacture's instruction. Briefly, whole blood was hemolyzed using tetradecyltrimethlyammonium bromide. Next a buffer containing anti-HbA1c was incubated with the hemolyzed sample which formed soluble antigen-antibody complexes. Then a buffer containing polyhaptens was added to bind unbound anti-HbA1c in an insoluble complex which was quantified turbidimetrically. At the same time total hemoglobin was measured spectrophotometrically. An HPLC method (Tosoh, Tokyo, Japan) was used as a reference method.

Results: The immunoassay was linear over the range of 3.89 to 16.23% with an analytical recovery ranging from 97.6-101.7%. Intra-assay precision was evaluated by running 10 replicates using Roche HbA1c controls N (Lot#618500) and P (Lot#610804) with mean concentrations of 5.70% and 11.12%. The intra-assay coefficients of variation (CV) were 1.2%, and 1.1%, respectively. Inter-assay precision was evaluated by running 58 replicates over 29 days using Control N (Lot#623414) at 6.05% and Control P (Lot#623415) at 13.67%. The inter-assay CV was 2.0% and 2.2%, respectively. The correlation (Deming regression) with the HPLC method using 45 de-identified normal HbA1c patient samples ranging from 4.80 to 10.07% showed a slope of 0.908 (95%CI: 0.868 to 0.948), an intercept of 0.616 (95%CI: 0.358 to 0.874) and an r of 0.9898. Correlation with the HPLC method using 18 de-identified HbA1c patient samples ranging from 5.3 to 15.8% with abnormal hemoglobin variants other than hemoglobin F showed a slope of 0.986 (95%CI: 0.931 to 1.041), an intercept of 0.085 (95%CI: -0.338 to 0.507) and an r of 0.9945.

Conclusion: The immunoassay was appropriate for measuring HgA1c in whole blood. The high throughput on a random access analyzer makes it ideal for a high volume clinical laboratory.

E-11

Stability of 1,25-dihydroxyvitamin D2 and 1,25-dihydroxyvitamin D3 in human serum

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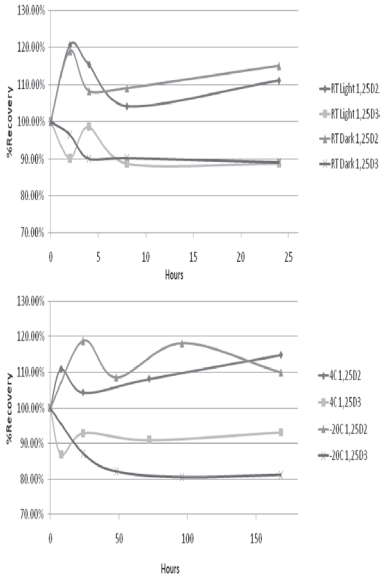
Background: 1,25-dihydroxyvitamin D (1,25(OH)2D) is the biologically active metabolite of vitamin D that plays a key role in the regulation of calcium and phosphate homeostasis. Its normal physiological concentration ranges from 15 to 60 pg/mL. Measurement of 1,25(OH)2D (1,25(OH)D2 and 1,25(OH)2D3) in serum is important in managing patients with renal failure, hyperphosphatemia, hypomagnesemia, hypoparathyroidism, pseudohyperparathyroidism, vitamin D-dependent rickets, granulomatous disease, lymphoma, primary hyperparathyroidism, and hypercalcemia. However, there is little information available in literature regarding the stability of 1,25(OH)2D in human serum.

Objective: To determine the stability of 1,25(OH)2D2 and 1,25(OH)2D3 in human serum under different conditions encountered in a typical clinical laboratory.

Methods: A commercial charcoal stripped human serum was spiked to 20 pg/mL of 1,25(OH)2D2 and 1,25(OH)2D3, then aliquoted into 0.6 mL vials. One sample (t =0h) was stored at -80°C immediately, while the remaining samples were stored either at -20°C, 4°C, room temperature (RT) in the dark or RT under light. Samples were then moved to -80°C storage at different time intervals: 2h, 4h, 8h, and 24h for RT, 8h, 24h, 72h, and 168h for 4°C, and 24h, 48h, 96h, and 168h for -20°C storage conditions. Samples were thawed and analyzed in one batch using an internally developed and validated liquid chromatography-tandem mass spectrometry method. Stability of the extracted samples (final composition 70%:30% methanol:water) in the autosampler (4°C) was also tested by re-injecting the whole batch 2 days later.

Results: As presented in Figure 1, both 1,25(OH)2D2 and 1,25(OH)2D3 were stable in spiked charcoal stripped human serum for the duration of the study (24h at RT, 168h at 4°C and -20°C). Extracted samples were also stable for the entire study period (2 days at 4°C).

Conclusion: 1,25(OH)2D2 and 1,25(OH)2D3 are stable in spiked charcoal stripped human serum and in 70%:30% methanol:water extracts.



E-12

Manufacturer Standardization of C-peptide Assays to an Isotope-Dilution Mass Spectrometry Candidate Reference Method

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Background: C-peptide measurement is an important indicator of endogenous insulin secretion. Currently C-peptide results vary widely among assay methods despite documentation of traceability to the World Health Organization (WHO IRR 84/510) standard. Here we report the results of a study designed to determine if manufacturers can effectively standardize C-peptide assays by using matrix-appropriate pooled-serum calibrators with values assigned by a candidate reference method.

Methods: Forty serum samples were sent to six manufacturers (using a total of 9 different methods) along with six concentrations of pooled serum calibrators with values assigned by a 2-dimensional reversed-phase liquid chromatography-isotope dilution-mass spectrometry (IDMS) candidate reference method. Manufacturers were instructed to analyze the samples and provide results from calibrations performed both according to their normal procedures and with the pooled-serum calibrators. One-way analysis of variance with Kramer-Tukey adjustment for multiple tests was used to compare means for the different assay methods (n=9, resulting in 36 pairwise comparisons for each calibration); P values < 0.05 were considered statistically significant. Statistical analyses were performed using SAS V9.

Results: Between-lab variability increased with increasing C-peptide concentration. Normalization of individual serum results using the pooled-serum calibrators significantly improved comparability among lab/methods (figure). Using the usual calibration, mean results for each individual method ranged from 1.04 to 1.99 and 23 of 36 paired-means showed statistically significant differences. After pooled-serum calibration the means ranged from 1.33 to 1.48 and 14 of 36 paired-means showed statistically significant differences. Between-laboratory coefficients of variation were 20.3% vs. 7.6% for the usual and serum calibrations, respectively.

Conclusion: These data confirm that standardization of C-peptide assays can be achieved by providing manufacturers with pooled-serum calibrators with values assigned by a candidate reference method.

E-13

Determination of levels endogenous melatonin associated with clinical improvement with treatment of severe obstructive sleep apnea with CPAP

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Background: The sleep-wake cycle is regulated by interaction of endogenous circadian and homeostatic processes. The strong primary agents of synchronizing the circadian system are reported to be light and melatonin secretion. Patients with sleep disorders can trigger problems with phase of sleep, sleep fragmentation, arousals, Jet lag and shift-work. Disturbances in circadian phase have been reflected in the rates of endogenous plasma melatonin as an important mediator in the pathophysiology of sleep. Our objective are to verify whether the laboratory alters the phase of the apnea patients' sleep, we measured the rates of endogenous melatonin in the morning a group of individuals with apnea treated with Continuous positive airway pressure (CPAP) group vs untreated apnea compared with control without apnea.

Methods: We selected 54 individuals (men and women) of the Heart Institute Sleep, with a mean age of 54.58 ± 9.63, BMI 27.85 ± 3.22, divided into 28 with severe obstructive sleep apnea but make use of CPAP (OSA + CPAP) for over a year, 16 with severe untreated sleep apnea (OSA) and 10 subjects without apnea (controls). All subjects underwent clinical, laboratory and polysomnographic evaluations. The patients were classified using the apnea and hypopnea index (AHI) for AHI > 30 individuals with severe OSA and with AHI<5 for controls. We excluded patients with psychiatric, neurological, cardiorespiratory and medical diseases, use of psychoactive drugs and weight BMI> 40 kg/m^2. All women were in menopause for more than a year. Determination of melatonin was performed on blood collected at 8:00 Am on all patients. The methodology used was enzyme immunoassay-ELISA Melatonin - International IBL-Hamburg, Germany. Statistics: We calculate the averages and standard deviations of melatonin concentrations in groups and use one-way ANOVA with post hoc Bonferroni to observe the differences between them.

Results: The groups (OSA + CPAP), (OSA) and control showed average and standard deviation respectively : 17.36 ± 11.3 pg / mL, 49.25 ± 37.7 pg / mL and 24.85 ± 19.0 pg/ mL (p = 0.01). The group (OSA + CPAP) had significantly reduced when compared with group (OSA without treatment) (p = 0.006). The group (OSA) had higher levels of melatonin in the morning when compared with controls

(p = 0.000).

Conclusion: With these data we found that the use of CPAP may influence the secretion of melatonin in the morning releasing quantities close to the control group (no apnea) while the group untreated OSA have increased levels of melatonin release favoring daytime sleepiness in patients.

E-14

Developement of a candidate reference measurement procedure for the determination of estradiol in human serum

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Background: Estradiol regulates a wide range of biological functions and fluxuations in estradiol levels can lead to disease and adverse health conditions such as cancer. Current estradiol measurements contain high variability and the comparability of the data obtained with these measurements is limited. This variability prevents the use of translation of research findings into patient care and public health activities and thus limits the use of estradiol measurements in the diagnosis and prevention of diseases. To overcome this problem a reference method is needed to harmonize value assignments for research, clinical and public health laboratories as well as test manufacturers. Such a reference method is not available in the U.S. As part of CDC's effort to standardize hormone measurements, a candidate reference measurement procedure for estradiol in human serum, involving isotope dilution (ID) coupled with liquid chromatography/tandem mass spectrometry (LC-MS/MS), has been developed.

Methods: The proposed method measures endogenous estradiol and an internal standard (estradiol-D_5) extracted from serum using a double liquid-liquid extraction prior to analysis by reverse-phase LC-MS/MS. A gravimetric approach is used to prepare two sets of calibrators with target concentrations of 25 pg/mL and 250 pg/mL with equal internal standard concentrations to provide a 1:1 ratio. Additional calibrators are prepared with mass ratios of analyte to internal standard bracketing around the target concentrations from 0.5-1.5. Measurements are made using an API5500 MS coupled with a TurboV electrospray ionization source in negative ion mode and an Agilent HPLC system. The HPLC separation is performed on a C6-phenyl column with a gradient of water and methanol with ammonium hydroxide as a pH modifier to aid in ionization. Quantiation by multiple reaction monitoring (MRM) of estradiol and internal standard is performed.

Results: The current limit of detection and limit of quantitation are 0.12 pg/mL and 0.32 pg/mL, respectively. The candidate reference method can adjust the volume of serum to accommodate samples with estradiol levels of less than 1.0 pg/mL. Certified reference materials from the Institute for Reference Materials and Measurements (BCR 576 at 32.9 pg/mL, BCR 577 at 199 pg/mL, BCR 578 at 386 pg/mL) were used to assess imprecision and bias. The within and between runs imprecision is less than 4.5% and the bias is less than 3.0% across assessed concentration ranges. Traceability to a certified reference method has been established and compared using Deming regression analysis showing no proportional or constant bias. Structural analogs have been tested for interference with the measurement of estradiol and quantitation and confirmation ion ratios are used to monitor samples for possible interferences during analysis.

Conclusion: This candidate reference method for estradiol in serum demonstrates good accuracy and precision, as such, this method can be used as a viable base for accuracy to which routine methods for estradiol can be compared.

E-15

Evaluation of Growth Hormone Suppression after Oral Glucose Tolerance Testing using the Beckman Coulter Ultrasensitive hGH Assay

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Background: Acromegaly is characterized by excess growth hormone (GH) production and leads to continuous and gradual acral tissue growth and many co-

morbidities. The diagnosis is generally based on clinical features and confirmed by laboratory testing. The later includes demonstration of increased insulin like growth factor 1 (IGF-1) and the lack of GH suppression to an oral glucose tolerance test (OGTT). Random GH measurement might or might not be elevated. Unfortunately, poor comparability between GH immunoassays has resulted in lack of uniformity for the criteria used in the interpretation of GH suppression following OGTT. Current proposed nadir level for normal GH suppression ranges from 0.25 to 1.0 ng/mL depending on the immunoassay used in particular studies. Recently, the Acromegaly Consensus Group published recommendations suggesting that using ultrasensitive GH assays a nadir cutoff of 0.4 ng/mL after OGTT is considered an adequate response for GH suppression in disease-free patients.

Objective: Determine whether the cutoff for nadir GH values after OGTT of 0.4 ng/mL compared to the previous cutoff of 1.0 ng/mL is appropriate in diagnosis and monitoring of acromegalic patients using the Beckman Coulter Ultrasensitive hGH Assay (Chaska, MN)

Methods: Results from patients who underwent OGTT testing from July 2007 through October 2010 were evaluated (n=90). Analyzed data consisted of GH values at baseline and at 30, 60, 90, and 120 minutes after a 75g oral glucose administration. GH measurements were performed using the Beckman Coulter Ultrasensitive hGH Assay. The final diagnosis was retrieved from the medical records and patients were categorized into active-disease or inactive-disease based on the clinical diagnosis at the time of testing. Patients who had unclear diagnosis, were on medication for their acromegaly, had multiple tests with no change in disease state, or found to have no disease but elevated IGF-1 were excluded (n=46).

Results: The active-disease group (n=21) included patients with newly diagnosed acromegaly (n=14), and persistent or recurrent disease (n=7). The mean baseline GH for this group was 2.04 ng/mL (range 0.17-7.76 ng/mL). The inactive-disease group (n=23) included patients with acromegaly in remission (n=11) or patients without disease (n=12) with a mean baseline GH of 0.60 ng/mL.(range <0.01-0.9 ng/mL). The mean nadir GH values for the inactive-disease and active-disease group were 0.18 ng/mL (range <0.01-0.9 ng/mL) and 1.53 ng/mL (range 0.1-6.97 ng/mL), respectively. Using a cutoff of GH nadir value after OGTT of 0.4 ng/mL would have correctly classified 21 out of 23 (91%) patients with inactive-disease and 17 of 21 (81%) patients with active-disease. Using a cutoff of 1.0 ng/mL would have correctly classified 23 of 23 (100%) patients with inactive-disease and 10 out of 21 (48%) patients with active-disease.

Conclusion: The nadir GH value after OGTT indicates that the newly recommended cutoff of 0.4 ng/mL using the ultrasensitive GH assay more accurately correlates with clinical diagnosis of acromegaly than the previous cutoff of 1.0 ng/mL. Future prospective studies on a larger sample size should be performed to verify these findings.

E-16

Dedicated Semi-automated Serum Aldosterone Method for the ABSciex API-5000 Mass Spectrometry System

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Background: It is well-documented that serum aldosterone, as determined by antibody-based assays, is subject to methodological bias and various interferences. This has hampered attempts to create a standard definition of what constitutes a positive screen for primary aldosteronism using the ratio of aldosterone to plasma renin activity. LC-MS/MS methods for aldosterone represent a major step forward in solving this problem. To this end we sought to develop a simple, accurate, precise, robust serum aldosterone method for the ABSciex API-5000 triple quadrupole tandem mass spectrometer.

Methods: Ionization conditions were determined using ABSciex Analyst software protocols for compound optimization during direct infusion of aldosterone in methanol solution into the MS. Two transitions were selected for monitoring: quantifier *m/z* 359.09→188.90 amu and qualifier *m/z* 359.09→331.10 amu. All pipetting was performed on the Hamilton Starlet robotic liquid handling system. Standards were prepared by spiking a weighed-in stock aldosterone solution in MeOH into double-stripped steroid-free serum. Calibrators ranged from 56.0 pmol/L (2.02 ng/dL) to 3460 pmol/L (124.9 ng/dL). Five Hundred microlitres of specimen or calibrator was prepared by spiking 60 µL of 110 nmol/L d7-aldosterone solution in 50:50 MeOH/H_20 which was then extracted with 2500 µL of methyl tert-butyl ether (3 min vortex) followed by 5 min of centrifugation (3000 rpm, 5 deg C). Two mililitres of the organic layer was then transferred to a 2.2 mL Eppendorf tube for dry-down. Samples were reconstituted with 125 µL of 50:50 MeOH/H_2O solution and vortexed. HPLC was performed using NH_4Ac (2 mmol/L) buffered H_2O (A) and MeOH (B) on a Shimazdu LC-20AD/CT-20A/SIL-20ACHT UFLC using a Phenomenex Gemini-NX 3u C18 110A 100 x 2.0 mm with the oven heated to 55ºC. Gradient was determined for an elution time of approximately 6.5 min and a total run time of 10 min/specimen. Recoveries were determined by four-fold serial dilution of a high human sample (1156 pmol/L) with steroid-free-serum. Precision was determined by modification of CLSI EP-5A2 on human serum pools, limit of quantitation and detection by S/N ratios, and method comparison by analysis against Siemens Coat-a-Count radioimmunoassay (n=138). Interferences were determined by spiking high levels of related steroids (cortisol,corticosterone,11-DOC,11-deoxycortisol,18-OH-corticosterone,18-OH-11-DOC) and running samples by this method. Statistics performed in R v.2.10.0.

Results: Calibration curves demonstrated linearity up to the highest standard with no evidence of deviation (R^2: 0.9984-0.9998). Recoveries were 97-109%. Total precision was 120 pmol/L and 10% at 50 pmol/L. LOQ based on median S/N of 10:1 was 50 pmol/L and LOQ based on median S/N was <22 pmol/L. Regression (Passing-Bablok) between LC-MS/MS and RIA was determined to be LC-MS/MS= 1.14xRIA-59.2 pmol/L after exclusion of 4 outliers, 3 of which had much higher results by RIA and were, incidentally, noted to have chronic kidney disease. No interference was found except as shouldering in the qualifier transition caused by 18-OH-corticosterone.

Conclusion: We have developed a robust, liquid-liquid extraction LC-MS/MS method for aldosterone on the ABSciex API-5000 system with excellent sensitivity and test characteristics suitable for implementation in a clinical laboratory.

QC1 ($25OHD_3$ 110 pg/mL, $25OHD_2$ 135 pg/mL)				
	Accuracy n=30	Intra-Run Imprecision n=5	Inter-Run Imprecision n=10	Total Imprecision n=30
$25OHD_3$	101%	2.83-4.25%	6.04%	5.66%
$25OHD_2$	92%	2.79-5.79%	5.44%	6.52%
QC2 ($25OHD_3$ 310 pg/mL, $25OHD_2$ 310 pg/mL)				
	Accuracy n=30	Intra-Run Imprecision n=5	Inter-Run Imprecision n=10	Total Imprecision n=30
$25OHD_3$	106%	3.91-5.11%	4.61%	5.67%
$25OHD_2$	88%	2.03-6.49%	3.08%	4.16%

Conclusion: We have developed a simple and sensitive method to quantify $25OHD_2$ and $25OHD_3$ in urine specimens in a single analytical LC-MS/MS run.

E-19

Performance Evaluation of a New IMMULITE® Erythropoietin Assay

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Objective: To evaluate the performance characteristics of a new IMMULITE erythropoietin (EPO) assay* (Siemens Healthcare Diagnostics, Tarrytown, NY, US).

Relevance: EPO measurement is useful in the diagnosis of anemias and polycythemias. The new IMMULITE EPO assay uses a 1-step monoclonal-monoclonal sandwich format and a single 30-minute incubation. In contrast, the previous assay used a monoclonal capture antibody, a polyclonal detection antibody, and two sequential 30-minute incubations. The new formulation provides a faster time-to-result and extends the upper end of the range (to 750 mIU/mL). Replacing the polyclonal detection antibody with a monoclonal antibody may also reduce lot-to-lot variability.

Methods: The IMMULITE EPO assay, a solid-phase enzyme-labeled chemiluminescent immunometric assay, has a reportable range of 1.0 to 750 mIU/mL and is standardized to WHO 67/343. Method comparison (IMMULITE EPO assay vs. Beckman Access® 2 EPO assay) and reproducibility studies were conducted at 3 external independent laboratories, using 2 IMMULITE 2000 reagent kit lots. The method comparison study used 217 endogenous disease-state samples (anemia or polycythemia). The reproducibility study included 7 patient pools with EPO values distributed across the assay range. Several validation studies were conducted at Siemens: method comparison to Beckman Access 2 using 186 disease-state samples; precision (using 7 patient pools with EPO values spanning the assay range); linearity (using 6 patient samples with EPO values spanning the assay range); limit of blank (LoB) and limit of detection (LoD) in accordance with CLSI EP17-A; functional sensitivity (defined as the concentration with 20% coefficient of variation [CV]); potential interference and cross-reactants; and reference interval (using 170 apparently healthy donors). Finally, IMMULITE EPO assay results on the IMMULITE 1000,

2000, and 2500 instruments were compared using the 186 disease-state samples.

Results: IMMULITE 2000 Results. The method comparison study resulted in the following linear regression equations: external study: IMM2000 = 0.96 x Access + 2.57 mIU/mL, r=0.98 (Deming); internal (Siemens) study: IMM2000 = 1.07 x Access - 1.87 mIU/mL, r=0.98 (OLS). The reproducibility total %CV ranged from 6.6 to 16.2 (3.4 to 8.8 within-run), and the precision total %CV ranged from 6.4 to 10.3 (3.6 to 6.8 within-run). The dilution linearity demonstrated a mean recovery of 100% (range 90% to 130%). The assay LoB and LoD were 0.5 and 1.0 mIU/mL, respectively, and the functional sensitivity was 1.5 mIU/mL. The interference and cross-reactant study indicated that the assay is highly specific for EPO. The reference range study yielded a central 95% range of 4.3 to 29 mIU/mL (median: 10.6 mIU/mL). IMMULITE instrument family results: Comparison of IMMULITE EPO assay performed with different IMMULITE instruments resulted in the following ordinary least squares equations: IMM2000 = 0.97 x IMM2500 + 1.63 mIU/mL, r=0.99; IMM2000 = IMM1000 x 1.06 - 1.2 mIU/mL, r=0.99.

Conclusion: The new IMMULITE EPO assay shows acceptable performance characteristics for EPO measurement on the different IMMULITE instruments.

* This assay has not been cleared by the FDA and is not available for sale in the US. The assay is CE marked.

E-20

Development of an LC-MS/MS method for 1,25 dihydroxy vitamin D for use in the clinical laboratory

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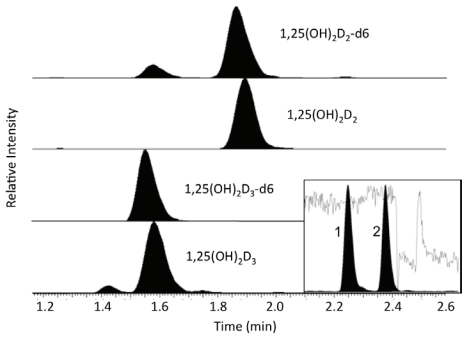
Background: The measurement of 25(OH)D is useful in determining vitamin D stores but provides no information about the active metabolite of vitamin D. The level of 1,25 dihydroxy vitamin D is important in monitoring patients with chronic kidney disease, oncogenic osteomalacia syndrome and acquired or inborn errors of phosphate homeostasis. $1,25(OH)_2D$ has been a challenging analyte to measure in the clinical laboratory due to low serum concentration. We developed an in-house method suitable for the clinical laboratory using a combination of immunoaffinity purification followed by high-performance liquid chromatography-tandem mass spectrometry (LC-MS/MS).

Methods: We used off-line protein precipitation of serum (methanol:acetonitrile) followed by an antibody-based extraction and 4-Phenyl-1,2,4-Triazoline-3,5-Dione derivitization. Analytes were resolved using reverse-phase HPLC and quantified using positive ion electrospray ionization-tandem mass spectrometry with hexadeuterated internal standards.

Results: $1,25(OH)_2D_3$ intra-assay and inter-assay imprecision was 5.6% and 8.0% (50pg/mL) and 8.7% and 13% (20pg/mL). Limits of detection and quantitation were 0.625pg/mL and 1.25pg/mL, respectively. $1,25(OH)_2D_2$ intra-assay and inter-assay imprecision was 8.7% and 11% (80pg/mL) and 11% and 13% (25pg/mL). Limits of quantification and detection were 0.625pg/mL. Comparison with RIA results spanning 5pg/mL to 125pg/mL had a proportional bias of 0.75, constant bias of -4.1 and Pearson correlation (r^2) of 0.31. A representative chromatogram from a patient sample for $1,25(OH)_2D_3$, $1,25(OH)_2D_2$ and the deuterated internal standards is shown in Figure 1. The resolution between $1,25(OH)_2D_3$ and $1,25(OH)_2D_2$ is 2.36, k'_{D3} of 5.21, k'_{D2} of 5.63 and a selectivity factor of 1.08. The final chromatographic method provided adequate separation of analytes from ion-suppresive matrix components (Figure 1, inset).

Conclusions: Protein precipitation with antibody-based extraction is an effective method for sample preparation in combination with LC-MS/MS detection for derivatized $1,25(OH)_2D$. This method has superior specificity over an existing clinically-used RIA with excellent limits of detection and good precision.



E-21

Simultaneous quantitation of Neuropeptide Y 1-36 and 3-36 in human plasma by time-of-flight electrospray mass spectrometry

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Background: Neuropeptide Y (NPY) is a 36 amino acid peptide which acts peripherally as a vasoconstrictor and is converted into the angiogenic NPY 3-36.

Objective: To develop a rapid, sensitive and specific HPLC-QTOF-MS method for the simultaneous determination of NPY 1-36 and 3-36 in human plasma.

Methods: The quantitation of the NPYs was carried out using an HPLC-QTOF-MS system (1200 Series connected to an 6530 QTOF mass spectrometer , Agilent Technologies, Santa Clara, CA) equipped with a reverse phase C_{18} column [2.1 x 15 mm and 1.8 µm particle size (Zorbax SB-C_{18})]. Column temperature was maintained at 50 °C. Mobile phases A and B were 2% methanol and 100 % methanol both containing 0.2% formic acid. The Jetstream ESI interface was operated in the positive mode, using the parameters: capillary voltage 3000 V; nebulizer pressure 35 psig; drying gas 9 L min^{-1}; gas temperature 370 °C; fragmentation voltage 200 V; skimmer voltage 65 V; octopole RF 750 V. LC/MS accurate mass spectra were recorded across the range 100-1700 *m/z*. The TOF was calibrated on a daily basis and subsequently operated at high accuracy (<5 ppm) without utilizing reference masses. Data were collected in centroid mode at a rate of 1 spectrum per s in the extended dynamic range mode (2 GHz). All data were acquired using MassHunter software (Agilent Technologies).

900 µL of human plasma containing 10 mM of DTT was placed in a Centrifree YM-30 ultrafiltration device (30,000 MW cut-off, Millipore), and centrifuged at 2700 rpm for 30 min at 37 °C. 30 µL of formic acid (10%) and 40 µL of 100 mM DTT were added to 400 µL of the ultrafiltrate. The latter was deproteinized with 700 µL of methanol containing deuterated internal standards. The resulting sample was centrifuged at 10,000 rpm for 5 minutes and loaded onto the LC column. NPY 1-36 and 3-36 peptide fragments were quantified in MS mode only. The ions chosen for quantification were 803.20380 for NPY 3-36(d_4-805.240810), 855.22480 for NPY 1-36 (d_2- 857.03450).

After sample injection the column was washed with 20% methanol for 6 minutes. All wash and gradient solutions contained 0.2% formic acid. The methanol gradient for the period 6.1 to 9.0 minutes was 20-90%, for 9.1-10 minutes 90-100% and for the period 10-13 minutes 100% methanol. The retention times for NPY 1-36, 3-36 and deuterated ISs were between 11.527 and 11.539 minutes.

Results: The LOQ for the NPYs were 0.1 ng/ mL. The recoveries were > 90% for both peptides. Linearity was good between 0.1-2 ng /mL. CVs < 10% between 0.2-2 ng/mL. Preliminary data indicate values for healthy normals are in the range of 0.23-0.67 ng/mL (NPY 1-36) and 0.19-0.65 ng/mL (NPY 3-36).

Conclusion: We developed a reliable, rapid procedure for the simultaneous quantitation of NPY 1-36 and NPY 3-36.

E-23

Simultaneous Measurement of 1,25-dihydroxyvitamin D_2 and D_3 in Human Serum by LC/MS/MS

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Background Vitamin D is a fat-soluble prohormone, the two major forms of which are vitamin D_2 and Vitamin D_3. The major role of vitamin D is to maintain normal levels of calcium and phosphorus in the blood. 1, 25-dihydroxyvitamin D_2 and D_3 ($DHVD_2$ and $DHVD_3$), the active form of vitamin D, is produced in the kidney. Measurement of circulating levels of DHVD is important in the diagnosis and management of disorders of calcium metabolism. This test is also used in the differential diagnosis of hypocalcemia and to monitor patients with renal osteodystrophy or chronic kidney disease.

The purpose of this study is to validate a method to precisely and accurately measure $DHVD_2$ and $DHVD_3$ in human serum. Here we report an accurate, rugged, and sensitive liquid chromatography-tandem mass spectrometry (LC/MS/MS) method for quantitation of $DHVD_2$ and $DHVD_3$ in human serum.

Methods Patient samples, calibrators, or controls were first delipidated and then spiked with deuterium-labeled internal standards. After affinity enrichment, the extracts were then derivatized using 4-phenyl-1,2,4-triazoline-3,5-dione (PTAD). 40 µL of the final derivative was introduced into the LC/MS/MS for analysis.

Results: The accuracy of the assay was conducted by performing correlation studies on clinical samples. This improved method demonstrates excellent correlation with our previous LC/MS/MS method (Y = 0.96X + 1.44 for $DHVD_2$ and Y = 1.00X - 1.02

for $DHVD_3$). The overall precision for $DHVD_2$ and $DHVD_3$ in serum was less than 7% across the range. The analytical reportable range was from 8 pg/mL to 200 pg/mL and the clinical reportable range was from 8 pg/mL to 1000 pg/mL. Linearity studies suggest that the analyte response was linear across the range of the calibration curve for both analytes. The limit of detection of $DHVD_2$ and $DHVD_3$ was determined to be 1.7 pg/mL and 2.5 pg/mL, respectively. No carryover was observed when 400 pg/mL was tested for both $DHVD_2$ and $DHVD_3$. We observed excellent reproducibility when this method was tested on different instruments with different reagent lots and different column lots.

Conclusion: This LC/MS/MS method provides an accurate, rugged, and sensitive way to determine $DHVD_2$ and $DHVD_3$ in human serum. This method has advantages over our previous method by using less sample volume which enables us to obtain results from many specimens that otherwise would have insufficient sample volume as well as less injection volume which allows repeated injections that may be necessary for confirmation.

E-24

Elevated concentrations of PTH 1-84 are related to survival in patients with chronic heart failure

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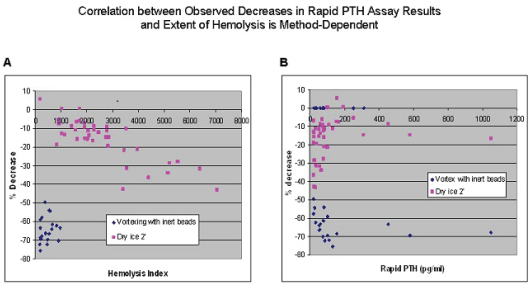
Background: Parathyroid hormone (PTH) is related to left ventricular hypertrophy in patients with end stage renal disease and secondary hyperparathyroidism. Furthermore, PTH is associated with worse prognosis in patients with overt HF and has an in vitro a hypertrophic effect on cardiomyocytes. The aim of the present study was to evaluate the relation between the circulating levels of bioactive PTH 1-84 and mortality in severe HF patients.

Methods: PTH 1-84 concentrations were determined in 76 patients with severe HF (20 women and 56 men; mean age of 68 years and mean ejection fraction of 23%) with the LIAISON third generation PTH assay (DiaSorin), a two-site automated sandwich immunoassay. Patients were followed for cardiac mortality from April 2004 till November 2010.

Results: Of the 76 patients enrolled, 41 died and 35 survived during follow-up. At baseline the mean PTH 1-84 concentration was significantly increased in the patients who died than in those who survived (41.7 vs 27.0 pg/mL, p=0.01). Kaplan-Meier curve analyses showed that mortality was increased in the the second tertile (15 deaths) and third tetile (18 deaths) in comparison to the first tertile (8 deaths; p=0.03). The risk stratification power of this assay was more efficient than with the second generation PTH assay.

Conclusions: Our results have demonstrated for the first time that bioactive PTH 1-84 levels are increased in heart failure patients. In addition, PTH 1-84 concentrations were higher in HF patients with the worst prognosis suggesting a potential use as a survival predictor.



E-26

Comparison of Agilent and Supelco C8 columns for plasma/serum estrogen measurement using liquid chromatography-tandem mass spectrometry

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Background HPLC tandem MS/MS is today regarded as the method of choice for the measurement of estrogens. The present study compares the C8 column (Rapid Resolution Cartridge, 2.1x30 mm, 3.5µ; Agilent Technologies, DE, USA) for the simultaneous measurement of estrogens with the C8 Supelco column (Supelco LC-8-DB, 3.3 cm × 3.0 mm, 3 µ particle size; Sigma-Aldrich, MO, USA) [published in Clin Biochem. 2008; 41:736-41].

Method An API-5000 triple-quadrupole mass spectrometer (AB-Sciex) with electrospray ionization source and Shimadzu HPLC system was used employing isotope dilution with deuterium labeled internal standards (ISs). 300 µL of acetonitrile containing ISs was added to 200 µL of serum. 1400 µL of deionized water was added to 350 µL of centrifuged supernatant and a 600 µL aliquot was injected onto the C-8 Agilent column. A 3 min wash with mobile phase A (methanol: water 2:98, v/v) at flow rate of 1 mL/min, was followed by the binary gradient program. The steroids were eluted at a flow rate of 600 µL/min. 80% A for 3 min, 48% B (methanol) to 58% B in 4.1 min, and 90% B for 0.9 min. The ionspray voltage was -4500 V and heater temperature was 600 oC. The declustering, entrance, and collision cell exit potentials were set at -120, -10, and -15 V, respectively; collision energy -55 V. curtain gas 20, collision gas 8, nebulizer gas 40, and heater gas 30.

Quantitation by multiple reaction monitoring analysis was performed in negative ion mode. Retention time (min) and ion pair for each analyte and its IS are: Estrone (5.32, m/z 269.2/145.2), Estrone-d4 (5.30, m/z 273.2/147), Estradiol (5.35, m/z 271.2/145.3), Estradiol-d4 (5.33, m/z 275.2/147.1), Estriol (3.89, m/z 287/171), Estriol-d2 (3.89, m/z 289/147), 16-OH Estrone (4.04, m/z 285.1/145.1), Aldosterone-d6 (m/z 365.3/337). Samples were analyzed using both the Agilent and previously published method using a Supelco C8 column. Column life was also assessed.

Results: The correlation between the two columns was: for Estrone: Agilent=1.07 Supelco +2.137, Syx=7.136, r=0.9942, n=17, Estradiol : Agilent= 1.008 Supelco +17.28, Syx=27.35, r=0.972, n=16 and for Estriol were Agilent= 1.106 Supelco +0.4412, Syx= 1.008, r=0.93, n=16.

The limit of quantitation was 2pg/mL for estradiol and estrone using either column. Other parameters were also comparable. Between-day CVs ranged from 4.5% to 9.5% (n=20). Recovery ranged from 88% to 108%. Accuracy for Estrone, Estradiol and Estriol was assessed by comparison of the Agilent C8 method with a commercial reference laboratory (CRR), and correlation between the laboratories was: for Estrone: GU= 0.9029 CRR - 1.651, Syx = 4.098, r = 0.996, n=63 , Estradiol: GU = 0.8523 CRR + 8.422, Syx = 51.58, r = 0.993, n=28 and for Estriol were GU=1.029 CRR +0.1448, Syx=0.6434, r = 0.991, n=28, respectively. In our laboratory the C8 Supelco column provides good chromatography for one day only, whereas the Agilent column can be used for at least three days and provides similar sensitivity, specificity and accuracy.

Conclusions The new estrogen method employing the Agilent C8 column provided reliable performance for the measurement of Estrone, Estradiol and Estriol by MS/MS.

E-28

First Evaluation Of Capillarys 2 Flex Piercing® (Sebia) As A New Analyzer For Hba_{1c} Assay

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Background: HbA_{1c} is a key biomarker for the monitoring of glycemic balance in diabetic patients. It may be measured by various methods, including high-pressure liquid chromatography (HPLC) and immunoassays. Here we report the results of the first evaluation of Capillarys 2 Flex Piercing®, a new analyzer using capillary electrophoresis for the separation and the quantification of HbA_{1c} from whole blood in primary capped tubes. Eight capillaries are used in parallel, allowing a throughput of 40 samples per hour.

Methods: The analytical performances of the assay have been tested under routine conditions. Blood samples collected in EDTA-containing tubes were obtained from the daily laboratory activity. HbA_{1c} values obtained in 500 samples ranging from 3.9% (19 mmol/mol) to 16.9% (161 mmol/mol) were compared to those of the HPLC assay routinely used in the lab (Variant II® analyzer, Bio-Rad). The influence of the most frequent analytical interferences on HbA_{1c} assay (labile HbA_{1c}, carbamoylated hemoglobin and hemoglobin variants) was also studied.

Results: Intra- and inter-assay CVs are respectively lower than 1.98% and 2.68%. The linearity is excellent for HbA_{1c} values ranging from 3.2% (12 mmol/mol) to 19.7% (192 mmol/mol) (r=0.999). The results are well correlated with those obtained by the HPLC

Methods: HbA_{1c}[Capillarys 2] = 0.941 x HbA_{1c}[Variant II] + 0.303 (r=0.993, n=500). Moreover, the use of external quality control samples (from the European Reference Laboratory for Glycohemoglobin Educational Programme 2010) indicated a good accuracy of the method, since the results are in agreement with IFCC targets. The presence of labile HbA_{1c} or carbamoylated hemoglobin did not affect HbA_{1c} measurement, as well as the presence of some hemoglobin variants, such as hemoglobin S, E and D.

Conclusion: This evaluation showed that the analytical performances of Capillarys 2 Flex Piercing® analyzer for HbA_{1c} assay fullfilled quality criteria necessary for clinical use, and allowed to recommend its implementation in clinical chemistry laboratories for a routine practice.

E-29

Putative Mechanisms That Link Cystatin-C With Development Of Coronary Heart Disease In Patients With Type 2 Diabetes Mellitus

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Background: Recent evidence suggest that Cystain C, a cysteine proteinaser inhibitor and marker of glomerular filtration rate, may play an important role in the pathogenesis of atherosclerosis and coronary heart disease (CHD). This study evaluates the associations of cystatin-C with traditional and non-traditional CHD risk factors and incident CHD in 250 patients with Type 2 diabetes.

Methods: Cystatin C, fasting, insulin, glucose, homocysteine, high-sensitivity C Reactive Protein (hs-CRP), adiponectin, leptin, leptin receptor , free leptin index, and full lipid profile were determined in T2DM patients with CHD (96) and without CHD (154) who were also classified as normo- or microalbuminuric. Clinical and anthropometric data were recorded and subjects were classified on the basis of the degree of insulin resistance (HOMA-IR) and serum cystatin C levels (1.4 mg/l was considered high). Linear and multivariate (with inclusion of age and smoking status as potential confounders) regression analyses were used to determine the associations of Cystatin C with variables and risk factors.

Results: Cystatin-C was positively correlated with age (r=0.24) and homocysteine (r= 0.35) but showed no correlation with the adipokines (adiponectin, leptin, free leptin index). Compared to those with normal levels, patients with elevated cystatin C had significantly ($p< 0.05$) higher homocysteine (18 Vs 13 µmol/L), HOMA-IR (9.8 Vs 8.2), free leptin index (3.3 Vs 2.1 ng/U), Triglycerides (2.2 Vs 1.7mmol/L); hs-CRP (0.97 Vs 0.62 mg/L)and significantly lower HDL cholesterol (1.1 Vs 1.3). 62% of subjects with CHD had elevated cystatin C. After adjustment for confounders, logistic regression analysis showed that elevated Cystatin C was significantly associated with hypertension (Odds Ratio (OR) = 1.2), HOMA-IR (OR = 1.3), hyperhomocysteinemia (OR = 2.8), microalbuminuria (OR =2.7), hs-cRP (OR = 8.9) and CHD (OR = 2.9).

Conclusion: Studies have shown that Cystatin-C plays a role in the inflammatory process that accompanies atherosclerosis and local cystatin C deficiency has been shown in atherosclerotic lesions where cystatin C has been postulated to be involved in the degradation of extracellular matrix. However, the associations of cystatin C with several CHD risk factors in this study suggest a multi-hit mechanism that goes beyond its local role in the pathogenesis of CHD. As Chronic kidney disease is known to be associated with substantial risk for cardiovascular morbidity and mortality, we conclude that estimation of Cystatin C has potential as a predictive marker for CHD in patients with Type 2 diabetes mellitus.

E-30

Development of the ADVIA Centaur® Systems Enhanced Testosterone (TSTO2) Assay

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Background: A total testosterone assay (Testosterone II)* with enhanced sensitivity and precision is being developed for the ADVIA Centaur (Centaur, Centaur XP, and Centaur CP) systems (Siemens, Tarrytown, NY, US) to quantify low levels of testosterone in serum and plasma.

Methods: The Testosterone II assay on the ADVIA Centaur Systems is based on a fully automated sequential, competitive immunoassay format. Serum or plasma sample (20 uL) is added to the cuvette followed by the addition of a testosterone-releasing agent and a biotinylated monoclonal antibody specific for testosterone. After an incubation of about 10 minutes, streptavidin-coated magnetic particles and a 5-β-DHT-acridinium ester conjugate are then added. After approximately 4 minutes, the reaction cuvette is washed and reagents are added to measure the chemiluminescent signal. The time-to-result is 18 minutes.

Results: The LoD was estimated to be 2.5 ng/dL for both the Centaur/XP and Centaur CP systems. The functional sensitivity at a 20% total CV was estimated to be less than 10 ng/dL. Imprecision (%CV) of the ADVIA Centaur/XP testosterone II assay measured over 20 days using commercially available serum controls and serum pools was ≤ 5% (within-run) and ≤ 7% (total). Imprecision (%CV) of the ADVIA Centaur CP was ≤ 6% (within-run) and was ≤ 8% (total). The analytical range/linearity was 2.5 - 1500 ng/dL. The reagent on board stability was 60 days with a 28-day calibration interval for the ADVIA Centaur/XP and a 14-day calibration interval for the ADVIA Centaur CP system. Correlation with LC-MS/MS for testosterone values between 3 and 1367 ng/dL yielded the following regression equation: ADVIA Centaur/XP = 1.02(LC-MS/MS) + 5.0 ng/dL, n = 98, r = 0.99.

Conclusion: The Testosterone II assay developed for the ADVIA Centaur Systems is acceptable for laboratory use with respect to sensitivity, precision, and accuracy.

* In development. Not available for sale.

E-31

The Assessment of Free Testosterone using the Beckman Coulter Access® Testosterone and Sex Hormone-Binding Globulin (SHBG) Assays

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Background: Most circulating testosterone is bound to sex hormone-binding globulin (SHBG). A lesser fraction is weakly bound to albumin and a small proportion exists as free hormone. Free testosterone can be estimated by a calculation using measured total testosterone, SHBG, and albumin providing an Apparent Free Testosterone Concentration by calculation (AFTC). The preferred method for measuring free and total testosterone is equilibrium dialysis and tandem mass spectrometry. This technology is unavailable at many clinical laboratories, so we investigated an alternative method.

Methods: We measured total testosterone, SHBG, and albumin in serum samples from a total of 67 male and female subjects. Testosterone and SHBG were measured by immunoassay on the Beckman Coulter Unicel DxI®. Albumin was assayed by the Beckman Coulter Unicel DxC®. Dynamic range is 10-1600 ng/dL (0.35-55.5 nmol/L) for testosterone and 0.33-200 nmol/L for SHBG. Test results are determined automatically by the system software. Total testosterone was also measured using tandem mass-spectrometry from a referral laboratory for the method comparison. The AFTC results were calculated from total testosterone, SHBG, and albumin results using the Vermeulen formula. The AFTC results were compared to free testosterone measured with tandem mass spectrometry following separation by equilibrium dialysis performed at a referral laboratory. Method comparisons were performed using Analyse-It* software. Concordance was determined by comparing interpretation of each method using each method's corresponding reference intervals, as the established reference intervals differed by method. We also investigated the performance characteristics of both immunoassays. Imprecision was determined using two levels of commercially available control material.

Results: Deming analysis yielded the regression lines y = 0.84x -1.68 and y = 0.97x -37.25 for free testosterone /AFTC and total testosterone respectively. Pearson correlation coefficients were 0.98 for both free/AFTC and total testosterone comparisons. Percent agreement was 87.9% for free testosterone v. AFTC. Total imprecision for total testosterone was 3.77% and 3.88% at levels of 104 and 880 ng/dL, respectively. Total imprecision for SHBG was 5.9% and 5.2% at levels of 105 and 10.70 nmol/L, respectively.

Conclusion: Although the actual values differed between each comparative method, any discordant result appeared to be due to the difference in reference interval rather than by a lack of agreement. Therefore concordance and performance characteristics were determined to be acceptable for total testosterone by immunoassay and calculation of the AFTC, for the routine measurement of free and total testosterone.

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**This does not constitute off label promotion or use by Beckman Coulter.

E-32

Indirect method for the estimation of Health Related Limits (reference intervals) for TSH, F4 and FT3 using Roche Modular in a large italian population aged 0-99 years

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Background: The development and the verification of reference interval for hormones and particularly for thyroid hormones is very relevant and many authors investigated the subjects. Since the intervals are method, genre and age dependent, we applied a well known indirect method in a large Italian population. The aim of the study was to verify the thyroid hormones genre and decades reference intervals suggested by the manufacturer and previously confirmed in our Area.

Methods: We downloaded all the TSH, FT4 and FT3 results obtained between July and December 2010 in our subregional laboratory which serves the entire Romagna with a population of about 1 million. All the assays have been carried out using Modular analyzer (Roche, Mannheim, Germany) and stored in our LIS (DnLab,

Noemalife, Bologna, Italy). 52171 TSH results, 38131 FT4 results and 20380 FT3 results have been divided in three different ways: I) in 9 groups according to decades; II) in the following four groups: 0-19 years, 20-49 years, 50-69 years, > 70 years; III) in two groups: 0-69 years, > 70 years. Indirect Health Related Limits (HRLs) for males and females were estimated according to the method proposed by Kairisto et al (Graph ROC).

Results: The Table summarizes Low (LHRL) and High (HRL) Health Related Limits of TSH, FT4 and FT3 in males and females aged 0-99 years.

Conclusion: TSH: reference interval suggested by the manufacturer is substantially confirmed in females aged 20-69 years while it seems necessary to select higher HHRLs for subjects aged over 70 years and under 19 years and especially in the first decade. FT3: HHRLs are lower in females and males while LHRLs are higher that those suggested by manufacturer especially in the years 0-19. FT4: the HRLs are moderately lower than those suggested by manufacturer both in females and males

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TSH (mu/L)	females			males		
years	subjects	LHRL	HHRL	subjects	LHRL	HHRL
0-19	1114	0,57	4,74	684	0.66	4.25
20-49	13442	0,32	4,04	4055	0.20	3.59
50-69	12450	0,19	4,05	4541	0.22	3.73
> 70	11603	0,18	4,63	4862	0.2	4.41
FT3 (ng/L)	females			males		
years	subjects	LHRL	HHRL	subjects	LHRL	HHRL
0-19	464	2.31	4.68	258	2.97	4.61
20-49	5735	2.17	3.58	1476	2.42	3.8
50-69	5121	1.99	3.38	1603	2.12	3.63
> 70	3971	1.74	3.43	1521	1.61	3.50
FT4 (ng/L)	females			males		
years	subjects	LHRL	HHRL	subjects	LHRL	HHRL
0-19	850	9.20	14.65	512	8.84	15.40
20-49	10374	8.62	14.8	2662	8.95	15.2
50-69	9428	8.42	14.9	2908	8.65	14.4
> 70	7803	8.53	15	2906	8.51	16

E-33

Evaluation of Calculated Free and Bioavailable Testosterone Using Beckman DxI Testosterone and Sex-Hormone Binding Globulin Assays

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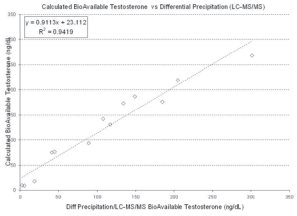
Background: Testosterone (T) binds non-specifically to albumin and specifically to sex-hormone binding globulin (SHBG). Free testosterone (FT) and Bio-available T (BioT) are better correlated with androgen activity than total testosterone. FT and BioT may be calculated using TT, SHGB, albumin concentrations, and known binding constants (Vermeulen, J Clin Endocrinol Metab 84: 3666-3672, 1999). Aim and

Methods: To validate the calculated FT and BioT in our institution, we evaluated T and SHBG assays on Beckman DXI® and compared calculated FT and BioT to equilibrium dialysis and differential precipitation, respectively, followed by liquid chromatography mass spectrometry (LC-MS/MS). FT (direct RIA) was also compared to equilibrium dialysis. Precision profile and reference range were examined. Consecutive serum specimens submitted for FT were analyzed. Data analysis used Microsoft Excel (Microsoft, Inc.) and EP Evaluator (Data Innovations, Inc.).

Results: For T, the imprecision expressed as % coefficient of variation (CV) was <4% (within-run) and <6% (between-run) at 94 and 830 ng/mL respectively. For SHBG, the CV was <6.3% (within-run) and <7% (between-run) at 9 and 100 nmol/L respectively. Mean recovery for both assays was 100% for concentrations spanning the analytical measurable range. Reference ranges for T, FT, and SHBG were within ranges recommended by the manufacturers. Calculated FT and BioT demonstrated excellent correlation with predicate assays: FT (calculated) = 0.5098 (equilibrium dialysis) + 0.5683, n=30 , r^2=0.929 ; BioT (calculated) = 0.9113 (differential precipitation) +23.112, n=30, r^2= 0.9419 (Figure); and FT (RIA) = 0.6791 (equilibrium dialysis) + 0.3753, n=30, r^2=0.8963.

Conclusions: Calculated FT and BioT provide an accurate, convenient and reliable alternative to direct analysis while avoiding limitations of the direct assay. Correlation and analytical performance data demonstrate that the T and SHBG immunoassays combined to calculated FT is an acceptable alternative to LC-MS/MS for the routine laboratory.



E-34

Analysis of Metanephrines in Plasma by LC-MS/MS

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Background: Metanephrine and normetanephrine are generated in the process of catecholamine metabolism, but are also produced excessively in pheochromocytoma tumors of the chromaffin cells, most of which are localized to the adrenal gland. The presence of a pheochromocytoma tumor is characterized by hypertension due to elevated concentrations of catecholamines. Historically, screening for such tumors by determination of catecholamines as well as conjugated and free metanephrines in urine has yielded false negative results in those with a genetic predisposition for the tumor and those with paroxysmal hypertension. Analysis of metanephrines in plasma, however, is of decisive diagnostic importance as it allows for more efficient exclusion or confirmation of the presence of a hyperplastic process. This test exhibits high sensitivity and specificity for the analytes produced by the tumor. The goal of this study was to develop and validate a high-throughput LC-MS/MS assay for measuring metanephrines in human plasma to aid in the diagnosis of pheochromocytoma and to monitor treatment outcomes.

Methods: Proteins are removed from plasma by weak cation exchange solid phase extraction using 2% formic acid in acetonitrile as the eluting solvent. Chromatographic isolation of the analytes and deuterated internal standards is achieved by elution on a Waters Atlantis HILIC silica column (2.1 x 50mm, 3.0 µm) connected to a Waters Acquity UPLC Xevo TQ MS/MS system using 100 mM ammonium formate(pH 3.0)/ acetonitrile(30:70) as eluent. Impurities are separated from metanephrines by gradient elution, and the tandem quadrupole mass spectrometer is operated in multiple reaction monitoring (MRM) mode with an electrospray ionization (ESI) probe in positive mode.

Results: Method validation results are displayed in the table below.

Conclusion: We successfully developed and validated an LC-MS/MS method for determining the concentrations of metanephrine and normetanephrine in human plasma that enables clinical diagnosis of pheochromocytoma and aids in monitoring treatment outcomes.

Validation Data

	Metanephrine	Normetanephrine
Method Comparison (HPLC/electrochemical detection; *n* = 252)	y = 0.933x - 0.015 ($S_{y/x}$ = 0.24; R^2 = 0.96)	y = 1.016x - 0.032 ($S_{y/x}$ = 0.34; R^2 = 0.99)
Linearity (1.0 - 100 nmol/L)	y = 1.004x + 0.013 (1.0% error)	y = 1.024x + 0.070 (3.0% error)
Recovery (mean of 5 replicates, 5 concentrations)	104.0 %	102.8 %
Analytical Measurement Range	0.1 - 5.0 nmol/L	0.1 - 5.0 nmol/L
Total Imprecision		
High control (mean)	4.0 %CV (1.54 nmol/L)	14.7 %CV (3.44 nmol/L)
Low control (mean)	7.0 %CV (0.43 nmol/L)	15.1 %CV (0.92 nmol/L)

E-35

Recovery of Insulin-Like Growth Factor-1 and Insulin-Like Growth Factor Binding Protein-3 after Liver Transplantation

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Background: The liver is the main source of insulin-like growth factor-1 (IGF-1). Cirrhotic patients present important reduction of plasma levels of IGF-1 and its binding protein (IGFBP-3). Hormonal changes after orthotopic liver transplantation

(OLT) have not been adequately evaluated yet. Our objective is to determine the changes of IGF-1 and IGFBP-3 in patients subjected to OLT.

Methods: A total of 21 adult males (age 49.7 ± 8.3 years; range 25-60 years) who underwent OLT for liver cirrhosis participated of the study. The main indications of OLT were alcohol abuse (n=9) and chronic hepatitis C infection (n=7). Fasting plasma levels of IGF-1 and IGFBP-3 were measured on the day before and 6 months after OLT by immunometric assay, enzyme-labeled chemiluminescent. Data were analyzed for fisher test.

MELD (Model for End-Stage Liver Disease) values varied from 12 to 30, with a mean± SD of 17.9± 4.5. Two patients were Child-Turcotte-Pugh class A, six class B, and thirteen class C.

Results: All patients had IGF-1 and IGFBP-3 lower than normal on the day before transplantation. The levels of IGF-1 and IGFBP-3 increased from 44.42± 34.12 to 198.88± 69.17 (p<.0001) and of IGFBP-3 from 0.96± 0.56 to 6.07± 9.05 (p=0.009). The levels of IGF-1 and IGFBP-3 increased in all patients after transplantation. IGF-1 and IGFBP-3 did not reach normal levels after transplantation in only one and three patients respectively

Conclusion: It is concluded that IGF-1 and IGFBP-3 are severely reduced in patients with hepatic cirrhosis. These changes are reversed after liver transplantation in almost all patients.

E-36

Evaluation of the Vitamin D Total Immunoassay on the ADVIA Centaur® System

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Background: The primary function of Vitamin D is to regulate normal blood levels of calcium and phosphorous. Vitamin D deficiencies are linked to rickets and osteomalacia. There are two major types of Vitamin D: ergocalciferol (D_2) and cholecalciferol (D_3). Both forms of vitamin D are metabolized by the liver to 25(OH) vitamin D and then converted in the liver or kidney into 1,25-dihydroxyvitamin D, the metabolically active version. Vitamin D deficiency can be best diagnosed using 25(OH) vitamin D versus the other vitamin D metabolites because 25(OH) vitamin D levels in serum reflect the body's storage levels of vitamin D and correlate with the clinical symptoms of vitamin D deficiencies.

Siemens Healthcare Diagnostics (Tarrytown, NY, US) has developed Vitamin D Total*, an automated equimolar 25(OH) vitamin D immunoassay for measuring both 25(OH) vitamin D_2 and D_3 on the ADVIA Centaur family of instruments. This is an 18-minute competitive immunoassay with an assay range of 3.7-150 ng/mL.

Methods: The performance of the Vitamin D Total assay was assessed at a US clinical site using two reagent lots.

Results: Imprecision was evaluated by assaying control materials and serum pools twice a day for 10 days, for a total of 40 replicates. On samples with vitamin D concentrations of approximately 35-130 ng/mL, the within-run CVs ranged from 2.4% to 7.0% and total CVs from 3.3% to 7.5% for lot A, and within-run CVs ranged from 2.4% to 6.7% and total CVs from 3.7% to 10.0% for lot B.

A total of 203 serum samples covering the range of the assay were assayed using two lots of ADVIA Centaur reagents and one lot of the DiaSorin 25-Hydroxyvitamin D RIA. Using a sufficiency cutoff of 30 ng/mL, the percent agreement of ADVIA Centaur was 96.1% with Lot A and 95.0% with Lot B. Method comparison on samples ranging from 0 to 50 ng/mL yielded these regression statistics: for Lot A (n = 75), ADVIA Centaur = 1.06 (RIA) + 3.5, r = 0.891; and for Lot B (n = 82), ADVIA Centaur = 1.04 (RIA) + 1.6, r = 0.880.

Conclusion: In conclusion, the results of this performance evaluation indicate that the ADVIA Centaur Vitamin D Total immunoassay can precisely measure vitamin D across a wide range of clinically relevant concentrations. The method comparison study showed equivalent performance to the DiaSorin 25-Hydroxyvitamin D RIA in the medically relevant range for aid in the determination of vitamin D sufficiency.

* This assay has not been cleared by the FDA and is not available for sale in the US. The assay is CE marked.

E-37

Performance evaluation of the Abbott Architect 25-OH Vitamin-D Assay

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Objective: To evaluate the performance of the newly introduced Abbott Architect 25-OH Vitamin-D assay by measuring imprecision, linear range, correlation with IDS-iSYS method and establishing reference ranges for our local population.

Relevance: There is an increasing recognition that a significant number of Australians and people from specific groups within the community are suffering from Vitamin-D deficiency. Because of this, testing for Vitamin-D as part of a routine check-up has become more common. At Medlab Pathology, approximately 400 Vitamin-D tests are performed per day (about 20% of patient requests/day). Due to high number of requests, performing Vitamin-D tests on the Architect analyser (MedLab's main stream analyser) is convenient, labor saving, economical and quick. Currently, Vitamin-D test is performed on an independent IDS-iSYS instrument as a batch.

Methodology: The Abbott Architect 25-OH Vitamin-D method is a one step polyclonal competitive chemiluminescent microparticle immunoassay standardised to an internal standard referenced to Diasorin-Liaison. 2 lot of reagents were used in this study. Within-run and total imprecision was assessed during a 7-day CLSI EP15-A2 protocol. For linear range, 2 sample pairs were prepared with 10 dilutions for each pair by mixing a high sample (493nmol/L) in specific ratios with a low sample (33nmol/L) following NCCLS-EP6-A protocol. Method correlation was performed over 5 days with IDS-iSYS Vitamin-D using 422 serum samples. Reference interval was calculated for both methods adopting inner 95th percentile method. Statistical software package 'Analyse-it' was used for all data analysis in this study. 5 sample results were submitted to DEQAS (EQAP Program for Vitamin-D) January 2011 program.

Results: Imprecision study resulted in highest total Imprecision of 5.6% at low level (50.8nmol/L) and lowest within-run imprecision of 2.9% at high level (194nmol/L). Method correlation in 422 samples over the range of 23-206nmol/L using Linear Regression with no special assumptions (Passing-Bablok) resulted in the following equation y = 1.1573x + 2.8994; R^2 = 0.9158. Linear dilution over the range of 33-493nmol/L gave a slope of 0.9985. Reference interval for Architect method is 33-174nmol/L and for IDS-iSYS method is 20-138nmol/L. The DEQAS-QAP report showed an average Positive bias of over 30% compared to ALTM.

Conclusion: Abbott Architect 25-OH vitamin-D is a more precise assay with a comparatively low imprecision at all 3 different levels of Vitamin-D. The linear range is satisfactory covering full range of calibration curve. There is a good correlation coefficient of 0.9158 between the methods. Architect method showed a positive bias of about 15%, however, Architect method had a good correlation with LC-MS/MS method as indicated in Architect kit insert. The difference in the reference interval between the methods is consistent with 15% positive bias by Architect method. A relatively low level of reference interval found in our population compared to general population is due to the ethnic background of the population MedLab covers. There were 4 Architect users in DEQAS-QAP program and Architect CV% was lowest of all the methods but there was a significant Positive bias.

E-38

Determination of Reverse Triiodothyronine Concentration by HPLC-MS/MS

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Background: Reverse triiodothyronine (rT_3) is an isomer of triiodothyronine (T_3) that is derived from thyroxine (T_4) through the action of deiodinase. Unlike T_3, rT_3 does not stimulate thyroid hormone receptors. Instead, rT_3 binds to these receptors, thereby blocking the action of T_3. Under stress conditions, the adrenal glands produce excess cortisol, inhibiting the conversion of T_4 to T_3 and shunting T_4 conversion from T_3 towards rT_3. As a consequence, there is a widespread shutdown in T_3 binding throughout the body, resulting in reduced body temperature and multiple enzyme dysfunction, in which the action of many enzymes is slowed. Symptoms are the same as those seen in hypothyroidism and include fatigue, headache, migraine, PMS, irritability, fluid retention, anxiety, and panic. rT3 levels also increase in sick euthyroid syndrome. This study validates a new method for measuring rT_3 to help in the diagnosis of multiple enzyme dysfunction and sick euthyroid syndrome.

Methods: rT_3 and an isotopic internal standard were extracted from serum or plasma. After protein precipitation, the clear extract was injected into an HPLC instrument with a tandem mass spectrometer. Method validation with serum was performed as follows: 1. linearity was determined using rT_3 standard solutions (range: 0 ng/dL to 200 ng/

dL); 2. limit of quantitation (LOQ) was determined using 3 serum pools with low rT_3 levels; 3. precision was determined by measuring rT_3 concentrations at 3 target levels; 4. recovery was determined at 6 rT_3 concentration levels (range: 2.5 ng/dL to 100 ng/dL); and 5. HPLC-MS/MS and radioimmunoassay rT_3 measurements were compared for 116 specimens. HPLC-MS/MS measurements for 3 other specimen types (serum collected with a serum separator tube (SST), EDTA plasma, and heparinized plasma) were compared with serum measurements for 30 individuals. The reference range was determined by analyzing 115 serum specimens from euthyroid individuals.

Results: Validation with serum specimens: 1. linearity: a correlation curve of measured concentrations vs target concentrations yielded a slope of 0.987, y-intercept of 0.936, and correlation coefficient (R^2) of 0.999; 2. LOQ: 2.0 ng/dL (defined as the lowest concentration within 20% CV); 3. precision: intra-assay CVs were 2.1-3.7% and total CVs were 2.3-4.1%; 4. recovery: a correlation curve comparing % recovery at the 6 rT_3 concentrations yielded a slope of 1.029, y-intercept of -1.400, and R^2 of 0.999; and 5. a correlation curve of HPLC-MS/MS vs radioimmunoassay measurements yielded a slope of 0.656, y-intercept of 1.910, and R^2 of 0.844. Correlation results for other specimen types vs serum were as follows: SST serum vs serum: slope=0.991, y-intercept=1.55, and R^2=0.997; EDTA plasma vs serum: slope=0.989, y-intercept=-0.45, and R^2=0.998; heparinized plasma vs serum: slope=0.984, y-intercept=-0.45, and R^2=0.996. The reference range was established as 7-26 ng/dL.

Conclusion: The HPLC-MS/MS method offers excellent precision and accuracy for routine rT_3 measurement. This new method correlates reasonably well with the radioimmunoassay and can be performed on 4 different specimen types.

E-39

Evaluation of Bone Turnover and Bone Mineral Density in Patients with Inflammatory Bowel Disease

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Background: Chronic inflammatory disorders such as inflammatory bowel diseases (IBDs), Crohn's disease (CD) and Idiopathic Ulcerative Colitis (IUC), affect bone metabolism and are frequently associated with the presence of osteopenia, osteoporosis and increased risk of fractures. The inflammatory process can increase the rate of bone turnover leading to bone loss, effects that are amplified by the deleterious skeletal effects of immunosuppressive drugs and decreased intestinal absorption of calcium. The objective of this study was to evaluate bone turnover and bone mineral density in patients with IBD.

Methods: We studied 100 patients, 20 to 50 years old, 38 men and 62 premenopausal women, 56 of those with IBD (36 CD and 20 IUC) and 44 age and sex-matched control patients. Clinical and anthropometric data were collected. Serum levels of isomerized C-terminal telopeptides of type 1 collagen (ß-CTx) were measured by electrochemiluminescence (ß-crosslaps, Roche Diagnostics, Mannheim, German). The reference value for premenopausal women and man was less than 0,58 ng/mL. Bone mineral density (BMD) was evaluated by dual-energy X-ray (DXA, Prodigy Advanced Plus, GE) at lumbar spine, proximal femoral neck and total hip. The criteria of low BMD was a Z-score equal to or lower than 2 SD. Absolute BMD values and the lowest Z-score for each patient were considered for analysis. Mann Whitney tests, univariate and multivariate analysis Kendals tau were employed, a two-tailed p value < 0.05 was judged to be statistically significant.

Results: There was no difference between groups regarding age and body mass index (BMI). The CD group had the lowest BMD values, with statistical significance versus controls in all regions examined and versus IUC in the lumbar spine. The CD group had lowest Z-score values in the total hip, with statistical significance versus the control group. The percentage of patients with Z-score $\leq$ -2 in control, CD and IUC groups were respectively: 6.8%, 30.5% and 15%. The median and range of serum CTx in the control, CD and IUC groups were respectively: 0.27 (0.07 to 0.86) ng/mL, 0.35 (0.08 to 0.99) ng/mL and 0.22 (0.04 to 0.55) ng/mL, with a significant difference between groups CD and IUC ($p = 0.025$). Univariate analysis showed a positive correlation between BMI and BMD at total hip and total body ($p = 0.038$ and $p = 0.001$). The correlation between BMD and CTx in the whole group was not significant.

Conclusions: 1) There was a high prevalence of low bone mass in IBD patients, especially those with Crohn's disease; 2) BMI was an important determinant of bone mineral density; 3) Crohn's patients had the lowest bone density and the highest CTx values, however bone remodeling did not appear to have a significant influence on the whole group bone density.

E-41

Performance Evaluation of the Dimension Vista® LOCI® Estradiol (E2) Assay

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Objective: To evaluate the performance of the Dimension Vista LOCI Estradiol (E2) Assay† in terms of linearity, onboard dilution, imprecision, functional sensitivity, and correlation with two existing commercial methods.

Relevance: Measurements of estradiol aid in the clinical assessment of various hormonal disorders and menstrual cycle irregularities. Estradiol measurements are also used to monitor follicular development during assisted reproduction technology (ART) treatment.

Methodology: The Dimension Vista E2 Assay is a fully automated homogeneous competitive immunoassay standardized to ID-GC/MS values; the method measures estradiol in serum or plasma samples using LOCI technology. Linearity of the analytical measurement range was evaluated using the CAP Linearity Survey LN8. Automated, onboard dilution results were compared to neat and manual dilution values using percent bias estimation. Within-run and total imprecision was assessed by ANOVA using a 20-day CLSI EP5-A2 protocol. Functional sensitivity, defined as the lowest measured concentration with 20% CV, was determined over 20 days using a panel of serum samples ranging from 8.8 to 33.7 pg/mL. Method comparisons were performed following CLSI EP09-A2 protocol and data analyzed by least squares linear regression. Results for 92 samples were compared for estradiol using the Dimension Vista E2 assay and the ADVIA Centaur® Enhanced Estradiol (eE2) Assay. A different set of 125 samples were compared for estradiol on the Dimension Vista E2 assay and the IMMULITE® 1000 Estradiol assay.

Results: Linearity was demonstrated across the analytical measurement range of 11 - 1500 pg/mL. Onboard dilutions demonstrated < 10% bias versus neat and manual dilution results. Method comparison of undiluted samples yielded the following regression statistics: Dimension Vista E2 = 1.02 * ADVIA Centaur® eE2 - 0.1 pg/mL (r = 0.997, n = 92) and Dimension Vista E2 = 0.95 * IMMULITE®1000 Estradiol + 6.0 pg/mL (r = 0.979, n = 125). Dimension Vista E2 within-laboratory (and repeatability) %CV estimates ranged from 2.1% to 20.2% (1.0%-11.8%) using three human serum pools and 1.8%-17.6% (0.8%-12.9%) with commercial controls. Functional sensitivity was determined to be 21.8 pg/mL.

Conclusions: The Dimension Vista LOCI E2 Assay method is a precise 10-minute assay with functional sensitivity at low concentrations and a wide reportable range of 11 - 7500 pg/mL. This new ID-GC/MS standardized method agrees well with accepted methods and is an attractive alternative for routine estradiol measurement.

† Product under development - not available for sale.

E-42

Do "dangerous" levels of hyperglycemia develop during 3-hour OGTTs when screening for diabetes in pregnancy?

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Background: Objective: Evaluate the frequency of hyperglycemia detected during 3-hr oral glucose tolerance tests in pregnancy. Relevance: Laboratorians may be asked by clinicians to cancel 3-hour OGTTs if the fasting plasma glucose on the day of the test is greatly elevated because of fear that the 100 g glucose load posses a risk to the fetus and/or the pregnancy. Hypothesis: We hypothesized that in pregnant women not known to be diabetic, peak glucose levels during the 3-hour OGTT would uncommonly exceed 200 mg/dL and would only rarely exceed 250 mg/dL.

Methodology/Results: Over a 1 year period (9-2-2009 to 9-2-2010), 261 3-h OGTTs were carried out in our system. Of these 261 tests, the fasting glucose was =>126 mg/dL in only 5 of 261 cases (1.9%). Of 1042 total results for these subjects, 56 (5.3%) were =>200 mg/dL. 9 results (0.9%) overall were =>250 mg/dL. The highest value was 276 mg/dL.

Conclusion: From our experience, massive hyperglycemia (e.g., glucose levels >300-500 mg/dL) was not recognized in pregnant women undergoing 3-h OGTTs. These data suggest that in nondiabetic women who are not acutely ill, cancellation of the 3-hour OGTT based upon a fasting plasma glucose of 200 mg/dL or greater would be an uncommon to rare event, and regardless of the fasting plasma glucose, the peak glucose during the 3-hour OGTT is only rarely =>250 mg/dL. While chronic hyperglycemia is certainly "toxic" to the mother and fetus, at this time, according to the literature there is no data to suggest that glucose tolerance screening in pregnant women not previously diagnosed with diabetes provides a danger to the fetus or the mother.

E-43

Circulating anti-Mullerian hormone levels as predictor of ovarian response in women undergoing IVF

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Background: To evaluate the clinical value of basal anti-Muullerian hormone (AMH) measurements compared with other available determinants in the prediction of ovarian response in women undergoing IVF treatment.

Methods: Basal levels of LH, FSH, Estradiol and AMH were measured in 65 subjects. All patients were followed prospectively and their cycle outcomes recorded.

Results: AMH values correlated the best with the number of retrieved oocytes ($r = 0.657$, $p<0.001$) relative to baseline FSH ($r = -0.351$, $p=0.007$) and baseline Estradiol levels ($r = -0.172$, $p=0.175$). Out of the 65 women, 33 were defined as normal responders (5-14 oocytes retrieved), 25 as poor responders (≤4 oocytes retrieved), and 7 as high responders (≥15 oocytes retrieved). Receiver operating characteristic curve analysis demonstrated that, for the prediction of poor responders, AMH had the largest area under the curve (AUC = 0.84) relative to age (AUC = 0.67), FSH (AUC = 0.65), Estradiol (AUC = 0.6) and LH (AUC = 0.54). Similarly, for the prediction of high responders, AMH had the largest AUC (AUC = 0.73) relative to age (AUC = 0.68), Estradiol (AUC = 0.72), FSH (AUC = 0.67) and LH (AUC = 0.53). A cut-off level <1.4 ng/mL AMH detected poor responders with a sensitivity of 80% and a specificity of 82.5% and a cut-off level >3 ng/mL AMH detected high responders with a sensitivity of 57% and a specificity of 84.5%.

Conclusion: Circulating AMH accurately predicts extremes of response, poor response especially. The ability of AMH to predict ovarian responsiveness is superior to basal LH, FSH and Estradiol.

E-44

State of the Art of Vitamin D Assays: Latest Generation Assays

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Background: The increasing demand for assessing vitamin D as a routine clinical check has resulted in increasing numbers of vitamin D requests. Radioimmunoassays do give accurate and reliable results but are labour intensive and therefore not suitable for high throughput laboratories. In contrast, automated vitamin D immunoassays can cope with the high throughput requirements but their accuracy and precision have been questioned. As a result, a number of pre-existing automated vitamin D assays have recently been modified and several new assays have been launched. This study aimed to compare latest generation automated vitamin D immunoassays with an established radioimmunoassay method.

Methods: We randomly selected 170 serum samples which displayed an even dispersion of vitamin D concentrations across the measuring range. Samples were divided into 6 aliquots, stored at -20°C and analysed in batches, with a freshly thawed aliquot used for each different assay. Vitamin D was measured using the Diasorin radioimmunoassay (RIA, Diasorin), which was previously demonstrated to be precise and have minimal bias compared to liquid chromatography-tandem mass spectrometry (Herrmann M, et al. Steroids 2010). Vitamin D was also measured with 5 automated chemiluminescent immunoassays from Abbott, Diasorin, IDS (iSys), Roche and Siemens. The Diasorin immunoassay was a pre-market assay. The Roche assay used was the recent monoclonal vitamin D assay which specifically detected 25-hydroxy vitamin D_3. All other tests detected both 25-hydroxy vitamin D_2 and D_3 and reported a total vitamin D result. To assess intra- and interassay variability we measured 5 replicates of a high and of a low serum pool over 5 consecutive days. A freshly thawed aliquot of each pool was used on each day.

Results: In the cohort tested, all automated assays correlated well with RIA with the exception of Roche: Abbott, $r = 0.932$; Diasorin, $r = 0.959$; IDS, $r = 0.947$; Roche, $r = 0.660$; Siemens, $r = 0.931$. Diasorin and Siemens showed very little bias between 25-120nmol/L, while the bias was variable in this range for Abbott, IDS and Roche. The Diasorin and IDS assays gave accurate results at vitamin D levels <25 nmol/L, while Siemens, Roche and Abbott showed substantial positive bias at these concentrations. Intra- and inter-assay precision differed between assays but was within the acceptable range for all assays.

Conclusion: Latest generation automated vitamin D immunoassays demonstrated significantly improved performance and compared well with RIA. While most assays produced acceptable results across the clinically relevant range Siemens and Diasorin demonstrated superior accuracy. The assays tested showed variable performance at low vitamin D concentrations and laboratories should adapt their reportable range accordingly.

E-45

Sutiable performance of serum LH and hCG assays for use in urine samples

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Background: Accurate assays are available for the analysis of serum reproductive hormones. However, obtaining daily serum samples to study patient profiles of these hormones is problematic. Therefore the performance of the Perkin-Elmer serum human chorionic gonadotropin (hCG) and luteinizing hormone (LH) assays in urine samples was examined to determine whether they would be suitable assays for daily urinary hormone analysis in large clinical trials.

Methods: LH and hCG (intact) AutoDelfia assays from Perkin-Elmer were tested on the 1235 AutoDELFIA automatic immunoassay system for their suitability to measure urinary concentration. The intra- and inter- assay variability, sensitivity, range, cross-reactivity profile, assay drift, performance on reference standards and dilution effects were all examined in accordance with FDA guidelines for assay validation.

Results: HGC Assay: The CV of the assay was less than 10% across all conditions (including intra- and inter- assay variability) and no substantial plate drift was observed. The sensitivity of the assay was at least 0.5mIU/ml and a linear range was seen from 0-5000mIU/ml, well within the required range for early pregnancy. Dilution of samples (as required if outside linear range) provided results that were 104% ± 3.13 from the expected result. The assay did not suffer from a high dose hook effect and showed no cross-reactivity with substances that share a strong homology with hCG (including LH, FSH and TSH). The assay was able to measure WHO hCG standards diluted in pooled negative urine samples correctly.

LH Assay: The analytical sensitivity was at least 0.5mIU/ml and assay CV was less than 10% across all conditions. Intra- and Inter- assay variability was less than 5% CV, and no assay drift was seen. Recovery of LH from pooled urine standards was 93.66% ± 8.8 and dilution of urine samples provided no deterioration in detection (101% ± 7.14). No cross-reactivity was seen to FSH or TSH and only very slightly to hCG (0.38% only at very high concentrations (50,000mIU) of hCG, so the assay should not be used in late pregnancy or post-partum). No high dose hook effect was seen in concentrations up to 1000mIU/ml.

Conclusion: The analytical performances of the hCG and LH assays in urine on the 1235 AutoDELFIA automatic immunoassay system were excellent. Therefore these assays can be used reliably in a laboratory environment to measure levels of hCG or LH in human urine.

E-46

Variations and Association of *NAT2* Gene with Type 2 Diabetes

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Background: N-Acetyltransferase 2 (NAT2) is drug-metabolizing enzyme that is genetically variable in human populations. Polymorphisms in the *NAT2* gene have been associated with drug toxicity and efficacy, as well as susceptibility to cancer and other complex diseases. Recently, an association of *NAT2* gene variation with diabetes risk has been also suggested. This is the first study performed in a population from Bosnia and Herzegovina (BH) in which the association of *NAT2* variations with the pathogenesis of Type 2 diabetes mellitus (T2DM) has been examined.

Methods: Here we analyzed the frequency of the NAT2*5 and NAT2*6 alleles, which code for a slow enzyme activity, in a group of 63 patients with Type 2 diabetes and 79 nondiabetic subjects. NAT2*5 and NAT2*6 were genotyped by using TaqMan® SNP Genotyping Assays, ID C__1204093_20 and C__1204091_10, respectively. Real-time PCR was performed on the LightCycler®480 Real-Time PCR System (Roche Diagnostics). Large-scale phenotype data were also collected, including fasting plasma glucose, HbA1c, insulin levels, triglycerides, total, LDL, and HDL cholesterol.

Results: Our data demonstrated that the frequencies of NAT2*5 (341T>C) and NAT2*6 (590G>A) polymorphisms in BH population were in line with the Caucasians genotype data. Strinkingly, there was a significant difference in genotype frequencies for NAT2*5 ($p<0.05$) and NAT2*6 ($p<0.001$) between diabetic and nondiabetic

subjects. NAT2*5 gene variation was associated with 2.4-fold increased risk for developing Type 2 diabetes (adjusted OR = 2.40, 95% CI = 1.10-5.25, p = 0.028). On the contrary, NAT2*6 variant significantly decreased by 5 fold susceptibility to the disease (adjusted OR = 0.20, 95% CI = 0.09-0.43, p <0.001). Furthermore, the NAT2*5 and NAT2*6 alleles were in high linkage disequilibrium (D' = 0.969) and there was a significant difference in the distribution of haplotypes (p = 0.01) and diplotypes (p = 0.002) between diabetic patients and nondiabetics.

Conclusion: Our data demonstrated that *NAT2* genetic variation appeared to be an important risk factor in Type 2 diabetes development. It would be pertinent to further explore the mechanisms of genetic polymorphisms of this drug-metabolizing enzyme in T2DM pathogenesis and its potential significance in optimal, individualized diabetes treatment.

E-47

Accurate and Fast Determination of Plasma Renin Activity with a Microplate-based Enzyme Immunoassay

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Background: Measurement of Plasma Renin Activity (PRA) is important for the clinical evaluation of hypertensive patients. PRA, in contrast to Renin concentration, is a very accurate indicator of hyperaldosteronism (5-13% of hypertensive cases) and can assist in the management of other forms of hypertension. However, until now, PRA tests are time consuming and rely on the use of radioactive isotopes. The main objective of this study was to develop an accurate and fast PRA assay using a generic microplate-based colorimetric enzyme immunoassay platform (EIA).

Methods: EDTA plasma was treated with protease inhibitors and a generation buffer to prevent degradation of angiotensin I (AI). The samples were split into two aliquots of the same volume. One aliquot was incubated at 0°C and the second at 37°C for 1.5h (AI generation step). After this step, 50 µL of standards containing a concentration of AI calibrated against WHO International Standards, controls and the aliquots treated as indicated above were mixed with 100 µL of solution containing biotinylated AI conjugate and a Renin activity inhibitor in the wells of a microplate coated with anti-AI antibodies. The microplate was incubated for 1h at room temperature with shaking, washed 5 times and 150 µL of buffer containing streptavidin-HRP was added. The microplate was incubated for 30 mins with shaking, washed 5 times and 150 µL of colorimetric substrate solution (TMB) was added, the reaction was stopped by adding 50 µL of stop solution to each well. The optical density was measured at 450 nm. Quantification of AI was done using curve fitting software. PRA was calculated from the difference in concentration of AI in samples incubated at 37°C or 0°C divided by the time of the generation step.

Results: The performance of the new EIA assay for PRA was comparable to that of RIA, however results were obtained much faster. Because the sensitivity of the assay was 0.038 ng/mL of AI, the generation step could be as short as 1.5h. Therefore, the total time of the assay was less than 4 hours. The dynamic range of the assay was between 0.04 and 25 ng/mL of AI. Linearity and accuracy (recovery) were 101 ± 9 and 98 ± 8 respectively (n=4). The intra and inter-assay precision, determined with 4 samples covering the measuring range of the assay were in the range of 6-8% and 4-7% respectively. No clinically significant interferences were found in samples containing elevated concentrations of haemoglobin, conjugated and unconjugated bilirubins, triglycerides or HSA. Crossreactivity with angiotensin II, III or peptides with structure similar to AI was less than 0.1%.

The present PRA EIA kit (x) was compared with the DBC PRA LIA kit (y). The comparison of 24 plasma samples yielded the linear correlation: y = 1.043x + 0.096, r=0.97. Also, a reasonable relationship, r=0.88, n=14, was found between PRA determined by the present EIA method and Renin concentration determined by Immunoradiometric technique (CisBio).

Conclusions: Our results demonstrate that this PRA EIA is rapid, accurate and can be use for the assessment of hypertensive patients.

E-48

Screening of Hypercortisolism in Patients with Resistant Hypertension

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Background: Resistant hypertension (RH) is an increasingly prevalent condition. About one third of patients have a secondary form of hypertension. Screening for hypercortisolism as a cause of RH has been traditionally indicated in patients with clinical features of Cushing Syndrome (CS). However, many of these features are highly prevalent in general population, such as centripetal obesity and hypertension. These features can easily be overlooked in routine clinical visits as signs of Cushing syndrome. Our objective was to obtain the prevalence of subclinical hypercortisolism in an outpatient population of patients with RH in a reference center.

Methods:Patients from 18 to 80 years old attending at a University Hospital were invited to participate in this study. We excluded those using glucocorticoids and pregnant women. Firstly, we did the overnight 1mg dexamethasone suppression test with measurement of serum cortisol at 8AM (CIA, Unicel, Beckman, USA). Subsequently, patients who didn't suppress cortisol levels to less then 1.8 µg/dL were requested to collect 2 samples of salivary cortisol (RIA, Siemens, USA) at 23PM. Two results accordingly higher then 3.6 nmol/L were considered positive. Samples accordingly below were considered normal and, in case of discordant results a third sample was requested. Patients were asked about the use of drugs that could affect the result of the suppression test, such as rifampicin, carbamazepine, phenytoin, phenobarbital and estrogens and examined for causes of pseudo-Cushing, such as obesity, psychiatric diseases and polycystic ovary syndrome (POS), as well as signs of Cushing syndrome. Statistical analysis was done using SPSS 17.0.

Results: A total of 400 patients were screened from August 2009 to September 2010. Seventy two percent were female. The mean age was 63 years (29-80), median of 65 years. Eighty-five patients (21.2%) had morning serum cortisol above 1.8 µ/dL, and 9 (2.5%) had values greater than or equal to 5 µg/dL. At least one possible cause of false positive was identified in 54.1% of these patients, being more frequent, in descending order: obesity (21.2%), psychiatric diseases (18.8%), drugs (5.9%), alcohol abuse (7.1%) and POS (1.2%). The nighttime salivary cortisol was definitely high in 22 patients (5.5% of the initial population). We had to retest 20 patients (23.5% of the 85 non-suppressors) for discrepant results and 4 patients due to insufficient salivary supply.

Conclusion: Our screening found subclinical hypercortisolism in a significant group of patients with RH, even though most of the cases initially suspected didn't confirm in subsequent tests. There are only few studies similar to ours in medical literature so far. Screenings conducted in obese patients with poorly controlled type 2 diabetes and no signs of Cushing syndrome using similar methodology found comparable results (subclinical hypercortisolism in 2 to 3% of patients). Further analysis should be performed to assess clinical significance and prognosis of subclinical CS in this population.

E-49

Comparison of Two Free T4 Immunoassays With A Direct Dialysis Method

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Introduction: The measurement of free T4 (fT4) is integral for diagnosing hyper- and hypothyroidism. Two methods are commonly used for measuring fT4: direct equilibrium dialysis (ED) and immunoassay using T4 analogues. Although ED is considered the gold standard, most clinical laboratories use FT4 methods that are available on automated platforms. However, the reliability of these immunoassay methods for detecting abnormal fT4 values has been questioned. The aim of this study was to compare the results of ED with those from the Roche Elecsys E170 and the Siemens Centaur XP.

Methods: ED was performed at 37°C for 16-18 hours using dialysis blocks with 200 µL of undiluted serum. After completion of the dialysis, the fT4 in the dialysate was measured using a sensitive radioimmunoassay (Calbiotech).

Results: FT4 was measured on 117 patient samples with fT4 levels ranging from 0.2 to 5.2 ng/dL (ED). Linear regression comparisons of the two immunoassays vs ED yielded a slope of 0.46 and an intercept of 0.05 for Centaur (R^2=0.79), and a slope of 0.48 and an intercept of 0.63 for Elecsys (R^2=0.56). The immunoassays performed relatively well with samples with normal fT4 results vs ED (92.9% concordance for Centaur and 96.9% for Elecsys), but not as well with abnormal samples. Compared to the reference range (0.8-2.7 ng/dL), ED yielded 6 samples (5.1%) below, and 13 samples (11.1%) above the respective limits. With the Roche Elecsys, only 1 sample (0.8%) was below the lower limit (0.8 ng/dL) and 7 samples (6.0%) were above the upper limit (1.7 ng/dL). With the Siemens Centaur, 9 samples (7.7%) had values below the lower limit (0.8 ng/dL) and only 1 sample (0.8%) was above the upper limit (1.8 ng/dL). For the 6 samples with results lower than reference interval by ED, the Centaur assay gave low results for 2 samples and the Elecsys gave a low result for 1 sample. For the 13 samples with results higher than the upper reference interval by ED, the Centaur gave a high result for 1 sample and the Elecsys for 4 samples. For the 98 samples with normal results by ED, the Centaur reported 7 as low and the Elecsys 3 as high. Thus, compared to ED, the sensitivities of the Centaur assay for detecting hypo- and hyperthyroidism were 33.3% and 7.7%, respectively. For the Elecsys, the

sensitivities were 16.7% and 30.8%, respectively. Overall, result concordance of the Centaur vs ED was 79.5%, and Elecsys vs ED 85.5%.

Conclusion:In samples with low fT4 (by ED), the Centaur was slightly better than the Elecsys (33.3% vs 16.7%) in detecting the abnormality. In contrast, samples with high fT4 (by ED), the Elecsys was slightly better than the Centaur (30.8% vs 7.7%). Our data agreed with previous observations that fT4 by immunoassays performs reasonably well for samples with normal fT4 (by ED) but are less reliable in samples from patients with abnormal fT4. We suggest using ED to measure fT4 if results by immunoassay do not correlate with the clinical picture.

E-52

Performance evaluation of Siemens ADVIA Centaur® enhanced estradiol assay and a split sample comparison with liquid chromatography-tandem mass spectrometry

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Background: Immunoassays generally have limitations on sensitivity and specificity for steroid hormones. Recently Siemens developed a next generation ADVIA Centaur® enhanced estradiol (eE2) assay and claimed it is isotope dilution gas chromatography-mass spectrometry traceable. The objective of this study was to evaluate the Siemens eE2 assay and compare it with a well-established estradiol liquid chromatography-tandem mass spectrometry (LC-MS/MS) method.

Methods: Siemens eE2 assay was evaluated by CLSI evaluation protocols. Split patient samples were compared between the eE2 assay and the current ADVIA Centaur E2-6 III assay (n=45), and between the eE2 assay and LC-MS/MS method by API 5000 mass spectrometer (n=30).

Results: Within-run (n=10 each) and total (n=20 each) imprecision of the eE2 assay demonstrated coefficient of variations (CV) of 5.7%, 3.2%, 1.5%, and 10.4%, 7.3%, 6.8%, at levels of 380 pmol/L, 752 pmol/L, and 2051 pmol/L respectively. The method demonstrates a dynamic linear response up to at least 9188 pmol/L and the dilution recoveries ranged from 85.4 to 100% with a mean of 94.7%.The lower limit of quantitation is 43 pmol/L. The method comparisons showed: eE2 = 0.9033 x E2-6 III - 16.2, R^2 = 0.9382, average bias = -12.3%, and eE2 = 0.9458 x LC-MS/MS + 19.5, R^2 = 0.9249, average bias = 0%. However, Siemens eE2 assay demonstrated high bias (up to ±50% vs. LC-MS/MS) at low levels (< 200 pmol/L) because of increased assay imprecision.

Conclusion: Siemens eE2 assay correlates well with LC-MS/MS. This method is reliable, rapid and appropriate for use in routine clinical laboratory for estradiol concentrations over 43 pmol/L. Post menopausal women and women receiving aromatase inhibitors could have estradiol concentrations <43 pmol/L. In these instances measurement by mass spectrometry is recommended.

E-53

Functional Sensitivity of Seven Commercial Thyroid Stimulating Hormone Immunoassays

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Background: Serum thyroid stimulating hormone (TSH) measurements are useful for detecting clinical and subclinical primary hypo- and hyperthyroidism in ambulatory patients. For diagnosis of hyperthyroidism, the functional sensitivity (FS) is an important performance criterion, and current guidelines recommend a FS of ≤ 0.02 mIU/L for "third" generation performance.

Methods: We evaluated TSH FS for the Access 2 (Beckman), ADVIA Centaur® (Siemens), Architect i2000 (Abbott), Dimension® ExL™(Siemens), Elecsys E170 (Roche), and the IMMULITE® 2000 and Dimension Vista® 1500 automated immunoassays (both from Siemens). Seven pools with sufficient volume for the imprecision study were each prepared by combining samples with comparable TSH concentrations. These 7 pools were aliquotted and stored frozen at -70 ^{0}C until time of testing. Imprecision was evaluated over 12 days of testing using two lots of reagent and two instrument calibrations. Seven aliquots per pool (one per immunoassay method) were thawed per day and assayed with one replicate per run, one run per day, two days per week, and three weeks per reagent lot for a total of 12 replicates. FS was determined by fitting a power function to the imprecision data using KaleidaGraph software

Results: The FSs (mIU/L) for Access 2, ADVIA Centaur, ARCHITECT i2000, Dimension ExL, Elecsys E170, IMMULITE 2000, and Dimension Vista 1500 were determined to be 0.039, 0.006, 0.007, 0.003, 0.008, 0.003, and 0.003, respectively. The mean TSH concentrations per pool for each immunoassay method are summarized in Table 1. The immunoassays we studied did not provide comparable mean TSH concentrations for the serum pools tested.

Conclusions: All assays showed excellent performance in FS consistent with a "third generation" claim except for the Access 2. Further harmonization of TSH immunoassays is required, especially at lower concentrations.

Table 1 Mean TSH concentrations (mIU/L) for seven serum pools by seven immunoassays

Pool	Access 2	ADVIA Centaur	Architect i2000	Dimension ExL	Elecsys E170	IMMULITE 2000	Vista 1500
1	0.022	0.016	0.008	0.005	0.007	0.015	0.008
2	0.077	0.014	0.010	0.007	0.009	0.016	0.010
3	0.046	0.028	0.021	0.018	0.024	0.024	0.021
4	0.038	0.039	0.029	0.028	0.033	0.027	0.030
5	0.120	0.133	0.114	0.115	0.127	0.117	0.119
6	0.180	0.164	0.142	0.151	0.187	0.161	0.153
7	0.284	0.310	0.263	0.279	0.310	0.243	0.283

E-54

New insights on the stability of the (1-84) PTH as determined with an automated 3rd generation PTH assay

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Background: Pre-analytical conditions for parathormone (PTH) determination are important for the overall confidence of the assay. Unfortunately, there are no clear recommendations regarding the use of serum samples or samples anticoagulated with EDTA for the best preservation of PTH. Moreover, conflicting results on the stability of the peptide at different temperatures have been published. We recently validated the Liaison (1-84)PTH (DiaSorin,Stillwater,MN), a 3rd generation PTH assay kit. Contrary to the 2nd generation ("intact") PTH assay kits, the antibodies used in this 3rd generation assay recognize the (1-84)PTH only and do not cross-react anymore with the amino-truncated C-terminal fragments which accumulate in CKD patients. As these fragments could not have the same stability than PTH (1-84) itself, the stability results obtained with intact PTH assay kits could not be extrapolated to the 3rd generation assays. The aim of our study was thus to evaluate the stability of the (1-84)PTH assayed with the 3rd generation PTH on Liaison at different temperatures and with different sampling tubes.

Methods: Blood samples were collected from 14 hemodialyzed patients at 0800 hours immediately before commencing renal dialysis. The samples were drawn into BD 5-ml EDTA and gel-separator tubes. One part of the samples was immediately spun and assayed. The results obtained constituted the "zero-point". The remaining volume of plasma and serum was immediately aliquoted as 28 pairs of plasma EDTA and serum. Three pairs of aliquots were kept at room temperature (+21°C,RT) and assayed after 8 hours, 1 and 2 days of conservation. Similarly, four pairs of aliquots were conserved at +4°C and assayed after 8 hours,1,2 and 7 days. Seven pairs of aliquots were conserved at -20°C and seven others at -80°C and assayed after 8 hours, 1 and 7 days, 1,3,6 and 12 months of conservation.

The other part of the samples (six pairs) was kept as whole EDTA or clotted blood at RT and +4°C. After 4,8 and 24 hours, one pair was centrifuged and assayed. The CV of the Liaison (1-84)PTH assay is <8%. We considered that the samples were not stable anymore if the PTH levels of one of the patients were decreased by more than 20% compared to the value obtained at the zero-point. We used the Wilcoxon and Mann-Whitney tests, with $p<0.05$ as the level of significance.

Results: There was no difference between EDTA and serum at zero-point. In the clotted blood, PTH was stable up to 8h at RT and 24h at +4°C. In the whole EDTA blood, it was stable 24h at RT, but only 4h at +4°C. In serum, PTH was stable 8h at RT, 24h at +4°C and up to 1 year at -20°C and -80°C. In EDTA plasma, PTH was stable 48h at RT and at +4°C and up to 1 year at -20°C and -80°C.

Conclusions: Our results show that (1-84)PTH is more stable than could be expected by the results previously obtained with "intact" PTH assays. This discrepancy could be linked to a difference of stability between the different N-truncated fragments.

E-55

Impact of the use of the Manufacturer's published reference range for PTH vs. the reference range established in the Laboratory for the classification of the haemodialyzed patients with the KDIGO Guidelines

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Introduction: The recommended target range for serum parathormone (PTH) in dialysis patients has changed from 150-300 pg/mL in the KDOQI guidelines to 2 to 9 times the upper limit of normal in the KDIGO ones. However, discussions about PTH reference values are needed. Indeed, reference values for serum PTH levels are generally obtained by measuring PTH in a population of apparently healthy subjects. Exclusion criteria for this population are highly important and should correspond to any potential cause of altered PTH secretion. In this study, we used the same reference population of vitamin D-replete normal subjects to establish reference values for 10 commercial PTH kits. We evaluated whether this may improve the classification of dialysis patients according to the KDIGO compared to the use of reference values proposed by the manufacturers.

Material and Methods: We studied 149 haemodialysis patients undergoing dialysis 3 times a week. Blood was obtained just before a dialysis session and centrifuged within 30 minutes of blood sampling. Serum was aliquoted and stored at -80°C until assayed. One hundred-twenty women aged 48.6±15.2 years, and 120 men aged 51.5±17.5 years served as a reference population. All were Caucasians, apparently healthy, and were supplemented with vitamin D3 at various doses. Inclusion criteria were a 25OHD concentration (DiaSorin Liaison) ≥75 nmol/L, serum calcium and phosphate levels comprised between 2.15 and 2.60 and 0.74 and 1.51 mmol/L, respectively, and an estimated GFR (MDRD formula) ≥60 mL/min/1.73 m². The use of drugs known to influence bone and calcium/phosphorus metabolism was an exclusion criteria. We tested 10 different commercial PTH assays, of which two were 3rd-generation assays. Seven assays were fully automated and 3 were immunoradiometric assays. With each of these assays, PTH was measured according to the recommendations of the respective manufacturers. First, we classifyed the dialysis patients according to the KDOQI guidelines. Second, they were classified according to the new KDIGO guidelines, by multiplying by a factor 2 and 9 the upper reference range of each PTH assay as provided by the manufacturers. Third, we used the upper value of the reference range that we have established in our reference population for each PTH assay to classify the dialysis patients according to the KDIGO guidelines. Finally, we took the measurement uncertainty into consideration to classify the patients with our established reference range.

Results: Our results show that, compared to the KDOQI, using the upper-normal limit provided by the manufacturers to determine the KDIGO range greatly improved the discrepancies in classifying the patients. Using the upper limit of our reference values to calculate the KDIGO target range moderately improved the classification of the patients. Taking the analytical variability into consideration, 8% of the patients only remained differently classified by the kits that yielded the most different absolute values.

Conclusions: With 10 different methods for PTH determination, a global overall agreement in the classification of stage-5 CKD patients could be achieved with the KDIGO guidelines when the same vitamin-D replete population was used to establish the reference range.

E-56

Measurement of urinary free cortisol by UPLC-tandem mass spectrometry

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Introduction: Free (unbound) cortisol represents the biologically active form of the circulating hormone. Urinary free cortisol measurement is useful in the evaluation of Cushing's syndrome. We developed and validated the urinary free cortisol assay on ultra performance liquid chromatography coupled to tandem mass spectrometry (UPLC-MS/MS).

Methods: 24 hour urine samples were treated with solid phase extraction using Waters Oasis MAX cartridge. The extract was quantified by UPLC-MS/MS (Waters ACQUITY UPLC and Xevo TQ MS). A reverse-phase C18 column (1.8 um, 2.1x5 mm) was used with gradient mode (run-time 3.4 min). The positive mode ElectroSpray Ionization was applied. Deuterated cortisol-d4 was used as the internal standard. The multiple reaction-monitoring transitions used for quantitation of cortisol and deuterated cortisol were m/z363.2 >120.9, m/z367.3 >121.0 respectively. The m/z363.2 >327.1 was used for confirmation of cortisol.

Results: Deming's linear regression was used to compare our UPLC-MS/MS method to the LC/MS/MS method at one reference laboratory with the following **Results:** (n = 89), r = 0.9989, y = 0.827x + 1.87, Syx = 7.9, bias= - 9.84, range 4.2 - 1311.6 µg/day. CAP survey results were 287.3 and 44.8 µg/L (within 1 SDI of peer group mean) for 2010-N10 and 2010-N11 samples respectively.

Precision Within-run (N=20)		
Mean (µg/L)	SD	%CV
46.9	2.0	4.2
303.4	10.0	3.3
Total Precision (N=20)		
Mean (µg/L)	SD	%CV
45.6	2.2	4.8
295.1	14.3	4.9

Precision was determined following CLSI Protocol EP5. We established analytical measurement range to be 2.0 µg/L to 800 µg/L. Measurements from 241 individuals without Cushing's syndrome were used to estimate a reference interval of 3 - 58 µg/day (nonparametric central 95% interval) and a mean of 23.6 ug/day. No statistical difference between urinary free cortisol results obtained from acidified and non-acidified urine samples was observed. No assay interference was demonstrated from structurally related compounds.

Conclusion: The urinary free cortisol assay by UPLC-MSMS demonstrated excellent precision, accuracy and short run-time that is suitable for implementation in our high volume laboratory setting.

E-57

Comparison of Agilent and Supelco C8 columns for the measurement of 11 steroids in human plasma/serum using liquid chromatography-tandem mass spectrometry

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Background: Column technology is changing rapidly and requires regular assessment and optimization, sometimes necessitating switching to the newer improved technology.

Objective: To compare the C8 column (Rapid Resolution Cartridge, 2.1 X 30 mm, 3.5µ; Agilent Technologies, DE, USA) for the simultaneous measurement of 11 steroid hormones (aldosterone, cortisol,11-deoxycortisol, androstenedione, DHEA, DHEAS, testosterone, progesterone, 17-OH progesterone, corticosterone, 25 OH Vitamin D3) with the C8 Supelco column (Supelco LC-8-DB, 3.3 cm X 3.0 mm, 3 µm particle size; Sigma-Aldrich, MO, USA) using LC-MS/MS previously reported in Clin Chim Acta 2006; 372:76-82.

Methods: An API-5000 triple-quadrupole mass spectrometer (Sciex, Canada) coupled with the PhotoSpray source and Shimadzu HPLC system (Shimadzu Scientific Instruments, MD) was used employing isotope dilution with deuterium labeled internal standards. 0.2 mL microliters of serum were deproteinized by adding 300 µL of acetonitrile containing internal standards. After centrifugation, 300 µL of supernatant were diluted with 1450 µL of water and 300 µL then injected onto the column.

Following a 3 min wash with mobile phase A (methanol: water 2:98, v/v) at flow rate of 1 mL/min, the switching valve was activated to initiate the binary gradient program which eluted the steroids at a flow rate of 500 µL/min as follows: 80% A for 3 min, 45% B (methanol) to 50% B over 2.4 min, and 50% B to 53% B over 0.01 min, 53% B to 58% B over 2.6 min, and 58% B to 90% B in 1 min and finally 90% B for 2.0 min. The atmospheric pressure photoionization (APPI) source was operated with ionspray voltage at -750 V and heater temperature at 400 °C.

Gas settings were as follows: ion source gas1 45, ion source gas 2 20, curtain gas 20, collision gas 6. Quantitation by multiple reaction monitoring (MRM) in 3 different periods was performed in positive ion mode for 10 analytes and in negative ion mode for aldosterone. Ion pair for each analyte and its IS are as follows.

Period I (positive ion mode) 3.0-3.8 min : DHEAS 271/213 DHEAS-d2 273/213.

Period II (negative ion mode): 3.8-4.33 min Aldosterone 359/331, Aldosterone-d6 365/337.

Period III (positive ion mode) 4.33-8.03 min, Cortisol 363/121 Cortisol-d4 367/121, Corticosterone 347/121 Corticosterone-d8 355/125, 11-Deoxycortisol 347/97, 11-Deoxycortisol-d2 349/97, Androstenedione 287/97, Androstenedione-d7 294/100, Testosterone 289/109,Testosterone-d2 291/99, 17 α-Hydroxyprogesterone 331/109, 17 α-Hydroxyprogesterone-d8 339/100, DHEA 271/213, DHEA-d2 273/213, Progesterone 315/109, Progesterone-d9 324/100

Period IV (positive ion mode): 8.03-10.72 min 25-Hydroxyvitamin D3 383/365, 25-Hydroxyvitamin D3-d6 389/371.

Results: The Agilent and Supelco C8 columns have similar limits of quantitation (LOQs) ranging from 1.5 to 10 pg/mL. Between-day CVs ranged from 4.5% to 9.8% (n=25). The results of the comparison study yield r values ranging between 0.908 and 0.999. Recovery ranged from 92% to 108%.

Accuracy was assessed by comparison of the Agilent method (n=25) with a commercial reference laboratory , and correlation coefficients ranged between 0.997 and 0.946.

Conclusion: The Agilent column can simultaneously measure 11 steroids in serum within 11 min with minimal sample preparation. The column life is superior to that found with the previously used Supelco column.

E-58

Analytical performance of the Abbott ARCHITECT *i2000* 25-OH Vitamin D immunoassay and establishment of seasonal reference values

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Background: Vitamin D deficiency and insufficiency are commonly found in the general population. Currently, the best way to establish the vitamin D status is to determine its major storage form, 25-hydroxy vitamin D, in the blood. In this study we evaluated the analytical performance of the Abbott ARCHITECT 25-OH Vitamin D (25-OH-VD) immunoassay that was recently introduced on the market. We also established the seasonal reference ranges for 25-OH-VD in a healthy population.

Materials and Methods: The determination of 25-OH-VD and PTH were carried out on the Abbott ARCHITECT i2000 and of the other parameters on the c16000 instruments. For the precision studies patient and control samples were used and the CLSI EP5-A2 protocol was applied. Method comparison with the Diasorin Liaison total 25-OH vitamin D (n=312) and LC-MS (n=70) was carried out according to the CLSI EP10 protocol. The accuracy of the assay was determined by using samples of DEQAS and SKML external quality assessment schemes.

Reference values were determined according to the CLSI Protocol C28-A3. Serum samples were collected throughout the year from individuals (n=525) without (chronic) diseases, vitamin D supplementation and with normal ARCHITECT iPTH, calcium and creatinin values. These samples were stored at -80^{0}C.

Results: The total run imprecision in the range 10 - 80 ng/ml was better than 6 %, whereas the functional detection limit (CV<20%) was appr. 5 ngl/ml. A good lot to lot consistency (n=3) of less than 6 % was observed for control and human serum samples .

The results of the Abbott 25-OH-VD assay was within the 1SD range for the DEQAS and SKML external quality control schemes for all control samples (n=18).

The ARCHITECT 25-OH-VD revealed a fair correlation with the Liaison assay of: [25-OH-VD](Abbott)= 1.23x [25-OH-VD](liaison) + 2.81, with r=0.90 (n=314) and a good with LC-MS of of: [25-OH-VD](Abbott)= 1.04x[25-OH-VD](LC-MS) + 0.04 with r=0.97 (n=70). Also, an excellent combined between-device (n=2) and between-lot (n=2) correlation was found of [25-OH-VD](device1,lot1)= 1.00x [25-OH-VD](devide2,lot2) - 1.13, with r=0.99 (n=80).

The observed seasonal reference values (2.5th and 97.5th percentiles) were 15.7 - 60.3 ng/mL) (mean 32.6 ng/mL) and 8.8 - 46.3 ng/mL (mean 24.8 ng/mL) in summertime and wintertime population, respectively. No gender related differences were observed

Discussion: The Abbott ARCHITECT 25-OH-VD assay shows good analytical characteristics, with a good reproducibility in the clinically relevant range of 10 - 80 ng/ml and correlation with LC-MS. The accuracy appears to be good, based on the results of EQAS samples.

The reference range determined in our study are concordant with results of other studies and may serve as guidelines for clinical use.

Conclusion: In conclusion, the ARCHITECT 25-OH Vitamin D assay proves to be a rapid, precise and reliable assay for the quantitative determination of 25-OH vitamin D in human serum.

E-59

Quantitative Analysis of Human C-peptide by LC-MS Isotope-Dilution Assay: Microheterogeneity of Internal Standards

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Background: C-peptide results from proteolytic cleavage of pro-insulin and is a useful indicator of endogenous insulin production. Methods used to quantify C-peptide include enzyme-linked immunoassays, radioimmunoassays and fluorescence assays, but the results generated by different methods do not always agree. Isotope dilution mass spectrometry (IDMS) has the potential to be a reference method for C-peptide quantitation, provided that the microheterogeneity of the internal standard is taken into account.

Methods: Quantitative analysis was performed on an API 4000 triple-quadrupole mass spectrometer (AB SCIEX, Foster City, CA); [2H16]C-peptide (Bachem, Bubendorf, Switzerland) was used as the internal standard. An unlabeled C-peptide standard (Sigma-Aldrich, St. Louis, MO) was also analyzed. Two-dimensional (2D) liquid chromatography, Ion Exchange (IE) - Reverse Phase (RP), was implemented using Shimadzu Prominence HPLC system (Shimadzu Scientific Instruments, Columbia, MD). At the initial isolation stage, a two-step sequential sample fractionation (cation exchanger followed by separation on an anion exchanger or vice versa) was employed in order to provide for rapid processing of high sample volumes.

Results: The C-peptide standards (3-33) synthesized by Sigma and Bachem (deuterium labeled) exhibited highly heterogeneous spectra. The proposed method for C-peptide quantification takes into account the real content of the isoform used as reference material (e.g. 1013.1 m/Z for the Bachem standard) resulting in increased measurement accuracy. Also In addition, the methods used in pretreatment and analysis have a significant impact on the degree of C-peptide heterogeneity observed.

Conclusion: Measurement of C-peptide using IDMS is connected with additional error due to heterogeneity of the labeled analogue. The existence of multiple C-peptide isoforms in the standards evaluated results in highly overestimated C-peptide concentrations in biological fluids when the concentration of standard is determined by nitrogen, amino acid analysis, or by radioimmunoassay. Accounting for the heterogeneity of the standard and use of methodologies that minimize the degree of heterogeneity results in more accurate quantitation.

E-60

Chromogranin-A is a good biomarker for octreotide sensitivity in patients with ectopic ACTH and GHRH secreting neuroendocrine tumors

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Ectopic hormone production is an uncommon complication of malignant disease. When functioning, these tumors are associated to inconsistant clinical and biological characteristics. Bronchopulmonary carcinoids are the most common neoplasm implicated in ectopic ACTH-dependent Cushing's syndrome. Neuroendocrine enteropancreatic tumors (NEEPTs) include tumors developing from the pancreatic islets and the duodenal loop, rarely are associated to GHRH production. Surgical removal of the ectopic tumor is the therapy of choice, but it is not always feasible. We present the effects of somatostatin analogs treatment on hormone and chromogranin A levels in three patients. Objetives: To evaluate the role of chromogranin A in disease control in patients with carcinoid tumors treated by octreotide.

Methods: We describe here three different patients, with diagnosis of ectopic secreting neuroendocrine tumors not included within the clinical and molecular spectrum of multiple endocrine neoplasia type-1 (MEN1). They were operated on and had partial control of the disease and treated by octreotide for at least one year.

Results. Case 1: Male patient, 26 years old, presented with severe skin pigmentation, hypokalemia, hypertension. He had the diagnosis of a bronchial ACTH secreting carcinoid. He was operated on with apparently removal of tumor, and partial remission of Cushing´s disease, but he had a recurrence 6 months after surgery. The octreoscan showed high uptake on tumor site and he was treated by octreotide LAR 50 mg/month for two years, with a reduction of 58,54%on chromogranin A and 26,6% on ACTH levels. Case 2: A 56 years old woman, had the diagnosis of acromegaly due to and ectopic secretion of GHRH in a context of a pancreas carcinoid with liver metastasis. She had MRI showed diffusely enlarged pituitary gland. She was treated by octreotide LAR 30 mg/ month for one year, with reduction of 35%on chromogranin A and 47% on GH levels. Case 3: A 25 years old woman with a glioblastoma producing excessive GHRH with subsequent GH hypersecretion and resultant acromegaly. MRI showed a pituitary global enlanrgement and an invasive cerebral tumor. After brain surgery, and debulking of the glioblastoma, she was treated by Octreotide LAR 30 mg and showed a reduction of 14%on chromogranin A and 87% on GH levels.

Conclusion: Surgical removal of the ectopic tumor is the therapy of choice, but it is not always feasible because patients often present with widespread metastases. Patients with GHRH-induced acromegaly benefit from the administration of octreotide, which reduces GH, IGF-I, and GHRH, and may shrink the ectopic tumor, its metastases, and the secondary pituitary enlargement. In addition to imaging techniques, whole body scintiscan with labeled octreotide may help in the localization

of ectopic tumors. Somatostatin analogs can play an important role in the treatment of neuroendocrine tumors, dependent on the somatostatin receptor subtype expression pattern. In these patients que chromogranin A was a good marker of disease control but the predictive factors of therapeutic effects are unclear. We propose life-long suppressive therapy with somatostatin analogs in cases with persisting elevated serum GHRH concentrations after removal of the primary tumor.

E-61

Prevalence of Diabetes Mellitus and Impaired Glucose Tolerance among Freshmen in a Nigerian University

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Background: The aim of the study was to identify young adults who had Diabetes Mellitus or impaired glucose tolerance as well as those at risk of these conditions among a group of young undergraduates in a Nigerian University

*T*wo hundred and twenty two freshly admitted undergraduates of the Obafemi Awolowo University, Ile-Ife, osun State, Nigeria, who were randomly selected during the registration process were involved in the study. Anthropometric and other demographic details were obtained using a standard questionnaire developed at the Harvard University. Participants were screened for diabetes mellitus (DM) by measuring their fasting serum glucose. Blood samples were collected from respondents by venupuncture, after an overnight fast. The samples were centrifuged and the plasma collected. Glucose was measured the same day by the glucose oxidase method.

This was carried out using the glucose oxidase method Ethical clearance for the study was obtained from the appropriate authorities.

Results: Most of the subjects (90.4%) were within the age range 15-24 years. Two hundred and seven (94%) participants had serum glucose within the reference range while 10 (4.6%) had impaired glucose tolerance. 3 of the subjects (1.4%) had serum glucose greater than 7.1mmol/L, which is indicative of Diabetes Mellitus. A proportionally large number of participants (91.4%) engaged in physical activity equivalent to a walk of at least 30 minutes/day. Most of them (93.2%) had BMI < 25.0 while 6.8% were overweight with BMI within 25.0-29.9. One hundred and three (103) subjects, representing 46.8% agreed to taking three to more servings of whole grain per day . Statistical analysis was carried out using the statistical package for social sciences (SPSS) version 11.

Conclusion: Most of the subjects studied are involved in healthy lifestyles .This has resulted in very low prevalence of impaired glucose tolerance (IGT) and Diabetes Mellitus (DM) among the group. It will be interesting to follow up the group and see if they are able to maintain this trend as the risk of developing Diabetes Mellitus is known to increase with age.

E-62

Retinol-binding protein 4 (RBP4)-to-adiponectin ratio is superior to adiponectin and RBP4 for the detection of the metabolic syndrome in Type 2 Diabetic Subjects and Their First Degree Relatives

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Background: Obesity promotes atherosclerosis via mechanisms that include the production of adipokines. Two of these adipokines (adiponectin and Retinol-binding protein 4 (RBP4), play important but different roles. Unlike adiponectin which has anti-inflammatory, antidiabetic and antiatherogenic effects, RBP4 increases insulin resistance and promotes atherogenesis. We hypothesized that the associations of RBP4-to-adiponectin ratio with the metabolic syndrome (MS) and associated variables would be superior to adiponectin and RBP4 alone.

Methods: Fasting RBP4, adiponectin, insulin, glucose, and full lipid profile were determined in 98 Type 2 Diabetic (T2DM) patients and 191 normoglycemic first degree relatives (FDR). Insulin resistance was assessed with the homeostasis model (HOMA-IR). Subjects were classified by the IDF criteria for the MS.

Results: RBP4 (mean 29.6 Vs. 24.5 µg/ml) and RBP4:adiponetin ratio were significantly higher in T2DM patients (mean 4.7) compared to the FDR (mean 3.6) and adiponectin was significantly lower (mean 7.4 Vs. 8.6 µg/ml) despite similar waist circumference (WC). The correlations of RBP4 improved after factoring with adiponectin in the RBP4:adiponectin ratio (WC (r = 0.17 Vs r = 0.38); Triglycerides (r = 0.41 Vs 0.49); HDL-Cholesterol (r = 0.16 Vs 0.41); glucose (r = 0.17 Vs 0.28); HOMA-IR (r = 0.10 Vs 0.39)). Similarly, the correlations of RBP4:adionectin ratio were better than correlations of adiponectin. RBP4 and RBP4:adiponectin ratio showed stepwise increase while adiponectin showed stepwise decrease with increasing number of MS criteria. Binary logistic regression showed that the odds ratio (OR) of MS as predicted by adiponectin was 0.82; RBP4 (OR = 1.1); RBP4:adiponectin ratio (OR = 1.5). Receiver Operating Characteristic analysis showed that RBP4:adiponectin ratio had significantly higher area under the curve (0.745) compared with adiponectin (0.676) and RBP4 (0.659) for detection of MS.

Conclusion: We conclude that RBP4 and adiponectin are additional and useful criteria for identification of the MS. Factoring RBP4 with adiponectin significantly improves the diagnostic performance characteristics.

E-63

Evaluation of the DAKO chromogranin-A assay for measurement on serum matrix

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Background: Chromogranin A (CgA) is essential for the formation of secretory granules and sequestration of hormones in neuroendocrine cells. Measurement of CgA levels is included in the diagnostic procedure of neuroendocrine tumors and pheochromocytoma. The aim of this study was to assess the reliability of the DAKO ELISA assay for CgA measurement in serum.

Methods: CgA concentrations were determined in eighty patients (mean age: 68±12 years) in both serum and plasma samples. Levels of CgA were measured with a simplified double antibody sandwich assay using rabbit antibodies to a 23 kDa C-terminal fragment of human CgA (Dako, Glostrup, DK). Reference values for CgA in serum were obtained from 50 healthy subjects. Imprecision of the CgA assay on serum was also determined for two levels of CgA concentration.

Results: Between run imprecision for the CgA assay were 6.5% and 6.9% for concentrations of 13.7 UI/L and 58.8 UI/L, respectively. CgA levels measured on serum were significantly correlated with CgA levels obtained with plasma, considered as the reference matrix ($r = 0.92$, $p < 0.0001$). For concentrations below 120 UI/L, Passing and Bablok regression analysis using CgA measured on plasma as reference showed a slope of 0.77 and an intercept of 4.9 with CgA measured on serum (n=68). The Passing and Bablok regression analysis for concentrations above 120 UI/L (n=12) showed a slope of 0.83 and an intercept of -36.9. The median of the reference population was 12.3 UI/L [3.4-22.6].

Conclusions: Our study showed that serum might be a reliable matrix for measurement of CgA but with slightly lowered values than with plasma. Our study of the reference values showed that the upper limit of 23 UI/L proposed by the manufacturer remains relevant.

E-64

Performance of the LIAISON 25 OH Vitamin D TOTAL Assay on DiaSorin's Newly Launched LIAISON XL

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Objective: The aim of the present study was to evaluate of the LIAISON® 25 OH Vitamin D TOTAL kit on the LIAISON® XL, DiaSorin's next generation analyzer, against the performance of the well established LIAISON® platform. This assay measures total 25 OH vitamin D in a competitive chemiluminescent immunoassay.

Methods: 1003 routine samples were collected and used in the evaluation: 159 (16%) with LIAISON® 25 OH Vitamin D TOTAL levels less than 25 nmol/L, 781 (78%) with levels between 25 and 75 nmol/L, and the rest of the samples with LIAISON® 25 OH Vitamin D TOTAL levels greater than 75 nmol/L. Samples were run with LIAISON® 25 OH Vitamin D TOTAL on the LIAISON® and LIAISON® XL analyzer on the same day. Correlations, Bland-Altman statistic test, and concordance between the two analyzers was calculated, as well as the inter- and intra- day precision, and throughput of the LIAISON® XL were determined.

Results: The correlation between the two analyzers including all 1003 samples revealed a slope of 0.95 and r= 0.97. Bland-Altman analyses displayed that there was no proportional difference between the results of the two analyzers. When examining the results at medical decision points there was a 93.2% concordance with a 50 nmol/L cut-off, and 94.4% concordance with a 75 nmol/L cut-off. For precision analysis 20 replicates were run in one day for intra-assay variability, and for inter-assay variability samples (high and low values) were assayed in duplicate, twice a day for 5 days. Intra-

run revealed 1.8 - 5.5 CV% and Inter-run: 3.5 - 7.3 CV%. The throughput with this assay was 170 tests per hour.

Conclusion: The LIAISON® XL offers higher throughput, long walk-away time and improved instrument reliability. The performance of the LIAISON® 25 OH Vitamin D TOTAL assay was demonstrated to be comparable between the two platforms, with precision and accuracy matching established claims for the assay.

E-68

Adult Reference Intervals for 3-iodothyronamine, thyroxine, triiodothyronine, reverse T3 and 3,3'-diiodo-L-thyronine measured by Isotope Dilution HPLC Tandem Mass Spectrometry in Human serum

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Background We have developed a new thyroid panel test utilizing tandem mass spectrometry which allows for the simultaneous measurement of thyroxine (T4), triiodothyronine (T3), reverse-T3 (rT3), 3,3'-diiodo-L-thyronine (T2) and 3-iodothyronamine (abstract submitted). It is essential to know the reference intervals for each of these analytes before the test can be applied in the clinical arena.

Methods: Serum/plasma was obtained from 130 healthy females and 130 healthy males within the age range of 20-60 years. The thyroid panel was measured in all the samples. The 2.5th and 97.5th percentiles were calculated using 3 different methods, including the Percentile, the Gaussian and the Hoffmann approaches.

Results: The results are shown in the table below.The 3-iodothyronamine reference intervals were below 5 pg/mL for both males and females. Only 4 of the 260 samples had values for 3-iodothyronamine between 5-10 pg/mL.

Conclusion: The reference intervals for T4, T3 and rT3 are similar to those found in the literature. Reference intervals for T2 and 3-iodothyronamine are new and not previously known. The narrowest reference intervals were found when employing the Hoffmann approach. Nevertheless the reference intervals obtained using these 3 approaches on the same sample set agree fairly well.

REFERENCE INTERVALS

UNITS		Percentile	Percentile	Gaussian	Gaussian	Hoffmann	Hoffmann
		2.5th	97.5th	2.5th	97.5th	2.5th	97.5th
pg/mL	Male T2	9.4	30.6	7.0	30.8	11.2	26.6
pg/mL	Female T2	7.2	24.4	6.7	22.6	9.6	19.7
ng/dL	Male T3	86.5	168.5	83.1	171.2	94.4	151.4
ng/dL	Female T3	79.8	187.0	74.0	167.7	87.1	144.5
ng/dL	Male rT3	8.8	23.1	8.5	24.4	11.3	21.5
ng/dL	Female rT3	9.5	25.1	7.7	23.1	10.5	20.3
ug/dL	Male T4	4.9	10.5	4.7	10.2	5.6	9.2
ug/dL	Female T4	5.1	11.3	4.2	10.9	5.4	9.6

E-69

Macroprolactin on the Roche Elecsys 2010: experience with126 patients in an Indian setup

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Background: Prolactin polymorphism in the human sera is a critical issue in the diagnostics of this very important hormone. In certain samples, the biologically active predominant monomeric form may be misestimated due to interference from the polymeric aggregates of prolactin-IgG complexes (macroprolactin). Of clinical relevance is the fact that macroprolactenemia may lead to misdiagnosis and mismanagement of true hyperprolactinaemic patients. In the Roche Elecsys 2010 immunoassay platform, the Prolactin II assay has a relatively low reactivity with most forms of macroprolactin. Albeit, clinician feedback regarding mismatch of prolactin levels with patient's clinical status, and the plausible variability in prolactin due to ethnicity and race differences in our Asian population compared to the reference population (Caucasian), prompted us to examine the requirement for polyethylene glycol (PEG) precipitation in the analysis of hyperprolactinaemia detected by the Roche Elecsys 2010 system, in a backdrop of our own reference range.

Methods: 1. Derive a post-PEG prolactin reference range: For the determination of reference interval, blood samples were drawn from 50 normal subjects (15 males and 35 females). Assay: We measured prolactin (ng/ml) with the Elecsys 2010 (Roche Diagnostics, prolactin II assay). All sera were estimated for total prolactin initially and after PEG precipitation using the manufacturer's specified protocol. Briefly, 250 µL of sera, mixed with an equal volume of 25% PEG 6000 (Sigma-Aldrich,Germany), was vortexed and the suspension was clarified by centrifugation at 5000g for 10 mins and the supernatant analyzed immediately for prolactin. We calculated the reference interval, defined as 95% confidence limits, using the Kolmogorov-Smirnov test on the Medcalc Statistical Software, Version 8.0 (Mariakerke, Belgium).2. Comparative Analysis: Data of 126 hyperprolactinaemic samples were collected from the laboratory information system for AMRI Hospitals-Dhakuria from Mar-Dec 2010. These samples had been tested for macroprolactin using the manufacturer's specified PEG precipitation protocol. Monomeric prolactin concentration post-PEG precipitation was compared with a reference range determined by PEG precipitation in the normal subjects.

Results: The male (17-75 yrs, median=44) and female (15-70 yrs, median=30) reference panel of samples on visual inspection revealed total prolactin and post-PEG distribution plots of normal gaussian distribution. Manufacturer-published reference intervals for total prolactin were similar to our newly derived ranges (1.4-20.47ng/ml, males) and (5.42-21.53 ng/ml, females). However, the upper reference limit for post-PEG prolactin range in healthy males (10.49 ng/ml) and females (15.38ng/ml) were about 30% lower indicating the precipitation of macroprolactin, big prolactin, and a portion of the monomeric prolactin present in sera. Of the126 patients who fell into the category of hyperprolactenemia (by the manufacturer's reference range) over the time period studied, macroprolactinemia was misreported as hyperprolactinaemia in 13% of patients (16/126) when re-assessed by the newly derived post-PEG prolactin reference range.

Conclusion: PEG screening is highly recommended even for assays with low macroprolactin immunoreactivity such as the second generation prolactin assay on the Roche Elecsys 2010 and this should be analyzed against a local population derived reference range for monomeric prolactin.

E-70

Development of an Enhanced Chemiluminescence Total 25(OH) Vitamin D Assay on the VITROS® ECi/ECiQ Immunodiagnostic System, the 3600 Immunodiagnostic System and the 5600 Integrated System

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Introduction: Vitamin D is a fat soluble steroid hormone that occurs in two forms, vitamin D2 (ergocalciferol) and vitamin D3 (cholecalciferol). Vitamin D enters the body through skin exposure to the sun or through dietary intake, enters the circulation and is hydroxylated in the liver to form 25(OH) Vitamin D. It is further hydroxylated in the kidney to form the biologically active hormone, 1,25(OH) Vitamin D. The active hormone is involved in the intestinal absorption of calcium and phosphorous and interacts with the parathyroid gland to act as a regulator of bone formation.

Vitamin D metabolites are bound to a vitamin D binding protein in the plasma and are distributed throughout the body. Because the concentration of 1,25(OH) Vitamin D is 1000 times lower than 25(OH) Vitamin D and has a 4 hour half life, plasma 25(OH) Vitamin D is the most reliable clinical indicator of vitamin D status. 25(OH) Vitamin D levels are also indicative of the body's storage levels of vitamin D and correlate with the clinical symptoms of vitamin D deficiency. In the late 18th century, Vitamin D was first recognized as an essential dietary component in prevention of rickets. Recently, research has indicated that vitamin D deficiency may be linked to chronic diseases such as cancer (breast, colon and prostate), cardiovascular disease, osteoporosis, osteomalacia and several autoimmune diseases.

An assay for total vitamin D (25-hydroxyvitamin D) with a measuring range up to 350 ng/mL on the VITROS® ECi/ECiQ Immunodiagnostic System, the 3600 Immunodiagnostic System and the 5600 Integrated System is being developed by Ortho Clinical Diagnostics.

Materials and Methods: The prototype VITROS Total 25(OH) Vitamin D assay is a competitive immunoassay design. Patient sample, dissociation buffer and conjugate buffer are added sequentially to a VITROS MicroWell which is coated with antibody. The Vitamin D in the sample competes with the Vitamin D conjugate for binding to antibody. After a 16 minute incubation period, unbound materials are removed by washing. Signal Reagent (containing the Enhanced Chemiluminescence substrate) is added and light emission is measured. The light signal generated by bound HRP conjugate is inversely proportional to the concentration of 25(OH) Vitamin D present in the patient sample. Time to first result is 24 minutes.

Results: Calibrator values for the VITROS Total 25(OH) Vitamin D assay are traceable to LC-MS/MS. The assay has a limit of detection (LoD) of $\leq$ 3.0 ng/mL and a linear range of up to 350 ng/mL. Total assay CVs were 6.9%, 5.6%, 5.5%, and 3.5% for samples at 29.0, 49.5, 71, and 113 ng/mL, respectively. A correlation study against

LC-MS/MS was performed with 80 serum human patient samples, yielding a slope of 0.98, intercept of 1.14, and regression coefficient of 0.92.

Conclusion: In conclusion, the VITROS Total 25(OH) Vitamin D assay combines good analytical and clinical performance with the operational simplicity of a rapid automated continuous random access immunoassay system.

E-71

Evaluation of the pituitary gonadotropins and testosterone on confirmed Prostrate Cancer patients attending National Hospital,Abuja

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Background: It is suspected that male sex hormone levels (LH, FSH, Prolatin and testosterone) play a significant role in the development of prostate cancer. Clinical studies aimed at elucidating this role have produced conflicting results, and it is not clear whether elevated male sex hormone levels are associated with an increased or decreased risk of prostate cancer.

Objective: This study was conducted to determine the clinical significance of the pituitary gonadotropins and testosterone on prostate cancer and to establish clinical correlation between total PSA, testosterone and pituitary gonadotropins on prostate cancer.

Methods: To test this hypothesis, we conducted a study on biopsy confirmed fifty-five (55) male subjects with prostate cancer. Fifty-two (52) apparently healthy individuals were also randomly selected for controls, with a mean age of 72 years and 50 years respectively. Serum levels of total testosterone, Luteinizing hormone (LH). Follicle stimulating hormone (FSH), Estradiol (E2) and Prostate specific antigen (PSA) - a tumor marker were performed, using the Roche diagnostic Elecsys 2010 Immunoassay System based on electrochemiluminescence.

Results: using correlation analysis, the subjects show significant, but variable hormonal differences for the gonadotropins: LH: P=0.001 and FSH: P=0.001; but when subject and control groups were compared, no significant differences were observed for the total testosterone, P=0.075 and Estradiol, $p < 0.10$. A positive, but statistically insignificant correlation was observed for the PSA and LH; r=0.473 and 0.231 and for PSA and FSH r=0.640 and 0.220 for the subjects and control groups respectively.

Conclusions: This study suggests that none of these hormones are strongly associated with prostate cancer.

E-72

Challenge with Free T4 Reference Ranges on Beckman DXI Analyzer

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During the implementation of the new Beckman Analyzer DXI, the company-recommended reference range of Free T4 (FT4) 0.61-1.12 ng/dl was accepted after testing 37 healthy volunteers (36 of which had FT4 levels within the aforementioned range). In collboration with endocrinologists, we realized that other large medical centers in the Chicago area, using identical equipment, had different FT4 reference ranges (approximately 0.6-1.7 ng/dl). Further investigation revealed that around 2005- 2007, the Beckman Company changed the recommendation for Free T4. The new reference ranges established by the Beckman Company, were based on recent studies on volunteers with normal TSH values.

Our studies performed on 104 healthy volunteers generated a FT4 reference range of 0.66-1.1 ng/dl, close to the new Beckman recommendation.

For further validation, a group of 800 patients with normal TSH values was analyzed. The study, (calculated 2.5 to 97.5 central percentile) revealed Free T4 range from 0.57-1.32 ng/dl.

A review of 130 patients' cases with Free T4 between 1.12- 1.7 ng/dl showed 87 cases with TSH above 0.1 mIU/L, while 75% of these cases had TSH in complete normal range.

Taking into consideration the results of this study, as well as the reference ranges in major Chicago hospitals, an old, wide free T4 reference range (0.6-1.7 ng/dl) was implemented, as it was concluded to best serve our patients in John H. Stroger Hospital.

E-73

An evaluation of hemoglobin A1c test ordering patterns in primary care setting: Pilot experience in Valencia Community (Spain)

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Background: To evaluate glycosilated hemoglobin (HbA1c) ordering patterns by general practitioners (GPs) in eight health care areas over two year period using the calculation of indicators of demand appropriateness.

Methods: The number of HbA1c tests ordered by GPs in year 2008-2009 was examined in a cross-sectional study of eight health districts. According to the guidelines in effect prior to July 2009, HbA1c testing was used only for diabetic patient management. A possible inappropriate HbA1c request was defined as any order for a given patient with an HbA1c result of < 6%. Two appropriateness indicators were calculated: (a) HbA1c with a result of <6.5% and <6%, in 2008 and 2009 and (b) Theoretical HbA1c orders, according to diabetes prevalence and American Diabetes Association recommendations (ADA) in 2009. The first parameter was used to assess probable use in nondiabetic patients and the second to estimate compliance with recommendations for use in diabetic patients. Total HbA1c orders and the percentage of HbA1c results <6.5% and <6% with respect to the total number of tests ordered for patients managed by GPs in each Department, for every annual period are collected. The data are collected from Laboratory Information System, and indicators calculated automatically through a data warehouse and OLAP cubes software.

Results: A progressive increase in the demand for HbA1c determinations was observed. Approximately 54% of the HbA1c values obtaines in seven of the eight depatments studied were lower than 6,5%. The number of theoretical HbA1c orders that would have been expected given the known prevalence of diabetes was higher than the number of HbA1c orders in all the departments.

Conclusion: The results seem to indicate taht HbA1c orders by the health departments studied were not always appropriate. It is likely that HbA1c determinations were overused in patients without diabetes and underused in patients with diabetes.

Health Care Department	Inhabitants	HbA1c requested by GPs		%HbA requested by GPs with results<6.5%		%HbA1c requested by GPs with results<6%		Theorical HbA1c orders according to diabetes prevalence and ADA recommendations (year 2009)
		2008	2009	2008	2009	2008	2009	
A	197029	18574	21325	48,5	43,2	35,6	28,4	22600
B	254233	24346	21719	54,6	53,1	38,2	34,9	29161
C	274233	14663	18098	65,1	48,8	54,9	48,8	31455
D	271218	21258	22519	54,8	55,9	37,9	38,5	31110
E	55282	4836	5455	48,8	53,2	29,3	33,6	6341
F	372138	ND	20705	ND	28,1	ND	10,9	42686
G	357267	ND	33776	ND	60,6	ND	46,6	40980
H	233075	14458	18641	54,6	66	33,4	46,5	26735

Population attended in each Health Area. HbA1c requested by GPs and percentage of determinations with results less than 6 and 6,5% in 2008 and 2009. Theorical HbA1c orders according to diabetes prevalence and ADA recommendations in year 2009.

E-74

Rapid Intact Parathyroid Hormone Validation for the VITROS 3600 Analyzer

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Background: Intact parathyroid hormone (iPTH) is an 84-amino-acid single chain polypeptide with functional importance in the biologic regulation of calcium. The extremely short half-life of iPTH (2-4 minutes) allows for accurate, intraoperative measurement of rapid changes in iPTH levels after the surgical removal of autonomously functioning parathyroid glands in the setting of hyperparathyroidism. Measurement

of intraoperative iPTH is currently recommended for patients undergoing surgery for hyperparathyroidism, parathyroid reoperation, and angiography-assisted venous localization of abnormal parathyroid glands. It is recommended that surgical candidates undergo baseline iPTH testing pre-operatively in addition to pre-excision intraoperative and post-excision intraoperative measurements. A greater than 50% post-excision drop in iPTH from the highest baseline value is compatible with surgical success.

Objective: The objective of our study was to perform an in-house validation of the iPTH assay on the Vitros 3600 analyzer and perform a three-way split sample comparison between the Vitros 3600, Siemens Immulite 1000, and Roche COBAS 6000 PTH assays.

Methods: Patient samples were analyzed via the FDA-approved, VITROS Immunodiagnostic Products Intact PTH sandwich assay to determine test performance compared with our current laboratory intraoperative PTH assay on the Siemens Immulite 1000 analyzer and routine PTH assay on the Roche COBAS 6000 analyzer. In addition, intraoperative patient samples were run in parallel on all three analyzers for real-time comparison under standard clinical testing conditions.

Results: The iPTH assay on the VITROS 3600 demonstrated acceptable within-laboratory imprecision of 18.5% at 8.7 pg/ml, 11.0% at 47.3 pg/mL and 12.3% at 1571.6 pg/mL. Patient sample comparisons between the VITROS 3600 and Roche COBAS analyzers yielded excellent concordance with a least squares linear regression equation of VITROS = 1.0767(COBAS) - 4.876, R^2= 0.99. In addition, comparison of the VITROS 3600 and Siemens Immulite 1000 assays yielded comparable concordance with a least squares linear regression equation of VITROS = 0.8393(Immulite)+ 18.544, R^2=0.98. Overall concordance between the VITROS and COBAS analyzers was an improvement over the existing relationship between the Immulite analyzer and the COBAS analyzer (Immulite = 1.249(COBAS) - 22.984, R^2 = 0.9646).

Conclusions: With the goal of increasing laboratory flexibility and staffing efficiency while providing reliable, expedient intraoperative iPTH testing, these data summarize our in-house validation of an FDA-approved rapid iPTH assay for clinical use on the VITROS 3600 instrument. The VITROS 3600 assay demonstrates excellent performance and concordance to our current institutional methodologies.

E-76

A comparison of direct immunoassays for serum estradiol

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Background: Measurement of serum estradiol is important for diagnosis of infertility, amenorrhea, menopausal status and precocious puberty. We evaluated the performance of automated direct E2 assay and tested the clinical usefulness for pediatric very low level E2 concentration.

Methods: We showed the imprecision of ADVIA Centaur Estradiol-6 III. We compared the data from 254 girls aged 4-14 with 3 direct immunoassay systems of ADVIA Centaur-Estradiol-6 III, Roche Modular E170-Roche Elecsys Estradiol 11, and Abbott Architect i4000-Abbott Architect Estradiol Assay (Rev 2004).

Results: Inter-assay imprecision for ADVIA Centaur assay were 5.1% at 116.5 pg/mL, 6.5% at 239.1 pg/mL and 2.3% at 548.1 pg/mL. The percentage of below detection limit were 49.3% for Centaur (<7 pg/mL), 6.7% for E170 (<5 pg/mL) and 69.5% for Architect (<10 pg/mL). Specimens with values over 7 pg/mL on Centaur were selected and analysed concurrently on Roche E170 and Abbott Architect analyzers. The correlation coefficients of E2 were 0.88 for Centaur vs. E170, 0.95 for Centaur vs. Architect, 0.94 for E170 vs. Architect. The regression analysis showed as follows; Centaur vs. E170; n=119, slope 0.98, intercept -2.48, r2=0.78, Centaur vs. Architect; n=62, slope 0.83, intercept 1.51, r2=0.90, E170 vs. Architect; n=68, slope 0.78, intercept 1.92, r2=0.89.

Conclusion: The imprecision of Centaur E2 assay were excellent at mid- to high ranges of E2. However, direct immunoassays are not precise and accurate enough for very low level E2 specimens.

E-77

Comparing Inverse log/linear Relationship of TSH and FT4 Between DxI 800 (Beckman Coulter) and E 170 Analyzer (Roche Diagnostics) with Patient Samples

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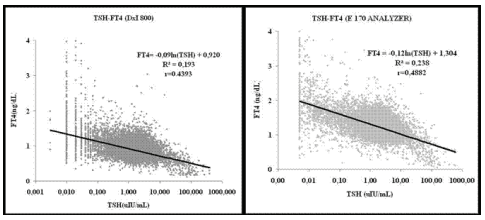
Background: Thyroid Stimulating Hormone (TSH) and free thyroxine (FT4) are used for diagnosis and treatment of thyroid diseases.There is an inverse log/linear relationship between TSH and FT4.This relationship is helpful to the clinician and laboratory specialists in the evaluation of TSH and FT4 tests.We compared the log/linear relationship of TSH and FT4 between two immunoassay analyzers.

Methods: 22400 TSH and FT4 results obtained from Access DxI 800 Unicel (Beckman Coulter, USA) and another 23250 TSH and FT4 results obtained from Modular E170 Analyzer (Roche Diagnostics,Germany) were checked and correlation coefficients (r) were calculated.

Results: The inverse log-linear correlation coefficients with their confidence intervals(CI%95) between TSH and FT4 were r=0.4393 (CI%, 0.429-0.449),(p<0.01) for DxI 800 Unicel and r= 0.4882 (CI%, 0.479-0.497),(p<0.01) for E170 Analyzer.



Conclusion: Despite the progress and development of the standardization studies, there are still differences between the results of analyzers. Considering the differences between the analyzers, clinicians and laboratory specialists should be careful in assessing the results of TSH and FT4 tests.

E-78

Measurement of Vitamin D Using the Abbott ARCHITECT 25-OH Vitamin D Assay

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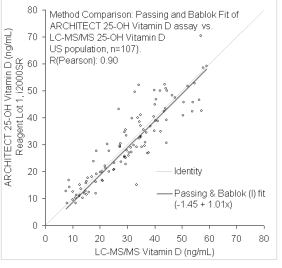
Background. Increased clinical awareness of the prevalence of vitamin D deficiency and insufficiency has led to a need for reliable automated immunoassays for 25(OH) vitamin D (25(OH)D). The aim of this study was to evaluate the performance of the Abbott ARCHITECT 25-OH Vitamin D assay.

Methods. The precision of the ARCHITECT assay was determined across the complete dynamic range using assay controls and samples pools in a 20 day protocol. Correlation with LC-MS/MS was evaluated on a set of 107 specimens on all ARCHITECT platforms (i1000SR, i2000 and i2000SR) and on 40 samples from DEQAS. In addition, eight specimens with high endogenous 25(OH)D2 were compared with Diasorin Liaison Vitamin D Total Assay and the Recovery of 25(OH) D2 was determined.

Results. The inter-assay precision (%CV) ranged from 6.0% to 2.9% for a samples with low (12.0 ng/mL) and high (142.2 ng/mL) Vitamin D concentrations close to the ends of the claimed measurement range (8.0 ng/mL - 160.0 ng/mL). The functional sensitivity (20% CV) was determined at 4.1 ng/mL. Mean Passing-Bablok Correlation versus LC-MS/MS was 0.99 (0.91-1.06) x LC-MS/MS -0.65 (-2.24 - 1.13), $R_{(Pearson)}$ = 0.89 (0.87-0.90). Furthermore for the 40 DEQAS samples the ARCHITECT results showed good agreement with the LC-MS/MS (proportional bias: 0.96). The ARCHITECT's mean recovery of 25(OH)D2 at 76.7% (95% CI: 58.2% to 95.2%) was comparable to Liaison (90.0%, 95% CI: 69.3% to 110.8%).

Conclusions. The fully automated ARCHITECT 25-OH Vitamin D assay exhibited excellent precision and sensitivity as well as a good correlation with LC-MS/MS. The assay is suitable for monitoring patients taking exogenous vitamin D providing appropriate clinical vigilance is applied for patients under 25(OH)D2 supplementation. The ARCHITECT 25-OH Vitamin D assay is a valuable tool in clinical laboratories for the accurate and precise determination of vitamin D status in human sera and plasma.



Thursday AM, July 28

Poster Session: 9:30 am - 12:00 pm
Hematology/Coagulation

E-80

A Sensitive ELISA Method for Detection of Antibodies to Factor IX

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Background: Alloantibodies against coagulation factor IX (FIX) complicate FIX replacement therapy in patients with hemophilia B. The clot-based Bethesda assay (BA) that is commonly used to detect and quantify FIX inhibitory antibodies lacks sensitivity at low titers and may not detect non-inhibitory antibodies. ELISA-based technology is becoming increasingly acceptable as a complementary method to detect antibodies to coagulation factors; we adapted this approach to develop a sensitive semiquantitative FIX antibody assay.

Objective: To develop an ELISA-based FIX antibody detection method and evaluate its performance relative to that of the traditional BA.

Methods: Our semiquantitative FIX IgG ELISA utilizes polystyrene microtiter plates coated with recombinant FIX. A sample containing a low level of FIX inhibitors serves as an assay calibrator. The assay results are expressed as a ratio of the calibrator and defined as an inhibitor index. The assay reference range was verified using 60 plasma and 60 serum specimens from healthy men (ages 18-65). Cross-reactivity was evaluated by testing samples positive for lupus anticoagulant (LA; n=10), FVIII inhibitors (n=10), rheumatoid factor (RF; n=10), and anti-cardiolipin (aCL) antibodies (n=10). The BA was performed on a coagulation analyzer, with results >0.4 Bethesda units/mL (BU/mL) taken to indicate the presence of inhibitors. For the assay comparisons, we used 5 FIX antibody-positive and 3 FIX antibody-negative samples purchased from a commercial source along with 24 de-identified residual specimens originally submitted for routine FIX inhibitor testing.

Results: In dilution studies of positive specimens, the FIX IgG ELISA showed acceptable precision (inter-assay CV <17%) at inhibitor index values as low as 0.11; this was defined as the negative cutoff value, and only one of the 120 normal subject results was above this value (0.12). ELISA index and BU values showed a linear relationship for samples with <1.4 BU/mL, and index values in this range agreed closely with BU values (R^2=0.962). The relationship was logarithmic for samples with higher inhibitor titers, leveling off at titers >15 BU/mL and showing no hook effect up to at least 180 BU/mL. Based on the correspondence of index and BU values at low inhibitor levels, we defined the positive index cutoff as ≥0.40; the index range of 0.11-0.39 was designated as equivocal, since dilutions of positive specimens falling into this category were negative by BA. The ELISA showed no cross-reactivity with LA, FVIII inhibitors, RF, or aCL; however, one aCL-positive specimen showed an equivocal result (index=0.12). The ELISA and BA methods yielded concordant results for 31/32 samples (97%), including 10 positive and 21 negative specimens; one specimen was discrepant (BA-positive [10.4 BU/mL], ELISA-negative). We believe this represents a false-positive BA result, as the specimen was from a patient with drug-induced LA and normal FIX activity as determined with a chromogenic assay.

Conclusions: The FIX IgG ELISA appears to be a valuable alternative method for detection of antibodies to FIX. Our results suggest that this method has potential as a screening tool for early detection of inhibitory and non-inhibitory antibodies and as a confirmatory method for excluding false-positive BA results.

E-81

Human megakaryocytes and platelets express Gas6 during differentiation of peripheral hematopoietic stem cells

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Background: It has been shown that Growth Arrest Specific 6 (Gas6) protein plays a role in the thrombus formation by affecting the functions of platelets in mice. Gas6, which is a vitamin K dependent protein, does not exhibit anticoagulant properties but rather may be an important regulator of vascular homeostasis and platelet signaling. While some of the publications indicated that gas6 was not present in human platelets, the others reported it otherwise. The presence of Gas6 in human platelets remains controversial. The objective of this study is to investigate whether Gas6 is present in human platelets. We believed that it would be inadequate if we only studied with platelets. That's why we planned to investigate the presence of Gas6 not only in human platelets but also in megakaryocytes, which are parent cells of platelets, obtained from differentiation of peripheral hematopoietic stem cells.

Methods: We developed a two stage liquid culture system to expand megakaryocytes from mobilized peripheral blood stem cells. CD 34+ hematopoietic stem cells were isolated from mononuclear cells taken from donors of patients undergoing allogenic bone marrow transplantation. Isolated CD34+ cells were induced for 21 days in order to differentiate them into megakaryocytes and platelets. Expanded cells were taken from the culture on days 7, 14 and 21, and were evaluated as morphologic, immunologic and flow cytometric. The presence of Gas6 in human megakaryocytes and platelets was assayed by the methods of Reverse Transcriptase (RT-PCR), Western Blot, Dot Blot and ELISA. In parallel experiments, we monitored the exogenous effects of Gas6 on the cell differentiation by adding it at different final concentrations: 100 ng/mL, 250 ng/mL and 500 ng/mL.

Results: We observed that the number of CD 34+ cells decreased and the number of CD 41+ cells, which are the indicators of the formation of megakaryocytes, increased during the processes of differentiation. According to morphologic observations, the initiation of megakaryocyte maturation began at day 7. The presence of Gas6 in human megakaryocytes and platelets was demonstrated by the methods of RT-PCR, Western Blot, Dot Blot and ELISA. When recombinant Gas6 was added into cell culture medium in parallel experiments in order to observe the effects of Gas6 on the differentiation process, on 7th day of the culture the number of CD 34+ cells and megakaryocyte progenitor cells increased depending on the Gas6 concentration. This increase was statistically significant with the Gas6 concentrations of 250 ng/mL and 500 ng/mL ($P<0.05$).

Conclusion: The presence of Gas6 was demonstrated in human platelets and megakaryocytes. In addition to this result, we observed that exogenous Gas6 has a positive effect in the early megakaryopoiesis. Further comprehensive *in vitro* and *in vivo* studies will be necessary to determine the precise role of Gas 6 during megakaryopoiesis.

E-82

Effect of time on automated complete blood count results obtained from under-filled tubes containing K2EDTA as anticoagulant

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Background. Clinical Laboratory Standards Institute (CLSI) and Becton-Dickinson manufacturer indicate that erroneous blood count results can arise from analyzing under-filled tubes containing K_2EDTA anticoagulant. A recent report from Xu et al. regarding the acceptability of under-filled K_2EDTA blood collection tubes for automated complete blood count (CBC) with leukocyte differential that were analyzed within 1 hour of collection, prompted us to asses if acceptability could be maintained for samples analyzed several hours after blood collection.

Methods. Phlebotomists from 3 patient service centers in different geographical areas of the city and 2 in other cities were instructed to obtain a full draw (FDT), 4 mL, K_2EDTA Becton-Dickinson Vacutainer™ tube and a second under-filled tube (UFT). Five patients in each PSC were selected daily for 5 days for a total of 101 duplicated samples. Samples were transported to the laboratory and analyzed in the same Coulter LH-750 hematology analyzer. FDT and UFT paired samples were integrated with the routine clinical samples. Blood volume for the UFT was estimated by comparison with a series of standards of known volumes. Descriptive statistics and mean % differences between FDT and UFT were calculated for each parameter. Statistical differences between the full draw and UFT were assessed by the Wilcoxon Signed-Ranked Test.

Results. Median transportation time to the laboratory was 7 hours and 47 minutes. Thirty five percent of the samples were analyzed within 2 to 4 hours from blood collection, 16% within 4 to 8 hours, 24% within 8 to 12 hours and 26% within 24 to 29 hours. The mean (± SD) volume for UFT was 2.9 (±0.4) mL. Mean % difference for red blood cells, hemoglobin and hematocrit was 0%, it was 0.01% for mean corpuscular volume, 0.1% for mean corpuscular hemoglobin, 0.1% for mean corpuscular hemoglobin concentration and 0.05% for red cell distribution width. For white blood cells (WBC) it was 0.4%; for the automated differential it was 0.4% for neutrophils, 0.3% for lymphocytes, -2% for monocytes, 0.1% for eosinophils and -1.3% for basophils. For platelets it was -0.04%, and for mean platelet volume (MPV) it was 0.5%. We observed significant differences only for WBC (P=0.02), monocytes (P=0.02) and MPV (P=0.002). The mean % of difference observed for these parameters was low (0.4%, -2% and 0.5% respectively) and probably would be clinically insignificant.

Conclusion. For most of the CBC parameters we did not observe a significant effect of time in UFT, significant differences for WBC, monocytes and MPV are probably clinically insignificant. Under-filled tubes may be acceptable for automated CBC even after prolonged time from blood collection.

E-83

D-Dimer Stability and Matrix Study in Whole Blood and Plasma on the ADVIA Centaur® Immunoassay System

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Background: The ADVIA Centaur D-Dimer assay* is a sandwich immunoassay intended for the quantitative measurement of cross-linked fibrin degradation products (XL-FDP) containing D-dimer in human plasma. The objective is to evaluate assay reproducibility using whole blood or plasma samples collected in different matrices, stored under different temperatures, and tested for stability at different intervals. Samples were also measured on a predicate device (bioMérieux VIDAS) to obtain baseline values.

Methods: Specimens were collected in EDTA, Na citrate, and Li heparin from 50 consenting patients with various clinical conditions. These whole blood (WB) or plasma samples were analyzed for D-dimer after storage under three conditions. Room temperature (RT; 22°C to 28°C) and refrigerated (Ref; 2°C to 8°C) specimens were tested at 24 and 48 hours; frozen (-20°C to -80°C) specimens were tested at 30 and 60 days. Storage values were compared to initial values to assess sample stability.

Results:

Condition	EDTA % Mean Recovery	Na Citrate % Mean Recovery	Li Heparin % Mean Recovery
WB: RT, 24 h	97%	Not tested	99%
WB: RT, 48 h	95%	Not tested	101%
WB: Ref, 24 h	99%	97%	99%
WB: Ref, 48 h	100%	99%	98%
Plasma: RT, 24 h	99%	Not tested	100%
Plasma: RT, 48 h	99%	Not tested	98%
Plasma: Ref, 24 h	98%	99%	100%
Plasma: Ref, 48 h	100%	98%	98%
Frozen, 30 days	101%	102%	104%
Frozen, 60 days	105%	105%	103%

The slope of the linear regression line obtained on initial and storage results was between 0.90 and 1.10. Average D-dimer storage values were ±5% of the initial value. Contingency table analysis of initial and storage values showed ≥95% agreement. However, Li heparin samples demonstrated significantly higher initial baseline recoveries: an average of 4030 ng/mL FEU (ADVIA Centaur) vs. 2318 ng/mL FEU (Na citrate samples, VIDAS).

Conclusion: EDTA samples were stable as WB or plasma at RT (22°C to 28°C) or refrigerated (2°C to 8°C) for 48 hours. Frozen EDTA and frozen Na citrate plasma samples were stable for 30 days (<-20°C) or 60 days (-70°C to -90°C). Na citrate samples were stable as WB or plasma when refrigerated (2°C to 8°C) for 48 hours. In view of higher baseline recoveries, Li heparin samples are unsuitable for the ADVIA Centaur D-Dimer assay.

* In development. Not available for sale.

E-84

Molecular Mechanism of Thrombin Mediated Activation of Coagulation Factor V

M. Na[1], J. Hirbawi[2], M. Kalafatis[1]. *[1]Cleveland State University, Cleveland, OH, [2]Cleveland Clinic Foundation, Cleveland, OH,*

The coagulation cascade represents a system of proteases that are crucially involved in maintenance of vascular hemostasis. Thrombin is responsible for the formation of the fibrin clot. The proteolytic conversion of prothrombin to thrombin is catalyzed by the prothrombinase complex which is composed of the enzyme, factor Xa (FXa), the cofactor, factor Va (FVa), assembled on a membrane surface in the presence of divalent metal ions. Factor V is a multidomain inactive procofactor composed of A1-A2-B-A3-C1-C2 domains and is activated by thrombin through proteolytic cleavages at Arg709, Arg1018 and Arg1545. Thrombin cleavage of factor V occurs in a sequential order. The last thrombin cleavage site at Arg1545 is kinetically less favored than the other two cleavage sites. To understand the significance of each cleavage for cofactor formation and prothrombinase function, we have created recombinant factor V molecules with two out of three cleavage sites mutated. We have generated a factor V molecule missing the Arg 709 and Arg1018 cleavage sites (FVQQR), a factor V molecule missing the Arg709 and Arg1545 cleavage sites (FVQRQ), a factor V molecule missing the Arg1018 and Arg1545 cleavage sites (FVRQQ), and a factor V molecule that is missing all three cleavage sites (FVQQQ). These recombinant factor V molecules along with wild type factor V (FVwt) were transiently expressed in COS7L cells, purified to homogeneity and assessed for their capability to interact with factor Xa and activate prothrombin to thrombin. Prothrombin activation by prothrombinase assembled with the mutant molecule was evaluated by SDS-PAGE and the kinetic parameters of the reactions were determined. Two-stage clotting assays revealed that FVaQQR ,FVaQRQ and FVaRQQ all have impaired clotting activites compared to fVawt and plasma derived factor Va (FVaPLASMA). FVQQQ was devoid of clotting and prothrombinase assembled with FVaQQQ had poor cofactor activity. Kinetic analyses demonstrated Kd values of FVaQQR for FXa of 0.75nM while fVaWT had a Kd of 0.25nM. FVaQRQ and FVRQQ were also impaired in their interaction with factor Xa. The kcat value for prothrombinase assembled with FVaQQR was slightly lower than the kcat obtained with prothrombinase assembled with FVaWT, while prothrombinase assembled with FVaQRQ and FVaRQQ had approximately 3-fold reduced catalytic efficiency when compared to the values obtained with prothrombinase assembled with FVaWT. Overall, the data demonstrate that cleavage at Arg1545 is a prerequisite for expression of optimum cofactor activity. A complete understanding of the mechanism underlying cleavage and activation of the procofactor resulting in the interaction of factor Va with prothrombin and factor Xa is required for the generation of small molecules that could be used to prevent thrombin generation in patients with thrombotic tendencies.

E-85

Comparison of Sysmex, Flow Cytometric, and Microscopic Bone Marrow Differentials in Wistar Rats

K. A. Criswell, J. H. Bock, R. P. Giovanelli. *Pfizer Inc., Groton, CT,*

Background: Preclinical drug trials frequently require evaluation of animal bone marrow to evaluate hematopoietic safety. Microscopic bone marrow evaluation is time-consuming and requires highly trained individuals. Flow cytometric determination of peroxidase activity, cell size, and lymphocyte immunophenotyping has previously been used for rodent bone marrows.

Methods: The Sysmex XT-2000iV hematology analyzer contains gates that may be manually set to capture individual populations. Therefore, it has the potential to further automate bone marrow analysis. This study was conducted to validate the cellular populations observed in Sysmex cytograms from rat bone marrow. Rats were treated with 0, 50, or 100 U/kg erythropoietin (EPO) or with 0, 5, 20, or 40 mg/kg cyclophosphamide (CP). Pharmacologic modulation of hematopoietic populations with these compounds was evaluated using flow cytometric, microscopic, and Sysmex technologies.

Results: Erythropoietin produced a dose-dependent decrease in M:E (myeloid to erythroid ratio). Sysmex and manual mean M:E values were 1.3, 0.9, 0.8 vs. 1.3, 0.8, 0.7, respectively for the EPO-treated groups. Linear regression of M:E ratio was 0.8090 and 0.8996 for Sysmex and flow cytometric differentials and Sysmex and microscopic differentials, respectively. CP produced a decrease in bone marrow cellularity. Total nucleated cell count (TNC) decreased from 59.4 in controls to 51.5, 33.7, and 16.1 x 10^3 cells in CP-treated groups. Sysmex and manual mean M:E values were 1.0, 1.1, 2.9, 9.0 and 1.1, 1.1, 2.1, 5.0, respectively in the CP cohort, demonstrating the instrument's ability to measure progressive changes in M:E. The linear regression between Sysmex and flow cytometry in CP-treated rats for M:E ratio reached 0.7819. Outliers in the flow method were due to extremely hypocellular bone marrow, resulting in flow results that failed to match microscopic differentials. In contrast, the linear regression for M:E between Sysmex and microscopic differentials was 0.9158 in CP-treated animals. This demonstrates that Sysmex technology can more accurately delineate bone marrow M:E ratio in rats under extreme hypocellularity conditions than flow cytometry. Lymphoid and erythroid populations overlapped in the Sysmex cytogram. Subsequent experiments depleted lymphocytes using a magnetic cell sorting (MACS) method (B cells with anti-rat CD45RA and T cells with anti-rat OX52). The positively selected populations were reanalyzed to demonstrate the specificity of each component cell type within the Sysmex cytogram. MACS effectively removed all lymphocytes from the samples and the recovered aliquots definitively linked Sysmex cytogram location for recovered lymphocytes. The average standard deviation from six bone marrows that were analyzed four times each for lymphocyte population was 0.37. An anti-myeloid MACS method was also used to verify the location of the myeloid population within the Sysmex histogram.

Conclusion: These studies demonstrate that the bone marrow populations achieved good separation when analyzed by the Sysmex XT-2000iV coupled with anti-

lymphocyte MAC. Sysmex gating into mature and immature populations of myeloid and erythroid cells, and of lymphocytes was comparable to those achieved by flow cytometry or microscopic evaluations. This suggests that the Sysmex technology holds significant promise in demonstrating increased reproducibility while reducing analysis time and cellular expertise required to evaluate rodent bone marrow.

E-86

Establishment and Evaluation of the new PLT measurement mode

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Background: In recent years, various automated hematology analyzers equipped with many functions have been developed and supplied us with a lot of clinically-useful information. New automated hematology analyzer (test model; Sysmex) has the new PLT measurement mode. That principle uses not only laser flow cytometry but also fluorescent dye which binds PLT. With the forward scatter light and side fluorescence, PLTs are distinguished from other blood cells and interference substances in 2-dimensional scattergram. Therefore PLTs are detected specifically. To establish this measurement mode, we evaluated the basic performance.

Methods: We confirmed 1) with-in run reproducibilities for 6 low value PLT samples ($\leq 100 \times 10^9$/L), including 3 samples with abnormal PLT histogram and 3 ones without such an abnormity, by electrical impedance mode (PLT-I), optical mode (PLT-O) or new measurement mode, 2) linearity of dilution for new PLT mode and 3) correlations between PLT-I, PLT-O or the new mode of Sysmex test model analyzer and immunological PLT counts method using anti-CD61 monoclonal antibody of Cell-Dyn Sapphire (automated hematology analyzer; Abbott).

Results: With-in run reproducibility C.V. for 3 low value PLT samples with normal PLT histogram by PLT-I, PLT-O and the new measurement mode were 5.0-14.0%, 5.4-11.6% and 2.2-4.4%, respectively, and the reproducibility with PLT histogram abnormality were 7.4-24.7%, 4.0-7.3%, 2.3-5.1%. In linearity of dilution test of PLT measured by the new method, it was linear up to 2400×10^9/L. Correlation coefficients between PLT-I, PLT-O or new measurement mode of Sysmex test model analyzer and immunological PLT counts method of Cell-Dyn Sapphire in 101 samples were r = 0.985 (*vs* PLT-I), r = 0.995 (*vs* PLT-O) and r = 0.998 (*vs* new measurement mode), respectively, and the correlation coefficients in 46 of those samples ($\leq 50 \times 10^9$/L) were r = 0.843, r = 0.945 and r = 0.964.

Conclusion: In this study, we confirmed that new PLT mode had the best reproducibility of three PLT modes in low value samples and that the new method correlated highly with the immunological method using anti-CD61. In platelet counts, compared with electrical impedance and optical method, immunological method is superior with thrombocytopenia. However, the reagent including monoclonal antibody is expensive for clinical laboratory testing. Therefore, the new PLT measurement method without antibody may be possible to perform it as part of routine tests.

E-87

Hemostasis Test Compatibility with a 1.8 ml Volume Sodium Citrate Blood Collection Tube

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Blood collection manufacturers have introduced small volume draw sodium citrate blood collection tubes for coagulation testing. The study was initiated to determine the substantial equivalence of routine hemostasis testing on a 1.8 ml volume draw tube versus the standard 2.7 ml tube manufactured by Greiner-Bio-One. The use of a small volume tube reduces the amount of blood that is need for collection for coagulation testing. With pediatric and elderly patients with fragile veins a full volume draw tube is difficult to obtain. Inadequate filling of the collection tube will decrease the proper ratio and will have a negative effect on the laboratory results. A small volume draw must be validated to ensure that a bias is not introduced. One hundred and ten volunteers donated two sodium citrate tubes (one each of 1.8 ml volume draw and a 2.7 ml volume draw) and the PT, INR, APTT and fibrinogen assays were performed on both collection tubes. Demographic data (age and sex) and anticoagulation history (heparin and coumadin) were collected.

Samples were drawn by routine venipuncture and processed for platelet poor plasma within 4 hours of collection adhering to CLSI H21-A5 guidelines. The PT, INR, APTT and fibrinogen were performed on a Stago STA®-Compact® with Stago reagents. The PT, INR and APTT were performed in singlicate, the fibrinogen was performed in duplicate and the values were compared between the 1.8 ml volume and the 2.7 ml volume collection tubes by linear regression. Linear regression analysis demonstrated a correlation between the 1.8 ml and the 2.7ml values ($R^2 > 0.96$) for all assays.

	Linear Regression N= 110		
Assay	Slope	Intercept	R^2
PT	1.01	0.06	0.998
INR	1.01	0.03	0.999
APTT	1.06	-2.01	0.969
Fibrinogen	0.99	-0.82	0.987

The results of this study indicate there is no significant difference for routine coagulation tests (PT, INR, PTT, FIB) between sampling from 1.8 ml and 2.7 ml draw collection tubes that were used in this study.

E-88

Evaluation of new parameters to assess leukocyte activation using a routine hematology cell analyzer

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Background: neutrophils, monocytes and lymphocytes participate in coordinated action in response to microorganisms. The last generation of routine hematology cell analyzers uses fluorescence flow cytometry and provides parameters based on cell morphology and function related to leukocyte activation in response to an external agent.

Objective: to evaluated parameters related to leukocyte activation and/or morphologic alterations as possible indicators of sepsis provided by a routine hematology cell analyzer.

Methods: blood samples collected in EDTA anti-coagulant from 55 patients with positive hemoculture (hemo+), 52 patients with clinical hypothesis of sepsis but negative hemoculture (hemo-) and 50 normal subjects were run on Sysmex XE5000® . Analyzed parameters: Neut-X and Lymp-X for assessing the granularity of neutrophils and lymphocytes, respectively; Neut-Y and Lymp-Y as indicatives of nucleic acid and protein intracellular content ; IMI-X as indicative of changes of cellular morphology or chemotaxis of neutrophils and monocytes; and WBC-X that has been suggested as representative of biosynthesis, cellular activity and exocytosis of leukocytes.

Results: hemo + group showed significantly higher values ($p<0.001$) in all parameters related to leukocytes, except Lymp-Y, when compared to normal group. When hemo- group was compared to normal group, there was no difference in Neut-X, Neut-Y and Lymp-X parameters. Lymp-Y was lower in hemo- ($p<0.001$). IMI-X ($p=0.025$) and WBC-X ($p<0.0001$) were higher in hemo- than normal group. These results suggest that changes in neutrophils and monocytes represented by the indices IMI- X and WBC-X are due to the presence of other infectious and/or inflammatory processes different from sepsis.

The comparison between patient groups showed higher values ($p\leq0.01$) of Neut-X, Neut-Y, Lymp-X and WBC-X in hemo+ group than in the hemo - patients. These data suggest that neutrophils of hemo+ patients have a higher granularity (toxic granulation) than neutrophils from individuals without sepsis. Lymp-Y proposed as indicative of the content of nucleic acid and protein of lymphocytes showed higher values in the normal group when compared to both groups of patients, but showed no difference between groups with and without bacteremia. This suggests that lymphocytes in infection and/or inflammation processes are activated and have undergone some change related to intracellular protein content. The values of WBC-X were higher in the group hemo+, being followed by the group with hemo- and with lower values in the normal group, suggesting that the activity of cellular biosynthesis and exocytosis is further exacerbated in neutrophils and monocytes of patients with bacteremia.

Conclusions: taken together, these data show a potential usefulness of these new parameters in the identification of morphological and functional changes of leukocytes in infectious states and can be used as aids in detecting these processes, serving as a rapid screening test in order to indicate the need for verification and identification of etiologic agent.

E-89

D-Dimer Method Comparison across Four Platforms: ADVIA Centaur, IMMULITE 2000, IMMULITE 2500, and VIDAS

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Background: D-dimer is a fibrin degradation product of clot formation. It is a combination of two "D" fragment moieties that are covalently linked through γ-glutamyl-ε-lysyl cross-linked by factor XIIIa. Increased D-dimer levels correlate with clinical conditions that relate to the formation of fibrin, mirroring an in vivo lysis of formed cross-linked fibrin. These clinical conditions include deep venous thrombosis (DVT), disseminated intravascular coagulation (DIC), pulmonary embolism (PE), postoperative states, malignancy, trauma, and preeclampsia. The purpose of this study was to compare D-dimer results across five platforms in subjects with disease states other than DVT and PE.

Methods: Residual Na citrate plasma samples (n = 155) were assayed for D-dimer on four different platforms (same day): ADVIA Centaur® D-Dimer (Siemens) assay* (sandwich immunoassay), IMMULITE® 2000 and IMMULITE® 2500 D-Dimer (Siemens) assays* (solid-phase, chemiluminescent immunometric assays), and VIDAS D-Dimer (bioMerieux) assay (two-step enzyme immunoassay sandwich method with a final fluorescent detection using enzyme-linked fluorescent assay). D-dimer analysis was performed on patients presenting with sepsis, pregnancy, DIC, and liver disease, as well as burn patients, cardiac patients, and normal subjects. All results were converted to ng/mL FEU for comparison.

Results: Comparison to VIDAS assay

Assay	Slope	Intercept	S_{yx}	R
ADVIA Centaur	1.370	227.19	1282.84	0.891
IMMULITE 2000	2.051	-914.11	1847.05	0.896
IMMULITE 2500	1.897	-776.75	1605.03	0.912

Conclusion: Despite the diversity of platforms and methodologies, concordance was good. Samples tested ranged from 193 to >20,000 ng/mL FEU. Among the groups tested, burn patient samples showed the highest D-dimer levels, and levels in pregnant patient samples, while elevated, were much lower. However, there were no conditions in which D-dimer results could be used as a predictive marker to aid in the diagnosis of a particular disorder.

* IMMULITE 2000 and IMMULITE 2500 D-Dimer assays are not available for sale in the US. ADVIA Centaur D-Dimer assay is under development; not available for sale.

E-90

Triclonality in immunofixation - can transiency be considered a constant feature? Evaluation of triclonal gammopathies detected by immunofixation in a 10-year series of patients

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Background: Triclonal gammopathies, characterized by the presence of three different clones of immunoglobulin (Ig)-secreting cells, have been described in the literature as being markedly rarer than monoclonal or biclonal gammopathies. Immufixation with high-resolution agarose gel (IF) detects paraprotein in the serum of patients with monoclonal or oligoclonal gammopathies; polymer formation can give false-positive results by simulating the appearance of multiple clones of the same Ig, which can be prevented by the addition of 2-mercaptoethanol. This study aims to characterize a population of patients with proven triclonality.

Methods:We analysed all 46249 IF requests from 29704 patients received by our lab between January 1st 2000 (introduction of our current archive) to February 14th 2011. Monoclonal Patterns (MP) were detected in 6233 (13.48%) serum samples from 2861 (9.63%) patients. There were 736 sera with Bi- or Triclonal IF patterns (11.8% of MP), from 440 patients (15.38%); triclonality was present in 51 sera (0.82% of MP), from 34 patients (1.19% of MP patients). Two of these 34 patients with triclonal MP were excluded from our analysis due to incomplete records; the remaining 32 patients were characterized.

Results: Twenty-one patients (65.6%) had a diagnosis of multiple myeloma (MM), 3 (9.4%) had lymphomas, 2 (6.2%) had monoclonal gammopathies of undetermined significance, 3 were immunossupressed (one HIV-positive and two solid organ-transplant recipients) and the remaining 3 had no identified malignancy. The triclonal component consisted of 3 different clones of the same immunoglobulin heavy and light chains in 14 patients (43.8%), most commonly IgA kappa (8 patients), followed by IgA lambda (4 patients). Fifteen patients (46.9%) presented with two different immunoglobulin molecules, with the most common double-clone being IgG lambda (6 patients), followed by IgG kappa and IgM lambda (3 patients each); the most common single-clones were IgG kappa (8 patients) and IgG lambda (4 patients). In the remaining 3 patients (9.4%) triclonality was composed of 3 different immunoglobulins, with an IgG kappa-IgG lambda combination being found in all three. Five patients (15.6%) underwent a single IF, which was triclonal. For the remaining 27 patients, sequential IF studies were obtained. A single patient (with MM) maintained a triclonal MP in all exams, while in 8 patients (25%) triclonalilty was identified in the last IF performed - 5 previously presented with a monoclonal pattern, while 3 had a pre-existing biclonal MP. The remaining 18 patients (56.2%) had transient triclonality, which was bidirectional - 7 patients were initially triclonal and de-escalated to biclonality (one case) or monoclonality (6 cases), while 11 evolved from monoclonality (8 patients) or biclonality (3 patients); all these 11 patients eventually lost triclonality in later studies.

Conclusion: Of the 32 patients analysed in this study, only 1 (3.1%) maintained a persistent triclonal IF pattern throughout multiple tests, while 56.2% of patients presented with transient triclonality, suggesting that transiency could be a feature of clonal evolution and involution, and is probably an indication to retest triclonal patients before establishing a definitive diagnosis of triclonal disease. Nevertheless, in 40.6% of patients in our series, neither persintence nor loss of triclonality could be demonstrated.

E-91

Determination Of Anti-Xa Activity Of Heparins And Fondaparinux

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Introduction: STA® - Liquid Anti-Xa (Diagnostica STAGO) is a new liquid reagent used to determine anti-Xa activity for unfractionated heparin (UFH), low molecular weight heparin (LMWH) and Fondaparinux. Calibrations are established with STA® - Multi Hep Calibrator for UFH and LMWH, and with STA® - Fondaparinux Calibrator for Fondaparinux.

Objectives: Compare the results obtained with STA® - Rotachrom® Heparin and STA® - Liquid Anti-Xa regarding anti-Xa activity for plasma samples from patients receiving therapeutic or prophylactic doses of UFH, LMWH or Fondaparinux.

Materials and Methods: 1861 plasma samples were tested during this international study with the two reagents, STA® - Liquid Anti-Xa and STA® - Rotachrom® Heparin, using the following calibration **Methods:** dedicated UFH (628 plasma samples), dedicated LMWH (541 plasma samples), dedicated Fondaparinux (116 plasma samples) and finally, the hybrid calibration method for UFH/LMWH (1179 plasma samples).

Correlation between the two reagents was analysed using a simple linear regression (STA® - Liquid Anti-Xa vs. STA® - Rotachrom® Heparin) and consistency was checked by the graphic representations of differences (Bland Altman plots) for each value obtained using either the dedicated or the hybrid calibration method.

Results: 1931 quality controls were performed during the study for all methods and 98.9% of results were within the control ranges. Day-to-day reproducibility for STA® - Liquid Anti-Xa was satisfactory and was equivalent to the reproducibility of the STA® - Rotachrom® Heparin (the inter-assay and inter-laboratories CV values obtained with the LMWH pathological control sample using the hybrid calibration method were 6.34% with STA® - Liquid Anti-Xa and 7.57% with STA® - Rotachrom® Heparin).

Regardless of the calibration used, the methods exhibited correlation over the working ranges tested (0.1-1.0 IU/ml for UFH, 0.1-2.0 IU/ml for LMWH and up to 2 µg/ml for Fondaparinux).

For the comparison of STA - Liquid Anti-Xa versus STA Rotachrom Heparin, the linear regression equations and the coefficients of correlation obtained are:

- Calibration specific for UFH: y=1.014x-0.052 (r=0.968)
- Calibration specific for LMWH: y=1.013x+0.004 (r=0.995)
- Calibration specific for Fondaparinux: y=1.088x-0.014 (r=0.992)
- Hybrid calibration for UFH/LMWH: y=1.021x-0.011 (r=0.994)

Performances of STA®-Liquid Anti-Xa were equivalent to that obtained with STA® - Rotachrom® Heparin.

Conclusion: The study showed that this new reagent is suitable for measurement of anti-Xa activity in patients being treated with UFH, LMWH or Fondaparinux.

Thanks to its liquid formulation, its good performances, and its association with STA-MultiHep Calibrator, which allows various calibrations to be performed easily using the same kit (dedicated UFH, dedicated LMWH and hybrid UFH/LMWH), this reagent presents real benefits in terms of day-to-day laboratory management.

E-92

Development of a mathematical model to predict the onset of iatrogenic anemia induced by repetitive phlebotomy in critical care patients

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Background: Critically ill patients (31-53%) admitted to intensive care units are frequent recipients of packed red cell transfusions in spite of use of lower hemoglobin thresholds while less than 30% of these patients have an obvious route of blood loss other than phlebotomy. Transfusion can alleviate the increased cardiovascular morbidity and mortality associated with anemia, but it confers risks of transfusion reactions, transmission of infective agents and immunosuppression in as many as 20% of transfusion recipients. The development of anemia among critical care patients is multi-factorial involving the rates of diagnostic blood loss, the erythropoeitic response and red cell senescence in addition to overt or occult bleeding and hemodilution. For individual non-bleeding patients it is difficult to estimate the relative contribution of each of these processes to the development of anemia because this involves the integration of rates and patient characteristics. Development of a mathematical model to predict the rate of onset of iatrogenic anemia in critical care patients would be useful tool to recognize patients at risk of iatrogenic anemia and transfusion.

Objective: To predict the onset of iatrogenic anemia using an objective mathematical model that integrates the rates of phlebotomy, erythropoiesis and red cell senescence with patient characteristics of blood volume and initial hemoglobin concentration.

Methods: The net rate of change in hemoglobin concentration with time was defined as the sum of the rates of hemoglobin synthesis (zero order), hemoglobin degradation due to red blood cell senescence (first order) and rate of diagnostic blood loss (first order). Integration of this net rate equation allowed the concentration of hemoglobin at any time to be estimated. **Results:** Using initial parameters of average body weight (average total blood volume), 53mL per day diagnostic blood loss, active marrow synthesis of hemoglobin and initial Hb levels in mid-reference range: The 70g/L hemoglobin transfusion threshold was reached in 40-70 days. To mimic critical care patients with initial hemoglobin levels of 110 g/L and with suppressed erythropoiesis, the influence of the volume of blood lost per day and patient body weight (blood volume) were predicted. Lack of hemoglobin synthesis, low initial hemoglobin concentration, small body weight and large daily diagnostic blood loss >50 ml/day accelerated the onset of the 70 g/L transfusion threshold within 9-14 days when three or more of these factors occurred simultaneously.

Conclusions: This model objectively depicts how diagnostic blood loss influences the onset of anemia in adult critical care patients. The model shows that subsets of patients could benefit from more conservative test ordering practices with reduced diagnostic blood loss and subsequent fewer transfusions.

E-93

Assessment of Oxidative Stress in Patients with Sickle Cell Disease

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Background: Continuous Reactive Oxygen Species (ROS) production in individuals with Sickle Cell Disease (SCD) may alter their overall redox status and cause tissue damage. The aim of this study was to evaluate oxidative stress in patients with SCD.

Patients and Methods: A total of 40 patients with SCD and 25 apparently healthy volunteers (control group) were enrolled in the study.

Components of glutathione system (GSH_{total} GSSG and $GSH_{reduced}$, vitamins A, C, and E, and malondialdehyde were determined with reverse-phase HPLC, non-transferrin bound iron (NTBI) was assessed with atomic absorption spectroscopy using graphite furnace, superoxide dismutase (SOD), glutathione reductase (GR) and glutathione peroxidase (GPx) activities were determined spectrophotometrically in red cell lysates, nitric oxide (NO) was detected colorimetrically, while FORT (free oxygen radicals test) and FORD(free oxygen radicals defense) using colorimetric assays.

Results: The table summarizes the main results of the study. We found significant impairment of the glutathione system indicated by reduced GSH_{total} ($p<0.00001$), $GSH_{reduced}$ ($p<0.00001$) and GSSG ($p<0.001$) values of the SCD patients compared to the control group. ROS expressed as FORT were significantly increased ($p<0.00001$), while antioxidant defence expressed as FORD was significantly reduced ($p<0.02$) in SCD group compared to the control group. Age and genotype of the patients as well as therapy of their disease appeared to play no role in their oxidative status.

	Controls	SCD	Difference (P)
Oxidants			
NTBI (μmol/L)	0.12 ±0.02	0.70±0.73	0.001
MDA (μmol/L)	0.59 ±0.06	0.89±0.019	0.001
GSSG (μmol/L)	167.2 ±42.7	114.0±59.2	0.001
$GSSG/GSH_t$ (%)	12.0 ±2.0	16.2±7.5	0.001
NO (μmol/L)	52.0±8.0	74.2±30.5	0.001
FORT (mmol/L H_2O_2)	1.87±0.26	3.42±0.94	0.001
Antioxidants			
GR (U/gHb)	5.9±1.5	6.5±2.4	NS
GPx (U/gHb)	30.0 ±4.9	44.1±11.3	0.001
SOD (U/gHb)	1628.8 ±384.5	1861.8±435.4	0.05
Vit A (μg/dL)	1.52±0.51	1.19±0.39	0.01
Vit E (μg/dL)	29.4±8.1	23.5±8.4	0.01
Vit C (μg/dL)	61.1±18.5	40.2±21.4	0.001
FORD (mmol/L Trolox)	1.34±0.14	1.22±0.20	0.02

Conclusion: Since oxidative stress seems to play a major role in SCD, the development of novel therapies founded on free radical biology appears imperative. Hemolysis and oxidative stress are only partially reduced by blood aphaeresis and hydroxyurea therapy, and in our SCD group seems to play no significant role. Newer therapeutic agents that can target oxidative stress, such as NADPH oxidase inhibitors, NO based therapeutics, anti-inflammatory agents and antioxidants supplementation may constitute valuable means for improved manifestations and outcome.

E-94

Variations of platelet parameters and activation marker in native healthy Chinese Tibetan adults at high altitudes

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Background: Due to the heritable adaptations to the hypoxic environment at high altitudes, native Chinese Tibetans possess some physiological variations compared with Han population, for example, higher RBC counts as well as hemoglobin levels. However, the variations of platelet parameters and activation markers have not been well elucidated in Chinese Tibetans. In the present study, we aim to investigate the variations of these platelet-related indexes in native healthy Tibetan adults.

Methods: 332 native healthy Chinese Tibetan adults at high altitudes and 245 age- and gender-matched native healthy Han population in the plain were included in this study. Platelet parameters including PLT (platelet count), PDW (platelet distribution width), MPV (mean platelet volume) and PCT (plateletcrit) were measured with SYSMEX XE-2100 counter, while the platelet activation marker P-selectin (CD62P) expressed on peripheral platelets was determined by flow cytometry.

Results: Compared with healthy Han adults, PLT as well as PCT increased significantly in Tibetans ($P<0.01$), while PDW was obviously lower ($P<0.01$). MDW and CD62P were both decreased in Tibetans than those in Han population, however the differences were not significant between two groups ($P>0.05$). All data were shown in Fig.1.

Conclusions: Our data suggest that laboratories in different regions should establish their own reference ranges of platelet parameters, due to the physiological variations of these indexes among different races in different regions. In addition, Chinese Tibetans possess a higher level of PLT and PCT, which are risk factors of cardiovascular diseases (CVD). However, Chinese Tibetans have not been shown to have increased morbidity of CVD, which may be the effect of lower activation of platelets in the peripheral blood.

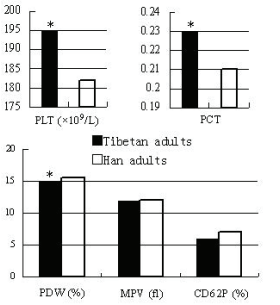

Fig.1 Analysis of platelet parameters (PLT, PCT, PDW, MPV) and activation marker CD62P in healthy Tibetan and Han adults. *P<0.01 *vs* Han adults.

E-95

Comparison of Rapid One Step Malaria HRP2 Rapid Test with Giemsa Stained smear in the Diagnosis of Malaria in Children

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Background: Malaria is a global health problem, responsible for nearly 3 million deaths each year, an average of one person (often a child aged less than 5 years) every 12s and on the increase worldwide. Improvements in malaria diagnosis should facilitate the identification of individuals infected with the malaria parasites and the treatment of such cases with appropriate drugs. Microscopy has historically been the mainstay of the diagnosis of malaria. A clinical diagnosis of malaria currently depends on the visualization of parasites by light microscopy of Giemsa stained thick and thin blood smears. This procedure is cheap and simple, but it is labor intensive and requires personnel who are well trained in the morphological differentiation of the Plasmodium species for successful diagnosis, which leads to proper treatment. In recent years, alternative methods for the identification of malaria infection have been developed based on antigen detection of plasmodium falciparum such as First Response Malaria Ag. (HRP2) Rapid Test (Premier Medical Corporation Ltd, India). The aim of this study was to compare First Response Malaria Ag. (HRP2) Rapid Test with Giemsa stained smears for the diagnosis of malaria in a clinical environment using an expanded gold standard.

Methods: 138 samples from patients attending the Child Health Department Emergency Room and OPD of Korle Bu Teaching Hospital in Accra, Ghana were examined with two different tests independently and blinded. The test methods employed in the study were Giemsa-stained blood smears and First Response malaria Ag. (HRP2) Rapid Test.Simple parametric statistics was employed in analysis.

Results: 56 (40.58%) and 59 (42.45%) samples were positive for the presence of P. falciparum by Giemsa Stain and First Response Malaria Ag Rapid Test respectively. Compared to the expanded gold standard the sensitivity of Giemsa Stain and First Response Malaria Ag Rapid Test was 94.7% and 95.6% at 95% confidence interval .The specificity was 100% and 95% at 95% confidence intervals .The positive predictive value was 100% and 97.3% and the negative predictive value was 96.2% and 96.7% respectively with good agreements k=0.95 and 0.94 .

Conclusion: The test performance of First Response Malaria Ag Rapid Test was very similar to Giemsa Stain. smears. However, the First Response Malaria Ag Rapid Test had added advantages; it was faster, easier to use, less expensive in terms of cost per test and test equipment than the Giemsa Stain. It is ideal for field work and places where there is no electricity. The First Response Malaria Ag Rapid Test can therefore be used as an alternative method for Giemsa Stain.

E-96

Platelet function monitoring and hematology after extended sample storage time using a novel stabilizing formulation

P. Guinto, D. Marchiarullo, C. A. Gelfand. *BD Diagnostics, Franklin Lakes, NJ,*

Background: With the growing number of patients diagnosed with cardiovascular disease and being treated with chronic aspirin or prescription therapy, platelet function testing is of increasing interest for routine monitoring of bleeding risk factors and management of antiplatelet medications. Citrate anticoagulant is the current gold standard sample for platelet aggregometry, however immediate (within 2-4 hour) analysis after blood withdrawal is deemed necessary for accurate monitoring.

Methods: We conducted aggregometry experiments with various anticoagulants and additives in order to identify a formulation that increases sample stability for longer times and minimizes the urgency of testing within 4 hours post collection. Blood from 12 subjects was drawn into tubes with citrate, potassium ethylenediaminetetraacetic acid (K_2EDTA), or a novel platelet stabilizing formulation (PSF). Whole blood impedance (WBIA) and light transmission aggregometry (LTA) were performed with collagen, ADP, and epinephrine as agonists and paired tests with *in vitro* spiked aspirin as an antagonist. Samples were immediately aliquoted and allowed to rest in whole blood state at room temperature until WBIA testing or platelet rich plasma (PRP) preparation for LTA at 1 (baseline), 8, 24, 32, and 48 hr. Routine hematology counts were also performed and compared to K_2EDTA at all time points.

Results: In general, WBIA and LTA observations with respect to stability are quite similar. At baseline, aggregation responses to collagen and ADP agonists were ~20% higher for PSF than citrate. At 8 hr, aggregation for PSF was similar to baseline agonist responses while citrate exhibited only 90% of baseline values. By 24 hours, citrated blood exhibited diminished collagen and ADP response (approximately 20% of baseline values) in comparison to PSF treated sample (approximately 80% of baseline values). By 48 hours, platelet function was better maintained by the PSF (65% of baseline values), while citrated blood showed almost no response to collagen and ADP. Epinephrine-induced aggregation varied considerably over time and from subject-to-subject for citrate and PSF, making comparisons difficult. The maintained platelet response with the PSF treated samples also retained sufficient aggregation potential to allow the aspirin antagonist effect to be observed at 48 hours, whereas aspirin efficacy in citrated blood was indistinguishable by 24 hours due to sample instability. Additionally, PSF, but not citrate, tracked well with K_2EDTA controls for complete blood counts at all time points.

Conclusions: Use of the novel PSF chemistry improved stability of whole blood samples for platelet aggregation measurement. Extended sample life may facilitate whole blood specimens to be transported to reference laboratories and potentially allow for widespread utilization of aggregation in routine clinical settings.

E-97

The clinical significance of immunoglobulin heavy chain (IgH) gene and T-cell receptor gamma (TCR-γ) chain gene rearrangements detected in plasma cell-free DNA from de novo B cell non-Hodgkin's Lymphoma (B-NHL)

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Background To evaluate the clinical significance of IgH and TCRγchain gene clonal rearrangement in de novo B-NHL with the specimens of plasma cell-free DNA instead of pathological biopsy samples from the lymph node or tumor tissue.

Methods Cell-free DNA extracted from 92 de novo B-NHL plasma blood samples were identified by house-keeping gene globin. We detected the IgH and TCRγclonal gene rearrangement by polymerase chain reaction (PCR).The results of plasma blood samples were compared with the results of pathological biopsy samples.

Results In 92 cases of de novo B-NHL, 91 cases had be extracted cell-free DNA successfully from their plasma blood samples, one de novo diffuse large B cell lymphoma(DLBCL) failed. In the study of 91 cases, a clonal IgH gene rearrangement was detected in 81 cases(89%), a clonal TCRγ gene rearrangement was detected in 1 DLBCL cases(1.1%).The 91 cases were consist of three groups, that is 66 de novo DLBCL,16 small lymphocytic lymphoma(SLL) and 9 follicullar lymphocytic(FL), the clonal gene rearrangement results of each groups separately were showing in Table1. Compared with the results of IgH and TCRγ gene rearrangement between plasma samples and biopsy samples in 31 de novo B-NHL, the IgH gene rearrangement positive rate of plasma samples and biopsy samples were 87%(27/31), 81%(25/31) respectively, P=0.5, there was no significant difference.

Conclusions For de novo B-NHL, the positive rate of IgH and TCRγchain gene rearrangement in cell-free DNA extracted from plasma blood samples was high. The clinical significance of plasma cell-free DNA gene rearrangement was as same as biopsy samples. Furthermore, it's more convenient and non-invasive. So, it's very useful for diagnosis,differential diagnosis and monitoring the curative effect.

Table1 The results of IgH and TCRγgene clonal rearrangement of 91 de novo B-NHL with the specimens of plasma cell-free DNA

Groups	cases	IgH(%)	TCRγ(%)
DLBCL	66	60(90.9)	1(1.5)
SLL	16	13(81.2)	0
FCL	9	8 (88.9)	0
Total	91	81(89.0)	1 (1.1)

E-98

Nitric Oxide Synthase (NOS) immobilized in electrospun fibers: towards novel nitric oxide (NO) release membranes

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Background: Nitric oxide is a molecule known to counteract platelet aggregation, and thus can stop the thrombosis cascade on the surface of blood-contacting medical implants. Nitric oxide synthases are enzymes (NOSs) responsible for catalytic conversion of the substrates L-arginine to NO and L-citrulline. By using NO releasing biomaterial in their closest native characteristics to mammalian tissue, one may be able to solve the issue of thrombosis and restenosis on the surface of foreign devices implanted or used as part of cardiovascular procedures. In the past we have tested the use of NOS enzymes in layer-by-layer thin films as source of *in-situ* NO synthesis and release. Our objective in the current work is to use NOS enzymes trapped in electrospun fiber matrices as biocompatible platform for NO release.

Methods: In this project, we investigate embedding of nitric oxide synthase (NOS) as a functional component contained in aqueous pockets of electrospun biopolymer matrices; namely, polycaprolactone (PCL) and Polyurethane (PU). A guided stream of polymer solution containing suspended aqueous pockets of enzyme solution is directed towards a collector drum in strong electric field. In its path of acceleration towards the target, the solvent evaporates and the charged jet thins-out leaving a fibrous membrane, devoid of solvent and containing 'nodes' of aqueous pockets with entrapped NOS enzymes.

Surface characterizations such as Transmission Electron Microscopic (TEM) and Atomic Force Microscopic (AFM) imaging are carried out on the newly formed NOS-containing electrospun fibers. Further, the NOS-modified membranes are tested electrochemically using a characteristic electrocatalytic reaction mediated by entrapped NOS enzymes. Finally, the NOS-containing electrospun membranes are subject to assays under various conditions to determine the structural integrity of NOS enzymes and their enzymatic activity.

Major Results: Morphology of the NOS containing nodes in individual microfibers imaged at different stages of electrospinning shows evidence of success of spin-trap process. Griess assay and hemoglobin deoxy assay shows quantitative release of NO from the NOS-modified fibers under physiologic conditions. This confirms electrochemical characterization using the NOS-mediated catalytic reduction reaction of exogenous NO of the same fibers.

Conclusion: Together, these results show that the native structure of the entrapped NOS in the aqueous pockets is conserved and is functional under physiologic conditions.

E-99

The significant difference in protein S activity between female and male stroke patients

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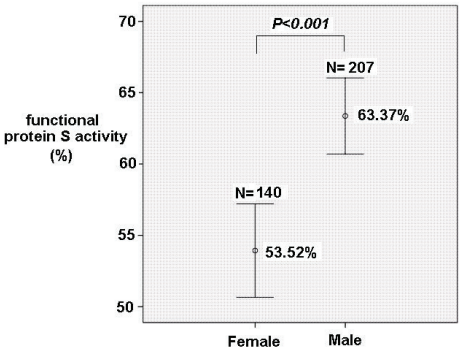
Background: Protein C inhibits coagulation by degrading activated factors V and VIII. Protein S participates as a cofactor in this degradation. As natural anticoagulants, functional activity and antigen tests for protein C and protein S are included in diagnostic algorithm of thrombophilia screening. However, the association with ischemic stroke was not fully evaluated yet. The aim of this study is to evaluate the protein C and protein S levels and verify their gender differences in Korean stroke patients.

Methods: A total of 347 admissions with acute ischemic stroke were enrolled for the study between July 2008 and December 2010. Patients were graded according to the National Institutes of Health Stroke Scale. Functional protein S and protein C activity were determined within 48 hours from admissions by STA STACHROM PROTEIN S and STA STACHROM PROTEIN C (Diagnostica Stago, Inc., NY, USA), respectively, using STA-R evolution (Diagnostica Stago, Inc., NY, USA). Total antigen tests were done by Enzyme Linked ImmunoSorbent Assay (Corgenix, Denver, USA).

Results: The median age of total patients was 49.0 years (range 31~86) and the male to female ratio was 207:140. In functional protein C and protein S activity, mean values were 100.6% (reference range 70-130%) and 59.15% (reference range 73.7-146.3%), respectively (in antigen tests, 79.39% (72-160%) and 77.63% (60-150%), respectively). In functional protein S activity, 73.96% of total patients showed decreased levels and female patients showed significantly lower means (53.52%) than males (63.37 %, $P<0.001$).

Conclusion: In this study, only protein S activity hass significantly decreased in acute ischemic stroke patients, especially for the female group. Therefore, a prospective follow-up study on the changes of functional protein S activity should be necessary to prove that screening of functional protein S activity can give some aid to recognize the risk of stroke in adult female patients.



E-100

Frequency distribution of Single Nucleotide Polymorphisms in p-selectin Gene in Chinese Tibetan and Han population

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Background Chinese Tibetans have lived in most extreme environments with altitudes exceeding 4000 m for 5000~7000 years, and there are several evidences show that they possess heritable adaptations to their hypoxic environment, such as high hemoglobin levels and high RBC counts, which may increase the risk of coronary artery disease. However, there are no evidences show that Tibetans have higher morbidity of atherosclerosis or venous thromboembolism than other ethnic group. Single nucleotide polymorphisms (SNPs) in P-selectin gene has been proved to be involved in coronary artery disease,stroke,venous thromboembolism and other diseases. Difference of frequency distribution of SNPs in p-selectin gene between Tibetan and Han population is still unkown. In this study, SNPs in 4 exons of P-selectin were investigated between Tibetan and Han population for the first time.

Methods 4 SNPs (Ser290Asn, Asn562Asp, Leu599Val and Thr715Pro) in P-selectin gene were analyzed in 306 Chinese Tibetan and 267 Han population. The high-resolution melting (HRM) was used to genotyping samples for the 4 exons SNPs. The HRM method was optimized on a LightCycler 480 machine, and genotyping was performed by GeneScan software.

Results There was a significant increase of the allele A frequency in Ser290Asn in Tibetans compared to Han population (p=0.003), but no significant differences in both the genotype and allele distribution of Asn562Asp polymorphisms of the P-selectin gene between Tibetan and Han populations (p >0.05). No polymorphisms of Thr715Pro and Leu599Val were found in this study.

Conclusion The results demonstrated that the genotype and allele distribution of Ser290Asn had significant difference between Chinese Tibetans and Han population. The difference of SNP frequency distribution in P- selectin gene may come from ethnic group difference.

Tab1. Gene type and Alleles frequency for 2 exons Ser290Asn and Asn562Asp of P-selectin polymorphisms for Chinese Tibetans and Han population control

306 Chinese Tibetans and 267 Han population control							
Ser290Asn	Tibetans	Han		Asn562Asp	Tibetans	Han	
Gene type AA	5	15		AA	272	242	
AG	80	87	*P=0.003*	AG	27	20	*p>0.05*
GG	221	165		GG	7	5	
Alleles A	90	117		A	571	504	
G	522	417		G	41	30	
A/G	0.172	0.280		A/G	13.927	16.8	

E-101

The Impact of the B Domain of Coagulation Factor V in Regulating Thrombin Generation

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The B domain of factor V (fV) is a central paradigm in its regulation of the cofactors participation in the conversion of prothrombin to thrombin and the crucial formation of a fibrin clot. In plasma, single chain factor V (fV) circulates as a 330-kDa quiescent procofactor consisting of multiple domains (A1-A2-B-A3-C1-C2) with nominal procoagulant activity. Following three sequential catalytic cleavages by a-thrombin at Arg^{709}, Arg^{1018} and Arg^{1545} the B-domain is liberated to generate the noncovalently associated light and heavy chains of factor Va (fVa). FVa binds to factor Xa (fXa) assembled on a phospholipid membrane in the presence of divalent metal ions to form the prothrombinase complex. Since single chain fV does not bind fXa, the proper removal of the B-domain is vital to generate procoagulant activity. Appropriate binding of fVa to fXa during prothrombinase function is essential to the proper activation of the substrate, prothrombin. Previous studies have determined the heavy and light chains of fVa to have fXa interactive sites. We hypothesize that a highly conserved and basic region of amino acids in the B-domain suggests a potential sheathing of either the heavy or light chain fXa interface sites. To verify this hypothesis we investigated the role of amino acid region 1000-1008 that contains seven basic amino acid residues. To ascertain the role of this region we have constructed a recombinant mutant fV molecule with all activation cleavage sites ($R^{709}/R^{1018}/R^{1545}$) mutated to glutamine ($fV^{Q3}$), a mutant fV molecule with region 1000-1008 deleted ($fV^{\Delta B8}$), and a mutant fV molecule containing the same deletion with all activation cleavage sites changed to glutamine ($fV^{\Delta B8/Q3}$). The recombinant molecules along with wild type fV (fV^{WT}) were transiently expressed in COS7L cells, purified to homogeneity, and assessed for their capability to bind fXa within prothrombinase prior (fV) and after incubation with thrombin (fVa). The data showed that fV^{Q3} and fVa^{Q3} were unable to interact with fXa. In contrast, the K_d values for $fV^{\Delta B8}$ (0.9 nM), $fVa^{\Delta B8}$ (0.4 nM), $fV^{\Delta B8/Q3}$ (0.7 nM) and $fVa^{\Delta B8/Q3}$ (0.5 nM), were similar to the affinity of fVa^{WT} for fXa (0.22 nM). Two-stage clotting assays revealed that while fVa^{Q3} was practically devoid of clotting activity, the mutant molecules $fVa^{\Delta B8}$, and $fVa^{\Delta B8/Q3}$ had clotting activities comparable to fVa^{WT}. Thus, unactivated $fV^{\Delta B8/Q3}$ has an affinity for fXa that is similar to the affinity of fVa^{WT} for the enzyme. In addition, $fV^{\Delta B8/Q3}$ that cannot be cleaved and activated by thrombin or activated during the course of the clotting assay, has similar clotting activity as fVa^{WT} (~3110 U/mg). The data presented in this study provide an important insight into one of the possible roles of the B domain of factor V, explicitly the fXa interactive sites on fVa are covered/inhibited by amino acids 1000-1008 of the fV B domain. These data strongly suggest that amino acid region 1000-1008 of fV contains a regulatory sequence protecting the organisms from spontaneous binding of the procofactor to fXa and unnecessary prothrombinase complex formation which will result in catastrophic physiological consequences.

Thursday AM, July 28

Poster Session: 9:30 am - 12:00 pm
Immunology

E-102

Detection of anti-aquaporin antibodies in human serum and CSF by ELISA based Assay

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Background: Neuromyelitis optica (NMO, Devic's disease) is an autoimmune demyelinating disease that attacks the spinal cord and optic nerves. NMO was considered a variant of multiple sclerosis (MS) until antibodies to aquaporin-4 (AQP4) were identified in the serum and CSF of 60%-90% of NMO patients; AQP4 is now used to help differentiate NMO from MS. Both indirect immunofluorescence assay (IFA) and ELISA have been used to detect AQP4 antibodies, but there is no consensus on the optimal method. This study was performed to evaluate the performance of an ELISA-based assay for measurement of AQP4 antibodies in human serum and CSF.

Methods: An AQP4 ELISA kit (Kronus, Star, ID) employing recombinant human AQP4 was validated. For method comparison, 46 de-identified sera were submitted for AQP4 antibody testing by IFA (Mayo Laboratories, Rochester, MN) and ELISA. To evaluate disease specificity, the ELISA was performed on 180 de-identified CSFs submitted for MS testing for oligoclonal bands, IgG synthesis rate, and myelin basic protein (MBP); 40 CSF and 120 sera from healthy donors were also tested.

Results: The AQP4 ELISA showed good precision and analytical sensitivity, but was non-linear (Table). The method comparison study showed 100% concordance between the ELISA and IFA: of the 46 sera tested for AQP4 antibody by ELISA and IFA, 5 were positive and 41 were negative by both methods. A CSF sample spiked with AQP4 antibody positive serum also tested positive in both assays. Of 54 CSF specimens with at least 1 marker consistent with MS (oligoclonal band, elevated IgG synthesis rate, or elevated MBP), none were positive for AQP4 antibody.

Conclusion: The ELISA provides results concordant with the IFA method. The ELISA is negative in individuals with markers consistent with MS, suggesting its specificity and ability to differentiate NMO from MS.

Table. Analytical characteristics of AQP4 ELISA

ELISA	Serum	CSF
Precision (overall CV)	7-11%	4-8%
Limit of quantitation	<5.0 U/mL	<5.0 U/mL
Limit of detection	5.0 U/mL	5.0 U/mL
Analytic measurement range	5-160 U/mL	5-160 U/mL
Clinical reportable range	<5-160 U/mL	<5-160 U/mL
Reference range	<5 U/mL	<5 U/mL
Specimen Stability		
18-22°C 2-8°C -20°C Freeze/thaw	3 days 7 days 21 days 3 cycles	14 days 14 days 49 days 3 cycles

E-103

Clinical Sensitivity and Specificity of Anti-CCP IgG for Rheumatoid Arthritis for Three Different Assay Methods

A. Wu[1], W. Ruan[1], S. Tu[1], J. Wang[2], T. Williamson[2]. *[1]UCSF-San Francisco General Hospital, San Francisco, CA, [2]Bio-Rad Laboratories, Benicia, CA,*

Background: Antibodies to CCP (cyclic citrullinated peptides) are a component of the new ACR/EULAR (2010) classification criteria for RA (rheumatoid arthritis). The performance of anti-CCP assays differ due to differences in antigens (CCP2 vs. CCP3) and a lack of calibrator standardization. The objective of this study is to determine the clinical sensitivity and specificity for three different anti-CCP IgG assays in previously diagnosed RA, blood bank, and non-RA connective tissue disease patients.

Methods: 175 unique samples from previously diagnosed RA patients (ICD-9 code:714.0); 150 unique blood bank samples, and 100 unique samples from non-RA connective tissue disease patients were analyzed for anti-CCP IgG with the BioPlex® 2200 Anti-CCP (Bio-Rad, Hercules, CA), the QUANTA Lite® CCP3 IgG ELISA (INOVA, San Diego, CA), and the Axis-Shield Anti-CCP (Axis-Shield, Dundee, UK). The data from the Axis-Shield assay were calculated with both the vendor recommended cut-off (≥ 6 U/mL = positive) and a modified cut-off (≥ 3 U/mL = positive) due to an extreme difference in clinical sensitivity as compared to vendor claims in their product's instructions for use. The data from one blood bank sample and from eleven non-RA connective tissue diseases samples were removed from the analysis because they were positive on all three methods and thus deemed to be positive.

Results: The clinical sensitivities for previously diagnosed RA patients were 78.9% (BioPlex 2200), 78.3% (QUANTA Lite), and 38.9% (Axis-Shield) with the vendor recommended cut-off or 63.4% with a modified cut-off. The clinical specificities for blood bank samples were 99.3% (BioPlex 2200), 96.6% (QUANTA Lite), and 99.3% (Axis-Shield) with the vendor recommended cut-off or 97.3% with a modified cut-off. The clinical specificities for non-RA connective tissue diseases were 97.8% (BioPlex 2200), 88.8% (QUANTA Lite), and 94.4% (Axis-Shield) with the vendor recommended cut-off or 83.1% with a modified cut-off.

Conclusion: The clinical sensitivity was highest for the BioPlex 2200 (CCP2) and the QUANTA Lite (CCP3) assays even though they utilize different antigen mixtures. The BioPlex 2200, QUANTA Lite, and Axis-Shield all had clinical specificities >96% for blood bank samples. The clinical specificity for non-RA connective tissue diseases was highest for the BioPlex 2200 and Axis-Shield with the vendor recommended cut-off and lower for the QUANTA Lite and Axis-Shield with a modified cut-off. The greatest difference between the assays was seen in the non-RA connective tissue diseases in which the anti-CCP assay is used to aid in the differential diagnosis between RA and other non-RA connective tissue diseases. It should also be noted that every laboratory should validate an assay's cut-off (reference range) against the claims in the products instructions for use.

E-104

Improved clinical sensitivity for rheumatoid arthritis by measuring subclass rheumatoid factors using a novel multiplex planar protein microarray assay

D. R. Bunch, S. Wang. *Cleveland Clinic, Cleveland, OH,*

Background: Rheumatoid arthritis (RA) affects 0.5-1% of the US population. Early diagnosis and aggressive treatment may mitigate or prevent damage and disability. Measuring rheumatoid factor (RF) may play an important role in RA diagnosis and management. Recently, a novel multiplexing immunoassay was developed (IgX PLEX RA qualitative assay, SQI Diagnostics, Toronto, Canada) that simultaneously measures subclasses of RF (IgA, IgG, IgM) and anti-cyclic citrullinated peptide (anti-CCP) IgG. The assay has a Health Canada license for use as a quantitative assay and received FDA 510(k) clearance for RF IgM and IgA.

Objective: To compare relative clinical sensitivity of the subclasses of RF with the RF II latex enhanced immunological agglutination turbidimetric assay (Roche Diagnostic, Indianapolis, IN) using patient specimens clinically diagnosed with RA.
Methods: RA samples (119 males with age 45-72 yrs and 131 females with age 25 - 72 years) were purchased from SLR Research Corp (Carlsbad, CA). The samples were assayed in duplicates using the RA assay processed on the SQiDWorks™ (SQI Diagnostics) analyzer, while one aliquot of each sample was assayed using the Roche RF II on a P modular system.

Results: The ranges of RFs in the patient sera were 9-222 IU/mL, 3-68 IU/mL, 6-175 IU/mL, and 6-155 IU/mL for Roche RF II, RF IgA, RF IgG, and RF IgM, respectively. Based on clinical diagnosis, the clinical sensitivity of the RF IgX 3-PLEX (99.2%) had an 8% improvement over the Roche RF II total RF (91.2%) in this patient population (Table 1).

Conclusion: The multiplexing assay provided a significant improvement with an 8% increase in clinical sensitivity compared to a total RF assay. Acknowledgement: This work was supported and analyzed in collaboration with SQI diagnostics.

Table 1. Clinical sensitivity of subclass RFs in a clinically diagnosed RA cohort (n=250)

	Clinical Sensitivity, %	False Negative Rate, %
RF IgX 3-PLEX	99.2	0.8
RF IgM	98.8	1.2
RF IgA	82.0	18.0
RF IgG	76.4	23.6
Roche RF	91.2	8.8

E-105

Erythrocyte Sedimentation Rate and C-Reactive Protein Test Utilization: Identification of Potential Waste in Laboratory Medicine

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Background: Erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) are non-specific tests for acute inflammatory processes. ESR is an antiquated, manual test that requires 60 minutes to perform by the Westergren method. CRP is an acute phase reactant that is widely measured on automated platforms. Studies have demonstrated equivalent performance of ESR and CRP in a majority of patient populations, with the exception of patients with temporal arteritis and/or polymyalgia rheumatica. At our institution, ESR and CRP are both high volume stand alone tests and are frequently ordered simultaneously. We identified and evaluated the patient populations where ESR and CRP were ordered concurrently to determine whether there was any added value to ordering both tests. Ultimately the goal was to reduce unnecessary ESR and/or CRP test requests.

Objective: Evaluate the analytical concordance and indication for testing of ESR and CRP to guide appropriate utilization.

Methods: Retrospective ESR and CRP results ordered alone (n_{ESR}=26,346, n_{CRP}=11,333) and simultaneously (n = 29,702) on the same patient on the same day were evaluated over a one year period (11/1/08 to 10/31/09). Patients were all seen at the Mayo Clinic (Rochester, MN). The concordance between positive and negative ESR and CRP results were evaluated based on ESR cut-offs for positive results of >22 mm/h for males or >29 mm/h for females, and CRP concentrations >8 mg/L. The diagnosis ICD-9 codes associated with each laboratory test order were obtained from the electronic medical record for the simultaneous ESR and CRP results and were analyzed for agreement. The diagnosis codes were then categorized by indication for testing and the groups were sorted to associate each set of patient results with a single indication for testing when multiple indications were found.

Results: In 95% of cases, ESR and CRP were ordered for the same medical indication. ESR was measured alone for 48% of total requests while CRP was measured alone for 27%. The overall concordance between ESR and CRP results was 81% (16% positive, 65% negative). The indications for testing with the greatest concordance included inflammation (86%, n = 3315) and neoplasm (83%, n = 571). The categories with the least concordance included polymyalgia rheumatica (73%, n = 368) and systemic lupus erythematosus (74%, n = 410). Prosthetic joint/device infection (n = 5572) and a variety of rheumatic diseases including rheumatoid arthritis (n = 2215) had ~80% concordance.

Conclusion: ESR and CRP are being ordered for the same purpose. ESR was ordered alone twice as frequently as CRP alone. When CRP was ordered, ESR was ordered concurrently 73% of the time despite an overall analytical concordance of 81%. These data demonstrate that antiquated tests such as ESR continue to be routinely utilized despite emergence of newer, more automated assays such as CRP. Furthermore, these findings suggest that there is redundancy in test orders for assessing acute inflammation for most clinical indications and identifies opportunities for the clinical laboratory to provide education to healthcare providers and subsequently guide proper test utilization.

E-106

Diagnostic value of anti-cyclic citrullinated peptide antibodies and HLA-DRB1 shared epitope in Andalusian patients with rheumatoid arthritis

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Background: Rheumatoid arthritis (RA) is a systemic inflammatory autoimmune disease of unknown etiology, characterized by chronic polyarthritis. Anti-cyclic citrullinated peptide (anti-CCP) antibodies have diagnostic value in RA but the role of shared epitope (SE) is unclear. We assessed the diagnostic value of anti-CCP antibodies and SE in Andalusian patients with symptoms of arthritis in their first visit to the rheumatologist.

Methods: We measured anti-CCP antibodies with QUANTA LiteTM enzyme-linked inmmunosorbent assay (ELISA) kit for the detection of IgG anti-CCP3 (Cyclic Citrullinated Peptide 3) antibodies in patient sera (cut-off value, 40 UI/ml). SE was determined with GenID® Reverse Hybridization kit for detection of SE in HLA-DRB1 alleles kit in patient plasma. They were tested for 211 patients with suspected rheumatoid arthritis. The American College of Rheumatology (ACR) criteria for RA were fulfilled for 106 patients. These patients were diagnosed of RA. The other 105 patients were diagnosed with other rheumatic disease. We also determined rheumatoid factor (RF) with BAYER® FR IgM immunoturbidimetric assay for ADVIA 2400 (cut-off value, 20 UI/ml). The study was a diagnostic test study. We determined the diagnostic value (sensibility, specificity and likelihood ratios) for anti-CCP antibodies, SE and RF. We determined the area under the curve (AUC) for anti-CCP antibodies and RF. Statistical analyses were performed using IBM SPSS Statistics version 19 for Windows (New York, USA).

Results: Sensitivity of anti-CCP antibodies, SE and RF for RA were 66.0%, 72.6% and 81.1%, and specificity were 96.2%, 38.1% and 76.2%, respectively. The AUC for anti-CCP antibodies was 0.875 with 95% CI of 0.828 to 0.922 and the AUC for RF was 0.864 with 95% CI of 0.815 to 0.913. The positive likelihood ratio for anti-CCP antibodies, SE and RF were 17.37, 1.06 and 3.41 respectively. The negative likelihood ratio for anti-CCP antibodies, SE and RF were 0.35, 0.72 and 0.25. Anti-CCP antibodies were positive in 30.0% of RF-negative RA patients. Anti-CCP antibodies and RF were positive in 60.4% of total RA patients and were negative in 13.2% of total RA patients.

Conclusion: In Andalusian patients with suspected rheumatoid arthritis, anti-CCP antibodies have a very good diagnostic value in their first visit to the rheumatologist. However, the SE doesn´t have diagnostic value in our population. The anti-CCP antibodies are a more specific test than the FR and hence they are very useful as a confirmatory test in a specialized doctor´s office.

E-107

Association of tumour necrosis factor-α gene polymorphism with prognosis of pulmonary sarcoidosis

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Background: Pulmonary sarcoidosis is an immune-mediated disorder characterized by the presence of granulomatous inflammation in the lung. It usually resolves spontaneously; however, in approx.20% of patients, it progresses to local/diffuse fibrosis. There is strong evidence for a genetic predisposition to sarcoidosis and associations have been reported between disease status and sharing of polymorphic alleles in various genes suspected of contributing to sarcoid susceptibility and/or clinical manifestation/prognosis. Polymorphic genes in the MHC classI/II loci have been repeatedly implicated in sarcoidosis, but also MHC classIII genes encode proteins (e.g. TNF-α) that are involved in immune response and thus may be relevant to inflammatory reaction in sarcoidosis.

Apart from the sarcoidosis predisposing genes, it is important to identify the genes modifying the disease. Recent data indicate that tumour necrosis factor alpha (TNF-α) may modify sarcoidosis development.. Presence of TNF-α -308A (rs1800629) variant allele was recently reported in conjunction with favourable prognosis in the Dutch population and its determination may, therefore, be used to clinically predict prognosis [1]. In the patients without the TNF-α -308A allele, in which the risk of progressing to more severe pulmonary involvement was higher, treatment should be started early to avoid irreversible damage.

Aim: To determine the distribution of TNF-α -308G/A SNP in Czech sarcoidosis patients and to replicate in the Czech population the results about association of TNF-α -308A variant allele with a favourable prognosis.

Methods: A *prospective* cohort *study has been conducted enrolling 122 patients with pulmonary sarcoidosis who were recently diagnosed at* Dept. Respiratory Medicine, Faculty Hospital Olomouc according the International Statement of Sarcoidosis (1999). Patients were further subdivided according to the presence/absence of Löfgren's syndrome (LS) and the chest X-ray stage (CXR). TNF-α -308G/A polymorphism was determined by PCR-SSP. Differences between allele/genotype frequencies were evaluated by Chi-squared test.

Results: The genotype and allele frequencies of 122 studied sarcoidosis patient were similar to the published frequencies in Czech population [2]. No association between TNF-α -308 polymorphism and sarcoidosis as a whole or with subgroups according the CXR-stage was found. Interestingly, the allele TNF-α -308A was more frequent in patients with Löfgren's syndrome comparised to those without LS ($p \leq 0.05$). When genotype/allele frequencies were compared between the patients with resolving and progressive sarcoidosis after 2 years follow-up, no significant difference was observed.

Conclusion: This pilot study showed overrepresentation of TNF-α -308A* in patients with Löfgren's syndrome, acute form of sarcoidosis. In the current group of patients we, however, could not replicate the original Dutch data about overall association of the investigated polymorphism with good prognosis. For the definite conclusion on

possible usefulness of genotyping for the TNF-α -308G/A polymorphism in predicting prognosis in Czech sarcoidosis patients, analysis of the second set of sarcoidosis patients followed clinically after 2 years is necessary.

Ref.1 Wijnen PA. The role of tumor necrosis factor alpha G-308A polymorphisms in course of pulmonary sarcoidosis. Tissue Antigens 2010;75:262-8. 2. Kubistova Z. Distribution of 22 cytokine gene polymorphisms in the healthy Czech population. Int J Immunogenet. 2006;33:261-7.

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E-108

Evaluation of assays for the measurement of Albumin and IgG in Cerebrospinal fluid on the Binding Site SPA PLUS turbidimetric analyser

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Measurement of cerebrospinal fluid (CSF) protein concentrations can be useful in the assessment of local immune responses in the central nervous system (CNS). Increased albumin and IgG levels can be indicative of blood-CSF barrier dysfunction and/or intrathecal immunoglobulin synthesis. Evaluation of barrier function and intrathecal synthesis in conjunction with other clinical findings can be a useful aid in the diagnosis of a variety of CNS disorders. Here we describe the evaluation of Albumin and IgG CSF assays for use on the SPAPLUS analyser, a small bench-top turbidimeter available from The Binding Site Group Ltd. The instrument is an automated, random access analyser with host interface capability, primary sample ID and reagent management systems. Precision is promoted through a combination of air-pressure reagent mixing and acid /alkali cuvette washing. The analyser was programmed to construct a calibration curve from a six point, serum based calibration set and validated by assay of control fluids. All dilutions were made with the instrument's on-board pipetting system, which was able to make dilutions between neat and 1/100. The main assay characteristics are summarised in the table below:

Assay	Albumin				IgG			
Range (mg/L)	170 - 3000				3.8 - 122			
Sample dilution	1/10				1/1			
Minimum sample dilution	1/1				1/1			
Sensitivity (mg/L)	17.0				3.8			
Precision % CV (CLSI EP5-A2)		2186 mg/L	417 mg/L	257 mg/L		101.6 mg/L	31.8 mg/L	6.0 mg/L
	Total	9.3%	9.1%	9.1%	Total	3.2%	7.9%	8.3%
	Within-run	2.0%	2.0%	2.4%	Within-run	2.0%	1.7%	3.5%
	Between-run	2.1%	2.3%	2.6%	Between-run	1.4%	7.7%	7.8%
	Between-day	8.9%	8.6%	8.4%	Between-day	2.0%	0.0%	4.3%

No significant interference (within ±10%) was observed on addition of bilirubin (200mg/L) or haemoglobin (5g/L; Albumin and 2g/L; IgG) to CSF samples with known concentrations. The assays showed a high degree of linearity when expected values were regressed against measured values of a serially diluted CSF sample:- Albumin: y= 1.01x - 0.02mg/L, R^2=0.999; IgG: y= 1.00x - 0.49mg/L, R^2=0.998. Comparison was made using the Siemens Albumin and IgG assays for the BN™II. Good agreement was demonstrated:- Albumin: y=0.90x +27.98mg/L, R^2=0.97 (n=72); IgG: y=1.01x +0.73mg/L, R^2=0.97 (n=73). We conclude that the SPA PLUS Albumin and IgG CSF assays are rapid and precise, and may be of use in laboratories were a large instrument may not be appropriate.

E-109

Recurrence assessment in papillary thyroid carcinoma associated with autoimmune thyroid diseases, after thyroidectomy and before radioiodine therapy

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Background: The incidence of thyroid cancer, in particular of well-differentiated papillary thyroid carcinomas (PTC), is increased in autoimmune thyroid diseases (ATD) such as Hashimoto's thyroiditis (HT) and Graves' disease (GD). These tumors may become aggressive and have a higher rate of distant metastases and relapse, than similar tumors occurring in euthyroid patients. The thyroidectomy followed by radioiodine (I-131) ablation of the residual thyroid tissue is considered the ideal treatment for these patients. Sensitive monitoring for thyroid cancer recurrence includes whole-body radioiodine scanning (I-131 WBS) and the measurement of thyroglobulin (Tg) serum concentration, after thyrotropin (TSH) stimulation. We assessed the relationship between some cytokines (interleukins IL-1β, IL-4, IL-10, tumor necrosis factor TNF-α), C reactive protein (CRP), Tg, anti-thyroglobulin (anti-Tg) antibodies and the recurrence in papillary thyroid carcinoma associated with autoimmune thyroid diseases (PTC+ATD), after thyroidectomy and before I-131 therapy.

Methods: The study included 83 patients with PTC+ATD (5M/78F, aged 40.2±19.6 years) - characterized by elevated serum levels of TSH (>30 mIU/l), low serum levels of free T4 (<0.45 ng/dl), elevated serum levels of Tg (>10 ng/ml - 27 patients) and positive titers of anti-Tg antibodies (41 patients). The serum levels of TSH, free T4, Tg, anti-Tg antibodies, CRP, IL-1β, IL-4, IL-10 and TNF-α were measured before the I-131 therapy by ELISA. For positive titers of anti-Tg antibodies, the Tg values were calculated using a Tg recovery test. I-131 WBS was performed in 3-5 days after the administration of I-131.

Results: I-131 WBS and Tg were concordant in 71.94% of cases. We found that 18 patients, of the 56 patients with undetectable Tg, had positive titers of anti-Tg antibodies and in 10 (55.5%) of these, the recurrence was confirmed. The rate of detectable CRP, IL-4 and IL-10 was higher: 1) in patients with recurrence, than without; 2) in patients with positive anti-Tg antibodies, than in patients with negative anti-Tg antibodies. There was a good correlation between the serum level of CRP and IL-4 ($r=0.42$, $p<0.001$), CRP and IL-10 ($r=0.47$, $p<0.001$). The anti-Tg antibodies activity, was directly correlated with IL-4 ($r=0.52$, $p<0.001$), IL-10 ($r=0.61$, $p<0.001$) and CRP ($r=0.51$, $p<0.001$). These positive correlations led to the idea, that IL-4 and IL-10 have a proinflammatory effect in PTC+ATD recurrence. It seems that IL-4 and IL-10, which exhibits multiple modulatory effects on the immune system, have an altered expression in recurrent PTC+ATD patients, due to the anti-Tg antibodies presence.

Conclusions: These data suggest that the elevated anti-Tg antibodies levels could be a potential marker of recurrence and poor prognosis in patients with PTC, even in patients with undetectable serum Tg values. Also, the anti-Tg antibodies have a role in the stimulation of T cells for cytokine production in these patients. This means that IL-4 and IL-10, produced by the Th2 lymphocytes, may contribute to the aggressiveness of the thyroid cancer in GD and HT patients. The antagonists to these cytokines or to their receptors may be helpful in the treatment of these aggressive PTC.

E-110

Double Positive CD4CD8 T Cells: A Key subpopulation in the pathogenesis of systemic lupus erythematosus

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Background: Double positive T cells(DPT)are a kind of progenitor T lymphocytes. The purpose of the present study is to investigate the role of DPT in the pathogenesis of systemic lupus erythematosus (SLE)through analyzing the relationships between DPT and the other indicators.

Methods: Samples from 175 SLE and 125 age and gender matched healthy controls were tested for DPT by flowcytometry. Antinuclear antibody (ANA) and anti-dsDNA antibodies were detected by indirect immunofluorescence (IIF); anti-RNP, anti-SSA and anti-rib-P by immunoblot; complement 3 (C3) and complement 4 (C4) by rate nephelometry. All data were analyzed by Mann-Whitney U test with SPSS 16.0. $P<0.05$ was considered statistically significant.

Results: 1. The median and 5%~95% intervals of DPT in SLE and healthy control were 0.5 [0.1-2.60] and 0.80 [0.2-2.74], respectively,and significant difference was found between two groups ($Z=4.557$, $P=0.000$).

2. DPT distribution in different SLE subgroups divided by diverse indicators showed that the percentages of DPT cells were significantly lower in the group of "ANA titer over 1:1000", the positive anti-dsDNA group, the positive anti-rib-P group, the positive anti-RNP group and the positive anti-SSA group, and the lower C3 or C4 concentration group (shown in Table 1).

Conclusion: 1.The DPT as progenitor T lymphocyte may have apoptotic dysfunction caused by the positive and negative selection process in SLE, that causes DPT lower than in healthy control.

2. In SLE patients with more autoantibodies, the DPT are low in percentages, suggesting that DPT may play "good" role in restraining the production of autoantibodies by interacting with B lymphocyte in SLE.

3.In patients with lower concentration of C3 or C4, the DPT are low,so DPT can be used as an indicator to evaluate the disease activity of SLE.

Table 1. Comparison of the percentage of DPT in different SLE subgroups

Indicators	Subgroup cutoff value	Count(%)	DPT median [5%~95% interval]	Mann-Whitney U test	
ANA	<1:1000	81(46.29%)	0.60 [0.10-3.16]	Z=2.140	P=0.032
	≥1:1000	94(53.71%)	0.40 [0.10-2.35]		
anti-dsDNA	-	140(80.00%)	0.50 [0.1-2.79]	Z=3.469	P=0.001
	+	35(20.00%)	0.30 [0-1.12]		
anti-rib-P	-	113(64.57%)	0.50 [0.1-3.35]	Z=2.067	P=0.039
	+	62(35.43%)	0.40 [0.02-1.37]		
anti-RNP	-	100(57.14%)	0.60 [0.10-2.79]	Z=2.374	P=0.018
	+	75(42.86%)	0.40 [0.10-2.52]		
anti-SSA	-	71(40.57%)	0.60[0.10-2.54]	Z=2.300	P=0.021
	+	104(59.43%)	0.40 [0.03-2.75]		
C3	<0.70	88(50.29%)	0.40 [0.05-1.90]	Z=3.824	P=0.000
	≥0.70	87(49.71%)	0.60 [0.10-3.82]		
C4	<0.14	79(45.14%)	0.40 [0.10-1.90]	Z=2.760	P=0.006
	≥0.14	96(54.86%)	0.50 [0.10-3.28]		

E-111

Comparision of intracellular localization and recycling route of mouse nepmucin and CD31 in endothelial cells

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Objective Mouse nepmucin, a novel adhesion molecule containing a classical mucin-like domain and a V-type Ig domain, expressed extensively in small vascular endothelial cells of peripheral lymph tissues and parenchymatous organs, but not in Peyer's patches (PPs). Nepmucin can bind to L-selectin, a crucial molecule for lymphocyte homing, and mediate L-selectin-depedent lymphocyte rolling, adhesion, and promote transendothelial migration (TEM) via its mucin-like domain and Ig domain. CD31/PECAM-1 molecule, a trans-membrane protein, was widely expressed on all vascular endothelial cells. It underwent recycling in endothelial cells and promote lymphocytes TEM. Nepmucin has similar distribution and also process recycling as CD31 molecule in LN HEVs. However, it remains unclear whether they share the same intracellular localization and recycling pathway. To address this issue, we compared the localization and recycling between nepmucin and CD31 on transfected endothelial cells (WT-F2 cells), and attempted to clarify the recycling mechanisms of nepmucin in endothelial cells.

Methods Recycling assay and internalization assay were employed to compare the localization and recycling pathway of nepmucin and CD31. To determine the distribution of recycling nepmucin and CD31 molecules in endothelial cells, WT-F2 cells mounted on slides were incubated with anti-nepmucin mAb (ZAQ5) or anti-CD31 mAb at 37°C for 1 h. After blocked by anti-rat IgG, the slides were incubated with Alexa488-anti-rat IgG for certain time periods (0, 15, 30, 45, 60 and 75 min) and observed under a confocal microscope. To detect the distribution of nepmucin and CD31 entered in endothelial cells. WT-F2 cells were incubated with Alexa-488-ZAQ5 or Alexa-594-anti CD31 mAb at 4°C for 30 min, then transferred to a 37°C, 5%CO_2 incubator for another 0, 30, 45, 60 and 75 min. The distribution of nepmucin and CD31 was analyzed by a confocal microscope.

Results CD31 molecules were found to recycle to the membrane from 15 min after incubation, while only few nepmucin molecules were observed at 30 min. The recycling rate of nepmucin was significantly lower than CD31. The CD31 and nepmucin molecules showed largely distinct localization in endothelial cells. CD31 was found mainly on the cell surface and also near the cell membrane. In contrast, nepmucin was found predominantly around the nucleus, and partly on the cell membrane.

Conclusion The distribution of mouse nepmucin in endothelial cells was distinct from CD31. Nepmucin underwent intracellular recycling like CD31 but may employed different mechanism(s). These results indicate that nepmucin carry out its biological function via a way different from CD31.

E-112

Association between pre-miRNA polymorphisms and chronic inflammation in rheumatoid arthritis in the Chinese Han population

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Objectives: The association between the single nucleotide polymorphisms (SNPs) in pre-miRNA and the chronic inflammation of rheumatoid arthritis (RA) is completely unknown. The aim of this study was to detect the association between the two single nucleotide polymorphisms (SNPs) --rs2910164 G>C and rs3746444 T>C in pre-miRNA (hsa-mir-146a and hsa-mir-499) and the chronic inflammation in the Chinese Han population with rheumatoid arthritis (RA).

Methods: 200 Han Chinese patients with RA were recruited in this study. The SNPs was genotyped by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). C-reactive protein (CRP) was measured by rate nephelometry. Erythrocyte sedimentation rate (ESR) was measured by photometrical capillary stopped flow kinetic analysis. The plasma concentrations of IL-6, TNF-α and TGF-β1 were measured by enzyme linked immunosorbent assay.

Results: There was a significant difference in the levels of CRP and ESR between different genotypes in rs3746444 (P=0.044 and P=0.036, respectively). The heterozygote CT had significantly higher levels of CRP and ESR compared with homozygote CC and TT. No significant associations was found between the SNP rs2910164 and the levels of CRP, ESR, IL-6, TNF-α and TGF-β1 (all P>0.05). There was no significant difference in the distribution of genotype in rs2910164 between active group and inactive group (P=0.291). However, as for the distribution of genotype in rs3746444, a significant difference was observed between the two groups (P=0.029). Genotype CT in active group was more common compared to the inactive group. (Fig. 1)

Conclusions: The results of this study show that the SNP rs3746444 in pre-miR-499 could affect the inflammatory reaction in patients of RA. The results can be used in the clinical assessment of inflammatory activity, which in turn may influence the therapeutic decision making.

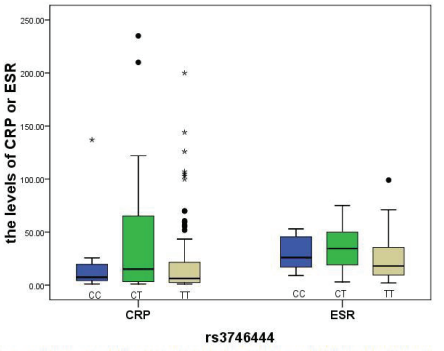

Fig. 1 Analysis of serum CRP and ESR level between different genotypes in rs3746444.

E-113

Evaluation of a Haptoglobin assay for use on the Binding Site SPA PLUS turbidimetric analyser

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Haptoglobin is an acid α2 acute-phase plasma glycoprotein and binds specifically to free plasma haemoglobin released during erythrocyte lysis. The high molecular weight haptoglobin/haemoglobin complex prevents filtering of haemoglobin by the kidneys. Low levels of haptoglobin are associated with haemolytic anaemias and liver disease. Here we describe the evaluation of a Haptoglobin assay for use on the SPA PLUS analyser, a small bench-top turbidimeter available from The Binding Site Group Ltd. The instrument is an automated, random access analyser with host interface capability, primary sample ID and reagent management systems. Precision is promoted through a combination of air-pressure reagent mixing and acid /alkali cuvette washing. The analyser was programmed to construct a calibration curve from

a six point, serum based calibration set. The calibrator set was standardised against the DA470k international reference material. The standard curves were validated by assay of control fluids supplied with the kit. Samples were initially measured at a 1/10 sample dilution and, if out of range, the instrument automatically re-measured the sample at an alternative dilution. All dilutions were made with the instrument's on-board pipetting system, which was able to make dilutions between neat and 1/100. The assay took 10 minutes and was read at end-point. The assay range was 0.26 - 4.0g/L using a 1/10 sample dilution, with a sensitivity of 0.026g/L when using neat sample. Analytical sensitivity was assessed by running sixty replicates of a blank sample and the lowest calibrator. Two distinct sets of data were generated with coefficients of variation of 1.9% and 1.0% respectively. Precision studies (CLSI EP5-A2) were performed at three levels in duplicate over 21 working days. Antigen levels of 3.4, 1.9 and 0.5g/L were assessed for total, within-run, between-run and between-day precision, using three different reagent lots on three analysers. The coefficients of variation were 3.7%, 2.1%, 2.6% and 1.8% for the high sample, 3.0%, 1.3%, 1.9% and 1.8% for the medium sample and 5.5%, 1.7% 2.4% and 4.7% for the low sample respectively. To assess assay linearity, serially diluted serum samples were measured and expected results were compared with actual results. The assay showed a high degree of linearity when expected values were regressed against measured values (y=1.04x + 0.0016, R^2=0.9967). No significant interference (within 10%) was observed on addition of bilirubin (200mg/L) or Chyle (1500 formazine turbidity units). Comparison was made between this assay and the Siemens Haptoglobin assay for the BN™II over a range of 0.26g/L - 8.3g/L. Good agreement was demonstrated: y=0.95x - 0.01 (r=0.983, n=59). We conclude that this assay measures haptoglobin precisely, accurately and rapidly, and may be of use in laboratories where a large instrument is not appropriate.

E-114

Evaluation of a latex-enhanced C-Reactive Protein assay for use on the Binding Site SPA PLUS turbidimetric analyser

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C-Reactive protein (CRP) is a 105 kDa member of the pentaxin family of proteins. It is synthesised in the liver and constitutive levels are low. It is reliably used as an acute phase indicator and concentrations rapidly increase during inflammation, with elevated levels being detectable after 6 hours. Here we describe development of a CRP assay for use on the SPA PLUS, a small, bench-top turbidimeter available from the Binding Site. The instrument is an automated, random-access analyser with host interface capability, primary sample ID and reagent management systems. Calibration curves are created from calibrator sets and validated by assay of control fluids supplied with the kits. The calibrator set was standardised against the ERM-DA472/IFCC international reference material - a newly issued, liquid stable standard produced to correct for a 20% loss of CRP observed upon freeze-drying in CRM470. Samples were initially measured at a 1/30 dilution and, if out of range, the instrument automatically re-measured the samples at an alternative dilution. All dilutions were made with the instrument's on-board pipetting system, which was able to make dilutions between neat and 1/100. The assay took 10 minutes and was read at end-point. The assay range was 4.2 - 300 mg/L using a 1/30 sample dilution, with a sensitivity of 0.014 mg/L using neat sample. Precision studies (CLSI EP5-A2) were performed at three levels in duplicate over 21 working days. Serum containing three different concentrations of CRP (205, 98 and 12mg/L) were assessed for total, within-run, between-run and between-day precision, using three different reagent lots on three analysers. The coefficients of variation were 6.5%, 2.0%, 3.2% and 5.3% for the high sample, 6.8%, 1.7%, 1.4% and 6.4% for the medium sample and 8.6%, 4.0% 2.1% and 7.3% for the low sample respectively. Linearity was assessed by assay of diluted serum samples and comparison of expected with measured **Results:** - CRP: y= 0.98x-4.8mg/L, R^2=0.9965. No significant interference (within ±10%) was observed with hemoglobin (5g/L), bilirubin (200mg/L) or Chyle (1500 FTU's). Comparison was made between this assay and Siemens cardiophase® hsCRP assay for the BNII™ using normal and clinical samples. Good agreement was demonstrated y=1.29 - 0.06 mg/L, R^2=0.998 (n=323). The difference in slope observed is largely due to the documented 20% difference between the new DA472 international standard to which the SPA PLUS assay is calibrated, and the CRM470 standard to which the Siemens assay is currently calibrated. We conclude that the CRP assay for the SPA PLUS measures CRP precisely, accurately, and rapidly and may be of use in laboratories where a large instrument is not appropriate.

E-115

The regulatory effect of calcineurine inhibitors on peripheral regulatory T cells and inhibitory costimulators in allo-renal recipients

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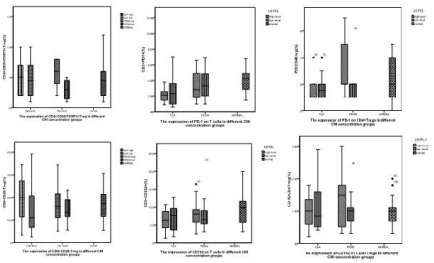
Objective: To explore the regulatory effect of FK506 and CsA on the expression of peripheral regulatory T cells (Tregs) and inhibitory costimulators CD152 and PD-1 on T cells and Tregs and the role of the effect in the induction and maitenance of transplant tolerance in allo-renal recipients.

Methods: The expression of CD4+CD25+FOXP3+Treg, CD8+CD28-Treg, CD152 and PD-1 in peripheral blood of stable allo-renal recipients receiving FK506 or CsA treatment was analyzed by flowcytometry. The pre-dose blood concentration of FK506 or CsA was detected by automatic analyzer (V-TWIN, SIEMENS).

Results: The expression of CD4+CD25+FOXP3+Treg in both FK506-treated and CsA-treated group were essentially normal(P>0.05); the expression of CD8+CD28-Treg remained normal in CsA treatment group but decreased a little in FK506 treated group (P>0.05). Compared with healthy controls, the expression of CD152 and PD-1 on peripheral T cells decreased, and the decrease in the CsA treated group was more significant (P<0.05). On CD4+CD25+FOXP3+Treg , the expression of CD152 and PD-1 in FK506 treated group were normal (P>0.05); and the expression of CD152 also remained normal (P>0.05) while the expression of PD-1 decreased significantly (P<0.05) in CsA treated group. The expressions of CD4+CD25+FOXP3+Treg and CD8+CD28-Treg, CD152 and PD-1 on T cells and PD-1 on CD4+CD25+FOXP3+Treg in allo-renal recipients with high FK506/CsA pre-dose blood concentrations (FK506>6ng/Ml, CsA>80ng/mL) were higher than in recipients with low FK506/CsA pre-dose blood concentrations (FK506≤6ng/mL,CsA≤80ng/mL).

Conclusion: The regulatory effect on Tregs and inhibitory costimulators are different between FK506 and CsA. These regulatory effects of CNI are concentration dependent. High level CNI exhibits stronger inhibitory effect on Tregs and inhibitory costimulators, which goes against the induction and maintenance of transplant tolerance.



E-116

Evaluation of the Bio-Rad BioPlex 2200 MMRV Multiplex Flow Immunoassay for the Detection of IgG-Class Antibodies to Measles, Mumps, Rubella, and Varicella-zoster virus

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Background: Vaccinations against measles, mumps, rubella and varicella zoster virus (MMRV) protect non-immune individuals from acquiring these infectious diseases. Healthcare workers and children are often required to have known immunity to these viruses and serologic evidence of immune status (positive IgG) is often used when documentation and medical history are not available. Serologic testing for MMRV IgG that is traditionally performed as single-analyte testing by enzyme immunoassay (EIA), chemiluminescent (CI) or immunofluorescence assay are now available as a consolidated multiplex flow immunoassay. This FDA-cleared, BioPlex 2200 MMRV IgG test (Bio-Rad Laboratories, Hercules, CA) is intended for the qualitative detection of IgG antibodies to MMRV in human serum and EDTA or heparinized plasma. This study evaluated the performance of the BioPlex 2200 MMRV IgG kit compared to the predicate FDA-cleared EIA assays to detect IgG antibodies for measles, mumps and VZV (Bio-Rad) and the FDA-cleared CI assay for Rubella IgG antibody (Beckman Coulter, Fullerton, CA) using 314 non-clinically characterized serum specimens submitted to our laboratory for routine testing.

Methods: All samples were tested prospectively by each of the predicate methods and using the BioPlex 2200 MMRV IgG kit. Discordant results were retested, with further discrepancies being arbitrated by additional referral testing with the respective FDA-cleared SeraQuest (Quest International, Doral, FL) IgG EIA assays for measles, mumps and VZV or the IMMULITE 2000 Immunoassay System Rubella Quantitative IgG (Siemens Healthcare Diagnostics, Deerfield, IL). Positivity was defined as requiring agreement between 2 of the 3 different serology tests used to detect IgG for MMRV, respectively. Testing was performed with approval of the institutional review board.

Results: Equivocal results were excluded from the analysis. Compared to the predicate serology testing for MMRV IgG, the BioPlex 2200 MMRV IgG test demonstrated an overall agreement of: 97.3% (CI 94.8 to 98.6%) for measles IgG, 95.9% (93.0 to 97.7) mumps IgG, 99.7% (98.1 to 99.9%) for rubella IgG and 95.1% (CI 92.1 to 97.0%) for VZV IgG. The positive/negative agreement for MMRV IgG was: 97.3/100%. 96.5/91.4%, 100/95.5%, and 94.9/100%, respectively.

Conclusion: These results demonstrate that the BioPlex 2200 MMRV IgG multiplex assay shows similar agreement with predicate serology testing for MMRV IgG. Concordance between serology testing exceeded the performance characteristics described in the package insert (665-0539A) for results obtained with prospective comparative testing. Use of the multiplex BioPlex 2200 MMRV IgG test allows for the simultaneous detection of MMRV IgG, potentially decreasing cost, turnaround time, and sample volume requirements.

E-117

Up-regulated expression of RANKL on T cells in pro-inflammatory microenvironment of rheumatoid arthritis

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Background: Rheumatoid arthritis (RA) is a chronic autoimmune disease with persistent inflammation of multiple synovial joints, which results in progressive destruction of bone and cartilage. Bone destruction is caused by mature osteoclasts differentiated from precursor cells, and RANKL is an important mediator that promotes differentiation of osteoclasts through interacting with RANK on osteoclast precursor cells. As some inflammatory cytokines can induce bone destruction by up-regulating RANKL, we aim to explore the change of the expression of RANKL and concentrations of three inflammatory cytokines IL-17A, TNF-α and IL-23 in peripheral blood of RA patients.

Methods: The expression of RANKL on peripheral blood T cell subsets was determined by flow cytometry, while the concentrations of IL-17A,TNF-α and IL-23 were evaluated with ELISA.

Results: The expression of RANKL on $CD3^+$T cells increased significantly in both active and inactive RA groups than that of healthy control group ($P<0.05$), while the expression of RANKL on $CD3^+CD4^+$T cells were just a little higher in all RA patients than that of healthy controls ($P>0.05$). However, the expression of RANKL on $CD3^+CD8^+$T cells increased significantly in active RA group than that of inactive RA group as well as control group ($P<0.05$) (Fig.1). In addition, plasma concentrations of IL-17A, TNF-α and IL-23 in active and inactive RA groups were significantly higher as compared with controls ($P<0.01$), and the differences of all these cytokines concentrations were not significant between two RA groups ($P>0.05$).

Conclusion: The elevated concentrations of IL-17A,TNF-α and IL-23 in plasma lead to the development of an pro-inflammatory microenvironment, which may induce the up-regulated expression of RANKL on peripheral T cells. This phenomenon is one of possible explanations that T cells induce bone destruction, which process is more efficiently augmented in inflammatory cytokines-abundant intra-articular environment of RA.

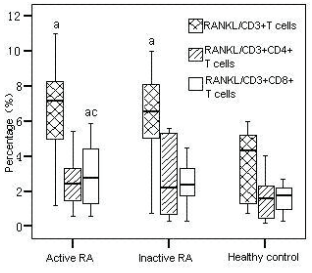

Fig.1 Expression analysis of RANKL on T cell subsets from RA patients and healthy controls. aP<0.05 vs health controls.cP<0.05 vs Inactive RA.

E-119

Laboratory Persistence of Small Monoclonal Gammopathies

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Background Monoclonal gammopathies often present with quantifiable M-spike on serum protein electrophoresis (SPEP). Significant diseases, however, may present with small, non-quantifiable abnormalities that appear only as restricted patterns of migration on SPEP but are confirmed as monoclonal proteins by immunofixation electrophoresis (IFE). Monoclonal gammopathy of undetermined significance (MGUS) is the most common plasma cell proliferation disorder and often presents with no quantifiable M-spike. These can be termed IFE MGUS.

Methods To determine the significance of IFE MGUS, we queried our dysproteinemia database (all Mayo Clinic Rochester patients with a plasma cell proliferative disorder) for patients whose initial MGUS diagnosis was based on a monoclonal protein that was detectable on IFE but not quantifiable by SPEP. Patients known myeloma, amyloidosis, and light chain deposition disease were excluded. Follow-up data was collected on those patients who had a second SPEP and IFE at least 1 month after initial diagnosis. We then looked at the laboratory course in terms of the IFE MGUS persistence and documented any hematological disease progression.

Results: The database query yielded a total of 1827 patients who had IFE MGUS on their first assessment. From these, 501 (27%) patients had follow-up laboratory data (average follow-up 1237 days) which included IFE. The majority (88%) of the monoclonal proteins were detected in a normal background, while 8% were detected in a polyclonal hypergammaglobulin background, and a remaining 4% detected in a hypogammaglobulin background. The distribution of immunoglobulin heavy chains in these patients (IgG, 59%; IgA, 20%; and IgM, 21%) was different from that reported for all MGUS patients. Notably, our population was enriched for IgA and IgM. The majority of the M-proteins persisted (67%) while the remaining either disappeared (29%) or had a variable course (4%) with inconsistent results. In all, 90% of IgA and 70 % of IgM IFE M-proteins persisted while only 35% of IgG IFE M-proteins persisted. Small M-proteins presenting in hypergammaglobulin backgrounds were approximately 2.5 times more likely to disappear than those presenting in normal backgrounds. A total of 14 (2.8%) patients progressed clinically to a hematologic disease at a median of 2.9 years. As in the persistence data, this group was over-represented by the IgA class. Eight of the 14 patients who had clinical progression had an IgA IFE M-protein while none had an IgM IFE M-protein.

Conclusions The results of this study demonstrate that the majority of IgG IFE MGUS are transient, but the majority of non-IgG IFE MGUS will persist. IgA IFE MGUS have the highest probability of progression. Given the overall persistence and progression, these patients should be followed as low risk MGUS patients.

E-120

Effectiveness of allergen panel versus single allergen testing in detection of allergy specific IgE

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Background: Allergies are a common chronic disease, with more than 50 million Americans being affected. In vitro serum testing for IgE antibodies provides identification of allergen(s) that may be associated with allergic disease. Testing is performed on single allergens or panels that include a mixture of 3-6 allergens. Panels are typically used as screens, with the expectation that follow-up with single allergen testing is performed when indicated. When results of the screening panel are negative additional testing is likely not necessary. However, results in the equivocal range can create confusion as to the best way to follow-up these patients.

Objectives: To assess the correlation between results in the screening panel and the single allergen tests and to determine the optimal reflex strategy based on screening panel results.

Methods: Two allergen panels were selected for the study: the mold panel (mx2) (MP) (penicillum notatum, cladosporium herbarum, aspergillus fumigatus, candida albicans, alternaria alternate, and helminthosporium halodes) and the nut panel (fx1) (NP) (peanut, hazelnut, brazil nut, almond, and coconut). These were chosen based on high test volumes (MP~4200/yr and NP ~1100/yr) and the percentage of positive results seen over three years (MP=4.4% and NP=20.8%). Serum samples were analyzed on an ImmunoCAP 1000 system (Phadia) and included 138 MP (52 negatives) and 53 NP (17 negatives) samples. Results were reported as a quantity (kU/L) and a class (negative, equivocal, positive, and strongly positive). For each sample, both the panel and all single allergen tests were run following manufacturers' protocols.

Results: For both multi-allergen panels, negative results predicted the absence of allergen-specific IgE based on single allergen follow-up testing. Only one false negative was identified in the NP and none in the MP. Positive results on the panels also agreed with follow-up, giving no equivocal or negative results on single-allergen IgE testing. Equivocal results, however, were not reliable in predicting the same in follow-up. For the MP, 6/13 (46%) were positive with follow-up. This appeared due to an overall tendency for the MP to be lower than single-allergen IgE testing, as evidenced by the movement of 10/64 (16%) positives to strongly positive. For the NP, 1/7 (14%) equivocal moved to positive and 4/7 (57%) moved to negative on follow-up testing. In contrast to the MP, NP results trended higher than follow-up where 2/7 (29%) strongly positives changed to positive. For both panels, sensitivity as a screen was increased treating equivocal as a positive (MP=100%, NP=97%) rather than a negative (MP=92%, NP=94%) and suggests that the appropriate reflex for these samples is to perform all relevant single allergen tests.

Conclusion: Allergen panels are a cost-effective option for screening related allergens (i.e. mold or nut allergies). The optimal strategy would begin with an allergen panel, reflexing all equivocal or positive results to relevant single allergen IgEs. As observed with the MP, a significant number of equivocal samples were classified as positive upon follow-up testing. Failure to follow-up on these reduces the sensitivity of the panels as a screen.

E-121

Evaluation of assays for the measurement of IgA and IgM in Cerebrospinal Fluid on the Binding Site SPA PLUS turbidimetric analyser

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Measurement of cerebrospinal fluid (CSF) protein concentrations can be useful in the assessment of local immune response in the central nervous system (CNS). Increased levels of immunoglobulin present in the CSF can be a positive indication for intrathecal synthesis. Quantification of CSF IgA and IgM in conjunction with other clinical findings can be especially useful in the diagnosis of bacterial and viral CNS infections. Here we describe the evaluation of IgA and IgM CSF assays for use on the SPA PLUS analyser, a small bench-top turbidimeter available from The Binding Site Group Ltd. The instrument is an automated, random access analyser with host interface capability, primary sample ID and reagent management systems. The analyser was programmed to construct a calibration curve from a six point, serum based calibration set. The standard curves were validated by assay of control fluids. All dilutions were made with the instrument's on-board pipetting system, which was able to make dilutions between neat and 1/100. The main assay characteristics are summarised in the table below:

Assay	IgA			IgM		
Range (mg/L)	1.4 - 44.1			0.3 - 7.0		
Sample dilution	1/10			1/1		
Minimum sample dilution	1/1			1/1		
Sensitivity (mg/L)	0.14			0.3		
Intra-assay and inter-assay precision %CV (Mean)		Intra-assay	Inter-assay		Intra-assay	Inter-assay
	40.5 mg/L	1.8%	2.3%	5.5 mg/L	1.7%	3.4%
	2.5 mg/L	3.7%	0.6%	0.8 mg/L	1.4%	5.5%

No significant interference (within 4%) was observed on addition of bilirubin (200mg/L) or haemoglobin (5g/L) to CSF samples with known concentrations. The assays showed a high degree of linearity when expected values were regressed against measured values of a serially diluted CSF sample:- IgA: y= 1.01x - 0.014mg/L, R^2=1.00; IgM: y= 0.985x - 0.280mg/L, R^2=0.99. Comparison was made using the Siemens IgA and IgM assays for the BN™II. Good agreement was demonstrated:- IgA: y=0.98x -0.004mg/L, R^2=0.988 (n=45); IgM: y=1.06x -0.08mg/L, R^2=0.996 (n=13. 37 additional samples were too low to be quantified by either assay and could not be correlated). We conclude that the IgA and IgM CSF assays for the SPA PLUS are rapid and precise, and may be of use in laboratories were a large instrument may not be appropriate.

E-123

Monitoring of serum free light chains in the treatment of light chain deposition disease with high cut-off haemodialysis

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Introduction: Light chain deposition disease (LCDD) is a rare entity where serum free light chains (sFLCs) are precipitated on the basement membranes of cells in the kidney and other organs. The severe renal failure is the common presenting feature. This disease hasn´t a clearly defined treatment and the patients have an adverse prognosis. We present the case of a patient affected of LCDD with severe renal failure and treated by chemotherapy and sFLCs haemodialysis with Theralite™ High Cut-Off technology. This new technology enable us an efficient and direct removal of sFLCs.

Case presentation: A 57 years old man was admitted to the hospital due to very intense bone pain. He was diagnosted with LCDD. The k/λ FLCs ratio was altered (43.00). The serum kappa free light chain (FLC) was 289.90 mg/l and the serum lambda FLC was 6.74 mg/l. During the progress, the intense bone pain improved but it was detected an acute renal failure (creatinine was 9.70 mg/dl) in the context of LCDD. The patient began treatment with Bortezomid and Haemodialysis (over a six hour period during six days) with high cut-off membrane (Theralite™) in order to remove FLCs in serum. We measured pre- and post-haemodialysis FLCs, the k/λ FLCs ratio and the creatinine in serum. The percent of serum kappa FLC removal was 60.80% and the levels of serum kappa FLC decreased from 90.13 mg/dl to 59.70 mg/dl during the treatment. The percent of k/λ FLCs ratio reduction was 57.38% (from 11.05 to 6.34 during the treatment). This treatment produced a improvement in the patient´s renal function with a decrease of 88.14% in the creatinine serum levels (from 5.90 mg/dl to 0.70 mg/dl). At present, the patient is in complete remission and he has undergone an autologous transplant.

Conclusion: A combination of the efficient and direct removal of the toxic excess of serum FLCs using high cut-off membrane (Theralite™) with effective chemotherapy with Bortezomid allow us to reduce the serum FLCs levels. The determination of the rate of reduction of FLCs with Freelite™ (The Binding Site) is a useful tool that allow us the accurate and rapid monitoring of serum FLCs levels to guarantee an optimum treatment of the patient.

E-124

Novel flash and glow 1,2-dioxetane chemiluminescent substrates and their application for clinical diagnostics platforms

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Background: Clinical diagnostic platforms rely heavily on luminescent substrate technologies as detection reagents. Direct chemiluminescent labels, such as acridinium esters or isoluminol, provide the shortest detection times due to the flash chemiluminescent signal generated immediately after addition of a second triggering solution. Enzyme-linked chemiluminescent substrates, including 1,2-dioxetanes or luminol, have longer substrate incubation times but benefit from enzyme signal amplification to provide ultrasensitive detection and also have greater substrate reagent stability. We have developed three new 1,2-dioxetane chemiluminescent alkaline phosphatase substrate molecules with the goal of providing faster assay kinetics to allow for read detection immediately after substrate addition and also providing a sustained maximum signal lasting over many hours. Each substrate reaches maximum signal within 3 minutes of substrate addition at 37 degree C and maximum glow persists for up to at least 1 hour.

Methods: Each substrate has been optimized for substrate and enhancer formulation and these formulations have been validated in model systems using both microtiter plates and Dynabeads® magnetic beads as solid phases. We have characterized each of these new substrates for assay performance based on a combination of signal intensity, background signal, enzyme kinetics and thermal reagent storage stability_all metrics that contribute to the assay performance parameters of sensitivity, dynamic range, precision and stability.

Results: Each of the flash and glow substrates described here can detect low attomole amounts of purified alkaline phosphatase. Subtle differences in the performance characteristics of each of these three novel substrates have been observed. For instance substrate B reaches maximum signal within 2 minutes compared with substrate C which reaches maximum signal immediately at 37 degree C, while substrate B attains the highest overall signal intensity when compared to the other substrates. We have also characterized these substrates in several ELISA model systems. Assay

sensitivity of a recombinant human IL-6 with these new substrates in a microplate ELISA model assay, as well as assay performance using several Dynabead® bead formats, will be presented. The glow signal can especially improve the assay precision of ELISAs performed in microtiter plates. Performance of these new flash and glow chemiluminescent substrates in comparison to other chemiluminescent substrate technologies will be shown. Ongoing accelerated and real-time stability studies predict shelf-life stability of at least one year at 2-8 degree C.

Conclusions: These new chemiluminescent substrates exhibit a combination of the greatest characteristics of both the direct label and enzyme linked chemiluminescent substrate detection systems. Each of these substrates has a fast signal ramp-up time rivaling that of traditional "flash" substrates, while maintaining the superior sensitivity, glow kinetics and substrate reagent stability typical of 1,2-dioxetane substrates. Assays using these substrates can be read within minutes after substrate addition providing new options for detection platforms requiring early read times and sensitive detection.

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E-125

New free light chain assays based on monolonal antibodies, a Performance Evaluation report

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Background: The International Myeloma Working Group has provided consensus guidelines for the use of immunoglobulin free light chain determinations in the diagnosis and management of clonal plasma cell disorders and emphasises their important role in today's Myeloma diagnostics. We herewith describe preliminary precision, comparability, lot to lot variation and patient case specific performance data of two new immunoassays for the detection of free Ig-light chains type kappa and lambda in serum and plasma. Both methods utilize latex-enhanced mouse monoclonal antibody reagents and are designed to be used on Siemens BN™ systems.

Methods: *Free Ig-light chain type kappa (N Latex FLC kappa), *Free Ig-light chain type lambda (N Latex FLC lambda).

Results: Performance evaluation studies were conducted at two sites. Site A used a BN™ II system and Site B used a BN ProSpec® analyzer. ANOVA studies according to CLSI guideline EP5-A2 were carried out to estimate precision performance using at least two sample pools and two control level each. Initial repeatability results for both methods at Site A expressed as min-max/median %CV were 2.1% - 4.3% / 3.2% and 1.1% - 3.5% / 1.8% at Site B. Within-laboratory results were 2.4% - 4.8% / 3.3% at Site A and 1.3% - 4.8% / 2.0% at Site B. Comparability studies were performed versus other commercially available FLC methods for the BN systems. Site A analyzed 213 samples for kappa and lambda and revealed agreement rates of 85.3% for kappa, 78.0% for lambda and 91.4% for κ/λ ratio. The results at Site B based on 86 samples were 88.9%, 82.6% and 95.3% respectively. Lot to lot comparison studies at Site A for two lots of kappa and lambda each revealed excellent agreements. Passing-Bablok regressions were y = 1.03x - 0.32 for kappa and 1.06x + 0.07 for lambda with Pearson correlation coefficients of r = 1.00 and r= 0.99 respectively. At Site B a preliminary evaluation of a myeloma patient type lambda undergoing dialysis using Gambro™ filter technique was conducted. Over a period of 14 days FLC determinations using the new N Latex FLC lambda reagent and another commercially available nephelometric FLC lambda method were performed twice a day before and after dialysis together with quantitative protein electrophoresis. The N Latex FLC lambda results were comparable with the quantitative results of the protein electrophoresis whereas the other FLC lambda method revealed results which were higher by a factor of 2 to 3.

Conclusion: The new FLC methods performed well under routine laboratory conditions on both BN platforms as stated by the evaluation sites.

*under development -not available for sale in the U.S.

E-126

Evaluation of a Commercial Multiplex Bead-Based Assay to Quantify Serotype Specific IgG Against Streptococcus Pneumoniae Polysaccharides in Clinical Specimens

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Background: Validation of a multiplex assay for clinical applications can be challenging. In this study, we validated a commercially available 14-plex bead-based assay for quantification of serotype-specific IgG against streptococcus pneumoniae polysaccharides in human serum. Streptococcus pneumoniae accounts for roughly 66% of diagnosed pneumonia and is the major cause of acute bacterial meningitis, acute bacterial sinusitis and acute otitis media. Host defense primarily depends on the ability to generate antibodies against the polysaccharide capsule of the bacterium. Detection of antibodies to capsular polysaccharides (Ps) is used clinically for vaccine status evaluation and immune function testing. **Methods:** The Pneumo 14 assay kit (Luminex Corp, Austin, TX), which quantitatively measures IgG levels against Ps serotypes 1, 3, 4, 8, 9N, 12F, 14, 19F, 23F, 6B, 7F, 18C, 19A and 9V, was evaluated in terms of accuracy, precision, sensitivity and linearity. Accuracy of the assay was assessed by testing the FDA standard reference serum lot 89SF as well as comparing it with in-house assays currently used by two large reference laboratories. Twenty pairs of pre- and post-vaccination (23-valent vaccine) serums and sixty two random serum specimens were used in the validation study .

Results: The imprecision (CV of total ten runs on five different days using kits from two lots) ranged from 9.5% to 24.9% for the 14 serotypes at low, mid and high levels of Ps IgG. Assay results of the FDA reference serum matched the assigned values for all nine serotypes with officially assigned values (9V, 14, 18C, 19F, 1, 3, 4, 6B, 7F). Percent recovery was within 100±20%. An acceptable correlation (R>0.7) with the two reference laboratories was obtained with Ps IgG levels ranging from 0.2μg/mL to higher than 20μg/mL for all serotypes, except 3 and 19F. The capsular Ps IgG values were further classified as "protective" (higher than 1.3μg/mL) or "not protective" (equal to or less than 1.3μg/mL) according to a commonly adopted cutoff value cited in the literature. The percentage of agreement for each serotype was higher than 70% when compared to both reference laboratories except 7F, which showed 62% agreement with one reference lab. Based on the ratio of post to pre vaccination Ps IgG levels, the paired specimens were classified as responders (with a ratio higher than 2.0 for at least 7 serotypes) or non-responders. The percentage of agreement between the Pneumo14 assay and one reference lab method was 100%. The assay was linear for most of the serotypes ranging from 0.2μg/mL to 20μg/mL or higher.

Conclusion: Precision and accuracy varied among serotypes. Although inter laboratory discordance was observed in serotype 3 and 19F, the other twelve serotypes showed good concordance with assays performed in two reference laboratories. In summary, this commercial assay produces reliable and accurate results and is clinically useful.

E-127

Comparison of HLA antibody detection results from the Luminex assays with different antigen composition (screen, identification and single antigen bead assay)

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Background: HLA antibody (Ab) detection and identification results can be different depending on the composition of HLA antigens. The introduction of solid-phase tests using single antigen bead (SAB) has increased the ability to detect unacceptable antigens in sensitized patients. However, many HLA laboratories use SAB assay only if luminex screen test is positive and SAB assay can be affected by technical variation or interference. Therefore, the lack of consensus in HLA Ab test strategy and reporting remains a problem in the transplantation laboratory. We compared the Ab detection and identification results from the luminex based bead assays with different antigen composition. Methods:In 71 sera from pre- or post-kidney transplant patients who had class I or class II HLA Abs by SAB, class I and class II HLA-specific IgG Abs were confirmed by luminex screen test with pooled antigens and luminex identification (ID) test with multiple antigens. The sera showing discrepant results were retested after SeraClean treatment to reduce nonspecific reaction.

Results: When the results reveal the presence or absence of HLA Abs, the concordance rates among the three luminex methods were 69.1% for class I and 72.7% for class II HLA Abs. Of the luminex-screen negative sera, 35.7% for class I and 35.0% for class II showed ID or SAB positive. Of the screen positive sera, 7.3% showed falsely HLA

class II positive results. While HLA Abs with median fluorescent index (MFI) >5000 were concordantly detected as positive results by three assays, much variability was observed in the identification of weak Ab (MFI <5000). In comparison of HLA Ab specificities results from the ID and SAB, 42.3% (30/71) of sera showed discrepancy. Of the 12 ID(+)/SA(-) sera, 6 (50%) were screen-positive and HLA Ab specificities were B72, B82, A31, A24, B61 Ag or multiple DQ antigens. Of the 18 ID(-)/SA(+) sera, 13 (72.2%) were screen-positive and HLA Abs specificities were B62, B44, A11, A1 or multiple DR, DQ antigens. The most HLA Abs showing discrepant results between ID and SA had weak MFI(<1000) or DQ specificity.

Conclusion: Detection of HLA Abs by ID or SAB assays is suggested even in the screen negative cases. As SAB assay alone may not be a reliable means to detect HLA Abs with low MFI values, the combination of currently available technologies will be needed to detect antibody specificities accurately.

E-128

Evaluation of a Prealbumin assay for use on the Binding Site SPA PLUS turbidimetric analyser

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Prealbumin is a 50kDa glycoprotein, the measurement of which is routinely used to assess nutritional status in critically ill patients. Here we describe the evaluation of a Prealbumin assay for use on the SPA PLUS analyser, a small bench-top turbidimeter available from The Binding Site Group Ltd. The instrument is an automated, random-access analyser with host interface capability, primary sample ID and reagent management systems. Precision is promoted through a combination of air pressure reagent mixing and acid/alkali cuvette washing. The analyser was programmed to construct a calibration curve from a six point, serum-based calibration set which was then validated by assay of control fluids. All dilutions were made with the instrument's on-board pipetting system, which was able to make dilutions between neat and 1/100. The main assay characteristics are summarised in the table below:

Assay	Prealbumin
Measuring range (g/L)	0.06 - 0.66
Sample dilution	1/10
Min sample dilution	1/1
Sensitivity (g/L)	0.006
Intra-assay precision %CV	1.2% (0.474 g/L)
(mean) (n=84)	1.7% (0.108 g/L)
Inter-assay precision %CV	1.2% (0.474 g/L)
(mean) (n=84)	1.6% (0.108 g/L)
Between-day precision %CV	2.6% (0.474 g/L)
(mean) (n=84)	5.2% (0.108 g/L)
Total precision %CV	3.1% (0.474 g/L)
(mean) (n=84)	5.7% (0.108 g/L)

No significant interference (within ±10%) was observed upon addition of bilirubin (200mg/L), haemoglobin (5g/L) or chyle (1500 FTU = formazine turbidity units) to samples with known Prealbumin concentrations. Linear regression analysis demonstrated a high degree of linearity of the assay when expected values were compared to measured values of a serially-diluted pool of high samples using the standard 1/10 sample dilution: $y = 0.9884x + 0.0041$g/L, $r^2 = 0.9994$. Comparison was made to the Siemens Prealbumin assay for the BN™II. Excellent agreement was demonstrated by Passing Bablok plot: $y = 0.99x - 0.00$g/L, $r = 0.992$ ($n = 60$). We conclude that the Prealbumin assay for the SPA PLUS is rapid and precise, and may be of use in laboratories where a large instrument may not be appropriate.

E-129

Comparison of Laboratory Methods for Monitoring IgA Myeloma Patients including the New Quantitative Heavy Chain/Light Chain Assay (Hevylite)

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Background. The current most sensitive method for detecting immunoglobulins in serum is the non-quantitative immunofixation electrophoresis (IFE). Other laboratory methods used to monitor myeloma patients include protein electrophoresis (SPEP) or M-spike, free light chain assay (sFLC), quantitative IgG, IgA, and IgM immunoglobulins (QIgs), as well as the new serum heavy chain/light chain (HLC) immunoassay. HLC is a quantitative analysis of intact immunoglobulin heavy chain (IgH) and light chain kappa or lambda. This assay provides concentrations of IgH kappa (k) and IgH lambda (λ) as well as the clinically useful IgHk/IgHλ ratio. The primary aim of this study was to evaluate the IgA HLC assay on the Siemens BNII instrument relative to the other laboratory methods.

Methods. The IgA k/λ HLC reagent kits, provided by The Binding Site, Inc, were evaluated on 108 multiple myeloma patient (69 IFE positive and 39 IFE negative) samples with known sFLC (The Binding Site), and QIgs (Siemens) results using the Siemens BNII instrument. A Sebia CAPILLARYS capillary electrophoresis system was utilized to generate SPEP profiles and utilized in tandem with total protein determination (Beckman-Coulter) to calculate monoclonal protein concentrations when possible. This sample set was obtained from specimens of both inpatients and outpatients previously diagnosed with IgA multiple myeloma, and monitored at the University of Arkansas Myeloma Institute.

Results: The published reference intervals for the HLC assay are; IgAκ 0.48-2.82 g/L, IgAλ 0.36-1.98 g/L and IgAκ/IgAλ ratio is 0.8-2.04. The HLC assay (g/L) showed strong correlation versus the quantitative IgA (g/L) assay ($y = 1.173x + 0.109$, $R^2 = 0.96$) and M-spike (g/L) ($y = 0.963x + 0.995$, $R^2 = 0.96$). Of the 69 IFE positive samples, 88% (61/69) were positive (abnormal) by HLC κ/λ ratio, 71% (49/69) were positive by sFLC κ/λ ratio, 58% (40/69) were abnormal for QIgA assay and M-spike was detected in 68% (47/69). Of the 66 samples positive for an abnormal HLC ratio, 88% (60/66) were positive for IFE, 73% were positive for sFLC κ/λ ratio (48/66), and 71% were positive for SPEP (47/66). All samples positive for SPEP (n=47) exhibited an elevated HLC κ/λ ratio.

Conclusion: In this study, the HLC assay appears to be more sensitive than SPEP for quantifying and detecting intact IgA immunoglobulins. Additionally, the HLC assay was strongly predictive of assayed SPEP monoclonal protein concentration determination. HLC assays can be potentially utilized in the monitoring of myeloma patients by providing numerical results for patients that are IFE positive but not readily followed by serum protein electrophoresis, and through calculation of the clinically beneficial IgAk/IgAλ ratio. Biological variations that may impact electrophoretic evaluation of the immunoglobulin protein should not affect this HLC ratio, for example changes in blood volume, hematocrit and metabolism. In this monitoring population, the cohort of specimens of elevated HLC assays did not perfectly overlap the cohort of positive sFLC κ/λ ratios indicating that the HLC assay may offer complementary analytical advantages to serum free light chain assays in some patients. Further clinical studies are required to fully assess the benefit of this assay in monitoring patients.

E-130

Performance evaluation of Elecsys analysis system for anti-cyclic citrullinated peptide detection in comparison with commercially available ELISA assays in Rheumatoid Arthritis Diagnosis

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Objectives: To evaluate the performance consistency of Electrochemical luminescence with ELISA for anti-cyclic citrullinated peptide (CCP) antibodies detection and define their diagnostic efficacy on Rheumatoid arthritis (RA).

Design and **Methods:** Serum anti-CCP antibodies of 121 subjects (93 RA, 17 non-RA autoimmune diseases and 11 bone inflammatory diseases) were simultaneously determined by Elecsys assay, Immunoscan CCPlus® Euro-diagnostica ELISA kit and Euroimmun anti-CCP ELISA kit.

Results: The consistent rate and Kappa coefficients among three assays were all more than 0.96. The spearman correlation coefficient (r_s) of quantitative results among the three assays were all more than 0.85. AUCs of ROC for Elecsys, Euro-diagnostica ELISA and Euroimmun ELISA assays were 0.790, 0.773 and 0.794, respectively (all of *P* value = *0.000*) (Figure 1), and their accuracy rate of diagnosis under the optimal cutoff values were 74.38%, 72.73% and 71.90%, respectively. High positive likelihood ratios of the three assays show that anti-CCP antibody detection was very useful for RA diagnosis no matter detected by ELISA or Electrochemical luminescence (ECL) on Elecsys analysis system. Under different cutoff values, the discrepant data among three assays would change, and with the cut-off value optimization, the accuracy of Elecsys assay was improved.

Conclusions: Elecsys analysis system is a convenient, quicker and real-time assay for serum anti-CCP antibody detection, which provides shorter turnaround times for clinicians. The diagnostic performance of Elecsys assay for anti-CCP antibodies was satisfactory and comparable to that of classical ELISA. Under the same specificity, Elecsys assay has a higher diagnostic accuracy and sensitivity.

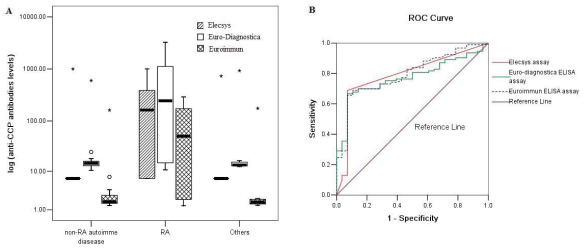

Fig. 1. Quantitative analysis of anti-CCP antibodies detected by Elecsys qassay, Euro-diagnostica ELISA kit and Euroimmun ELISA kit. A, It showed quantitative comparison between three different assays to detect anti-CCP antibodies in patients with RA, non-RA autoimmune diseases and other osteopathy. B, it showed ROC analysis of three different assays. Red full line indicated the AUC of Elecsys assay; Green full line indicated the AUC of Euro-diagnostica ELISA assay; Blue dash line indicated the AUC of Euroimmun ELISA assay. The difference of AUC among three assays was not significant.

E-131

Role of Anti-IgE Quantitation in Characterizing Autoimmune Chronic Urticaria

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Background: Chronic urticaria is a common skin disorder characterized by recurrent pruritic hives and wheals that persist for longer then 6 weeks. An autoimmune etiology is demonstrated in 30-50% of patients. In a majority of cases (30-40%), immune chronic urticaria is associated with antibodies to the high-affinity IgE receptor (FcεRIα) alpha subunit or, less frequently (5-10%) with IgG antibodies to IgE. Both antibodies can cause release of histamine by cross-linking FcεRIα receptor on basophils and mast cells by binding to the receptor or to bound IgE, respectively. Functional IgG antibodies can be demonstrated *in vitro* in the histamine release assay (HRA). Anti-IgE antibody can be detected using solid phase enzyme immunoassay (EIA). The aim of this study was to assess the prevalence of IgG autoantibodies to FcεRIα and IgE in samples from patients undergoing laboratory evaluation for chronic urticaria.

Methods: Two hundred sixty sera submitted for testing in HRA were also tested for presence of anti-IgE antibodies in an EIA developed in house. IgG antibodies specific for IgE were quantified using an omalizumab standard curve and values were expressed in ng IgG/mL serum. To perform HRA, patient sera were incubated with whole blood from normal donors and the amount of histamine released measured in an EIA (Rocky Mountain Diagnostics) according to manufacturer instructions. Results were expressed as % released histamine vs. total.

Results: 65 of 260 sera tested positive in either the HRA, anti-IgE antibody assay, or both. Of the 65 sera positive, 35 (53.8%) were HRA positive only; 22 (33.8%) were positive for anti-IgE antibody only; and 8 (12.3%) were positive in both assays. Of interest, 10.1% of HRA-negative sera were positive for anti-IgE antibody. Of 129 normal control sera tested for anti-IgE antibody, only 2.3% were positive. IgE antibody positivity with a negative HRA may be due to inability of the anti-IgE antibody to cross-link receptor when epitopes on bound IgE are obscured by receptor interactions in the *in vitro* assay. Normal cell donors for *in vitro* assays are selected for absence of atopy and may have low occupancy of FcεRIα by IgE or normal occupancy but a higher threshold for degranulation when cross-linked, or both.

Conclusion: Our data confirm that testing for anti-IgE antibody can aid in the laboratory evaluation of chronic urticaria patients. Addition of IgE antibody to the diagnostic algorithm may identify patients at risk for histamine degranulation not detected by *in vitro* functional assays.

E-132

IgM, but not IgA rheumatoid factor interferes with anti-cardiolipin and anti-β2 glycoprotein I measurements: A quantitative analysis

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Background: : IgM rheumatoid factor (RF) is sometimes referred to as capable of causing interference in the IgM anti-cardiolipin (aCL) testing. Published guidelines are, however, inconsistent, and evidence regarding the interference is limited. Laboratory personnel and clinicians do not have evidence-based information on how to interpret IgM aCL results when RF is present. To clarify this issue, we aimed at investigating IgM and also IgA RF cross-reactivity and/or interference in IgM and IgA aCL and anti-β2 glycoprotein I (aβ2GPI) measurements.

Methods: Serum specimens with high IgM and IgA RF levels were tested for IgG, IgA and IgM aCL and aβ2GPI antibodies to examine cross-reactivity. Samples containing various levels of IgG aCL and aβ2GPI antibodies were spiked with IgM (and IgA) RF, and samples with high RF levels were spiked with IgG aCL antibodies. The mixtures were tested for IgM and IgA aCL and aβ2GPI antibodies, and the bias of the IgM and IgA results was calculated as the difference between the obtained and the expected antibody results.

Results: Specimens with high IgM and IgA RF concentrations did not test positive for IgM or IgA aCL and aβ2GPI antibodies (except one weak positive IgA aβ2GPI result), indicating the lack of cross-reactivity. In the spiked specimens, addition of IgM RF caused significant and dose-dependent positive bias in the measurement of both aCL and aβ2GPI antibodies of IgM isotype in the presence of IgG aCL and aβ2GPI antibodies. In the measurement system that was used in the study, the threshold for triggering significant (> cut-off value of the IgM aCL assay) interference was 318 IU/mL for IgM RF, and 77 GPLU/mL for IgG aCL. Neither IgM, nor IgA RF, however, affected the IgA aCL and aβ2GPI antibody testing.

Conclusion: IgM RF interferes with IgM aCL and aβ2GPI antibody measurements in the presence of IgG aCL antibodies, and can cause false positive IgM aCL results. The mechanism of the interference is probably IgM RF binding to IgG antibodies bound to the solid phase antigen. Interference data (especially the threshold levels for interference) generated by other aCL and aβ2GPI assays may be different from these results, obtained with TheraTest kits. Researchers and manufacturers of antiphospholipid antibody assays are encouraged to obtain data about their own specific test systems to facilitate the interpretation of IgM aCL and aβ2GPI results in IgM RF positive subjects. In studies on the prevalence and clinical significance of IgM antiphospholipid antibodies RF interference should be considered and RF testing should be performed.

E-134

Cellular Immunologic Regulation Mechanism of HBV Recurrence in Liver Transplant Recipients

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Background: To investigate the causes and cellular immunologic regulation mechanism of HBV recurrence in liver transplant recipients.

Methods: A total of 40 liver transplant patients with end-stage liver disease secondary to hepatitis B were enrolled into this study. They were divided into two groups: HBV recurrence group (n=18) and no HBV recurrence group (n=22) after transplantation. CD3, CD4, CD8, CD28, Th1/Th17, Treg(CD4+CD25+Foxp3+) were measured by flowcytometry. The serum levels of IL-18 and IFN-γ, sCD28, sCD152, sCD80, sCD86 were determined by ELISA.

Results: There was no significantly difference in the subpopulations of T lymphocytes (CD3/ CD4/ CD8) between the two groups. The serum levels of the soluble costimulatory molecules, CD80, CD86, CD28, CTLA-4 were not different between the two groups. However, serum level of IFN-γ were lower in HBV recurrence group than no HBV recurrence group ($p<0.01$), but there is no difference in another inflammatory factor IL-18 between the two groups. There was no difference in the two kinds of Tregs (CD8+CD28-Treg and CD4+CD25+Foxp3+Treg) between the two groups, however Th17 were significantly decreased in HBV recurrence group, and they were associated with IFN-γ level.

Conclusion: The soluble costimulatory molecules, CD80, CD86, CD28, CTLA-4 are almost the same in patients with and without HBV recurrence, indicating that they don't have any effect in the recurrence of HBV. The activation of T helper cells are not the key factor in determining the outcome of liver transplantation. Inflammatory factor IFN-γ has important effect in anti HBV immunity. It can enhance the effect of cytotoxic T cells, helping them clear HBV. The balance of Th17/Treg may affect the outcome of liver transplant recipients. Th17 has much more important effect than Treg in HBV recurrence. The decrease of Th17 leads to down-regulation of anti-viral immunity. The decrease of inflammation factors such as IL-17 and IFN-γ limited the effect of cytotoxic T cell, suppressed the process of HBV clearance.

E-135

Intra-Assay Cv As A Tool For Quality Control Assessment In Flow Cytometry Enumeration Of T Lymphocytes Subsets

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Background: Internal quality control assessment in the quantification of subsets of T lymphocytes in Brazil is limited, due to the inexistence of health authority approved commercial kits. Its a common sense among Brazilian quality control auditors that standardized beads used for instrument calibration (as the Beckton and Dickinson

- BD - Calibrite) are not eligible for the assay quality evaluation, since they could not be used for two purposes. So, to monitor the assays and to fulfill the criteria of accreditation authorities, each laboratory need to create and validate its own criteria of acceptance. We proposed to monitor the coefficient of variation (CV) and intra-assay coefficient of variation (CVi).

Methods: We used a two-platform method for enumeration of T cell subsets. Fifty uL of whole blood collected in EDTA tubes was incubated with 20 uL of MultiTest CD3/CD8/CD45/CD4 (BD) fo5 15 minutes in the dark, and lysed with FacsLysing solution (BD) for 15 minutes. Samples were acquired in a BD FACSCalibur cytometer, using Multiset software and lyse-no-wash protocol. Cytometer maintenance program was followed as suggested by BD. We acquired one sample 5 times each day, for 4 consecutive months and analyzed month-by-month. A spreadsheet was created on Microsoft Excell, for calculations and creation of a graph to visual monitoring. Total lymphocyte counts were obtained in a Sysmex XE2100 cell counter. CVi was calculated as the duplicates standard deviations mean divided by the duplicates average mean. CD4 population was identified as CD3+CD4+CD8- cells and CD8 population as CD3+CD4-CD8+ cells.

Results: From September 1st to December 31st 52 samples were studied. CD3 analyses showed a CV variation between 0.5% and 4.14% and the maximum CVi was 1.91%. As predictable, after the 5th day we observed a stability in CVi values, and these values were then never above 1.80. CD4 CV was between 1.02% and 5.51% and the upper CVi was 3.83% after the 5th day each month. For the CD8 population CV values were between 0.60% and 4.60%. The upper CVi value was 2.81%. The difference observed in CD3 CVi and the others is probably because CD3 population is defined in two gates (CD45+ and CD3+) and CD4 and CD8 populations are defined in three gates (CD45+, CD3 + and CD4+ or CD8+), multiplying the variation of each gate.

Conclusions: We observed that CVi is stable in all the subsets studied after the 5th day of tests and so can be used as a tool to assess these assays quality control. We also observed that, despite a great variation in CV values in each population, a maximum could be established and the bigger values were not consecutive. So, we proposed two criteria of quality control violation: CVi higher than the observed in this 4 month period, after the 5th day of tests and; Two consecutive days with CV higher than the observed above. Both criteria are now used in our laboratory to initiate corrective measures

E-136

Lower serum C3 associates with microRNA146a rs2910164 heterozygosity in systemic lupus erythematosus

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Objectives. MicroRNAs(miRNAs) are noncoding RNAs that inhibit the expression of protein-coding genes by either translational repression or messenger RNA degradation. MicroRNA-146a is a negative regulator of innate immunity, and under-expression of microRNA-146a negatively correlated with clinical disease activity in SLE. Single nucleotide polymorphisms (SNPs) may significantly alter miRNA expression and function. Further analysis showed that G/C polymorphism of pre-microRNA146a rs2910164 within the sequence reduced the amount of pre- and mature microRNA-146a in papillary thyroid carcinoma (PTC). GC heterozygotes of rs2910164 differ from GG and CC homozygotes by producing 3 mature microRNA-146a one from the leading strand and two from the passenger strand each with its distinct set of target genes. Here, we hypothesize that mutation of pre-microRNA146a sequence may induce the reduction in miR-146a, leading to less efficient inhibition of target genes involved in the Toll-like receptor and cytokine signaling pathway in SLE.

Methods. The study recruited 213 SLE patients referred to West China Hospital of Sichuan University. We analyzed the genetic polymorphism of hsa-mir-146a rs2910164 G>C by PCR-RFLP, and evaluate the relationship between common laboratory tests and rs2910164 in SLE. Complement (C3 and C4), liver function, renal function and urine protein tests were also performed.

Results. We found that serum C3 in GC heterozygotes of rs2910164 was significantly lower than the GG and CC homozygotes (p=0.009), as shown in Figure 1. Liver function, renal function and urine protein tests were not different among the GG, CC and GC genotypes of rs2910164 in SLE patients.

Conclusions. Lower C3 may be an important risk factor of under-expression of microRNA146a. Thus, our data suggest that polymorphism in pre-miR-146a is associated with C3 concentration, contributing to the genetic predisposition to SLE .

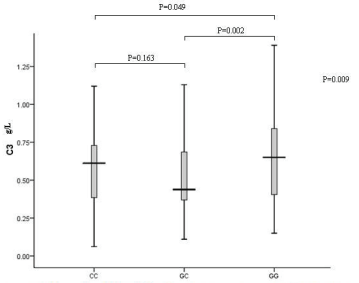

Figure 1. The results of C3 with GG, CC and GC genotypes of rs2910164 in SLE patients.

E-137

Utility of bronchoalveolar lavage findings in the diagnosis of patients with interstitial lung diseases

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Background: Interstitial lung diseases are a group of diseases in which the main pathological changes affect the alveolar structures. The diagnosis is based on clinical symptoms, pulmonary function tests and radiological studies. When we don´t get a diagnosis, invasive tests are used like bronchoalveolar lavage and pulmonary biopsy. The study of the bronchoalveolar lavage fluid in some interstitial lung diseases can reveal typical patterns to each disease that can support the diagnosis. The objective of this study was to perform a descriptive analysis of the cytologic study (lymphocytes, neutrophils, histiocytes and eosinophils) and the lymphocyte subpopulations in bronchoalveolar lavage fluid from patients with interstitial lung disease.

Methods: This is a retrospective study of the bronchoalveolar lavage fluids of 58 patients. The bronchoalveolar lavage fluid was analyzed to determine the distribution of cell populations and the lymphocyte subsets: $CD3^+$, $CD19^+$, $CD4^+$, $CD8^+$, $CD3^+CD4^-CD8^-$, and $CD3^-CD16\&56^+$.The cell populations and the lymphocyte subsets were determined in a FACS Canto II® flow cytometer. Values of cell populations and lymphocytes subsets were given in percentages (%). We studied the following interstitial lung diseases: sarcoidosis (SAR) (n=10), idiopathic pulmonary fibrosis (IPF) (n=12), non-specific interstitial pneumonia (NSIN) (n=20), cryptogenic organizing pneumonia (COP) (n=7), and extrinsic allergic alveolitis (EAA) (n=9).

Results: The distribution of cell populations in bronchoalveolar lavage classified the interstitial lung diseases in three groups. Isolated lymphocytic alveolitis was found in SAR and isolated neutrophilic alveolitis was found in COP and IPF. Mixed alveolitis was the most common pattern in EAA and NSIN. The CD4:CD8 ratio was the most useful parameter in our study. The ratio was high in SAR (median, 5.80) and it was inverted in EAA (median, 0.19). It was low in the other interstitial lung diseases, with median values of 1.03 in IPF, 1.00 in NSIN and 1.07 in NOC. NK cells populations were higher in NOC (median, 28.00) than the others diseases with median values of 3.00 in SAR, 2.00 in EAA, 4.50 in IPF and 3.00 in NSIN.

Conclusions: The study of the bronchoalveolar lavage fluid parameters in association with clinical and radiologic data, help us to discriminate between interstitial lung diseases. The CD4:CD8 ratio can discriminate sarcoidosis from the other interstitial lung diseases. NK cell populations can discriminate NOC from the others interstitial lung diseases. The bronchoalveolar lavage fluid should be considered a very useful tool in the diagnosis of the patient.

E-139

CNI Induces Th17/ Treg Imbalance and Susceptibility to Renal Dysfunction in Renal Transplant recipients

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Background: Calcineurin inhibitors(CNI) prevent graft rejection by blocking interleukin-2 (IL-2). IL-2 is required for the development and function of $Foxp3^+CD4^+CD25^+$ regulatory T cells (Treg). As an important cytokine, IL-2 was recently reported to inhibit Th17 cells. The renal transplantation recipients treated by

CNI might have Th17/ Treg imbalance, which would cause renal dysfunction even rejection.

Methods: we evaluated 103 renal transplant patients (55 male and 48 female) and 27 healthy volunteers. The patients were divided into stable group (60 patients) and renal dysfunction group (43 patients) according to SCr (female SCr≥110μmol/ml and male SCr≥140μmol/ml). The 103 renal transplant patients were further classified into four groups according to the blood levels of CNI (FK506>6 ng/mL and CSA >80 ng/mL). Of all these subjects, Th17 and Treg frequencies in the peripheral blood were analyzed by flowcytometry (FCM).

Results: Patients treated by CNI revealed obvious increase in peripheral Th17 cells and significant decrease in Treg cells when compared with healthy controls. Even more, the transplant patients with renal dysfunction had the highest TH17 cells and lowest Treg cells among all the study subjects (P<0.05). In different drug concentrations groups, the Tregs in the low FK506 and the low CSA concentration groups were higher than the high FK506 and high CSA concentration groups. There was no obvious difference in Th17 between different drug concentration groups (figure 1).

Conclusion: CNI is associated with Th17/Treg imbalance, which was characterized by higher Th17 and lower Treg cells. CNI influenced Treg cells expression in concentration-dependent way. Due to the inflammation effect of Th17 cells, high percentage of Th17 cells may play an important role in renal injury in renal transplantation recipients.

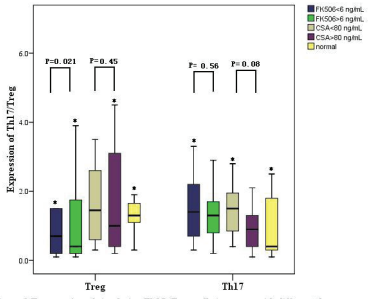

Figure 1 Frequencies of circulating Th17 /Treg cells in groups with different drug concentrations in renal transplantation patients. Whole blood from renal transplantation patients and healthy subjects were stimulated with PMA, ionomycin and monensin for 5 h, and then stained with labeled anti-human antibodies. *P<0.05 vs healthy control.

E-142

Redefinition of the cut-off value in the Phadiatop Infant screening test

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Background: The cut-off (c.o.) is decided in relation to the sensitivity and the clinical specificity of the test based on the issues of which the analysis is a part, on the local case studies or by verifying the cut-off in the literature. The objective of this work is to investigate the grey area of the screening test Phadiatop Infant (Phinf), in which seemingly sick and healthy patients overlap, and to define a reliable cut-off value.

Methods: 1751children (0-4years) have been assessed in this study for allergic diseases; they were initially studied with the Phinf (Phadia-Sweden), whose c.o. value is 0,35 KUa/L. The patients who result positive in this test are subsequently studied with the application of a panel of single allergens - if negative, the research is halted. Specific IgE (S-IgE) c.o.=>0,10KUa/L.For research and measuring of S-IgE produced vs a specific allergen or Phinf, a FEIA method is used with a new high sensitivity curve 0-100 KUa/L. The different c.o.values applied on single allergens and vs the Phinf have pushed us to study the patients who demonstrated Phinf values between 0,10 and 0,35 defined as negatives by manufacturing company.

Results: The allergy expansion panel has been applied to children (176) who have demonstrated a Phinf with concentrations > 0,10 and <0,35 KUa/L with the following results.

Phadiatop Infant KUa/L	Total Patients	Positive Patients	% Positive Patients
0.10-0.15	210	15	7%
0.16-0.20	154	64	39%
0.21-0.25	85	47	55%
0.26-0.30	59	34	57%
0.31-0.35	41	16	39%

The concentrations of S-IgE found in the "new positive patients" varied from 0,20 to 5,12 KUa /L; moreover, patients with S-IgE concentrations of 5,12 KUa/L or 4,72 KUa/L were found in the 0,16 -0,20 range vs peanut allergens (f13) and wheat (f4) respectively.

Conclusion: Using the contingency table we have established the new cut-off at 0,15 KUa/L increasing the sensitivity of the method and avoiding 32% of false negatives responding to moral responsibility not to miss a substantial part of false negatives.

E-143

Evaluation Of Igg Lambda &Amp; Igg Kappa Nephelometric Assays For The Quantification Of Serum Immunoglobulins In Monoclonal Gammopathies

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Introduction: Detection and accurate quantification of IgG monoclonal immunoglobulins by serum protein electrophoresis (SPE) can be difficult depending upon the concentrations of the monoclonal protein and the polyclonal immunoglobulin background. The addition of immunofixation electrophoresis (IFE) offers increased sensitivity over SPE although it is not quantitative. Immunoglobulin measurements by nephelometry are accurate at low concentrations. However, they inherently measure polyclonal immunoglobulins which will reduce the accuracy, particularly at low concentrations of monoclonal protein. In addition, monoclonal IgG measurements can be affected by the catabolic rate of the FcRn recycling receptors. Antisera recognizing epitopes in the quaternary structure between the kappa and lambda light chains and their associated intact heavy chains (heavy / light chain) have been produced. The objective of our study was to evaluate IgG kappa and IgG lambda heavy / light chain (HLC) ratios in our laboratory and to comment on their clinical utility in routine laboratory investigations.

Material and Methods: The patient population included samples from the Marshfield campus and its regional centers, encompassing Northern Wisconsin and upper Michigan. Patient eligibility was based on an initial order of SPE and serum immunoglobulins, with an IFE demonstrating an IgG paraprotein. Samples analysed included 464 serial sera; MM patients included 39/269 IgG kappa and 42/195 IgG lambda. Electrophoresis was performed using a SEBIA Hydrasys 2 in accordance with manufacturer's instructions. Total serum IgG, beta-2 microglobulin (B2M), albumin (Siemens HealthCare Diagnostics), IgG kappa and IgG lambda and serum free light chains (The Binding Site, Inc) were measured on a Siemens Healthcare Diagnostics BNII nephelometer.

Results: The International Staging System (ISS) for myeloma patients in this study was based on B2M and albumin results classifying the IgG kappa involved patients in Stage I (24), II (10) and III (5) and the IgG lambda involved patients as Stage I (21), II (13) and III (8). Pearson correlation was performed using SPE results (g/L) vs. total IgG (g/L) (r = 0.93 (95% CI 0.92-0.94), 2 tailed P< 0.0001), and total IgG (g/L) vs. summed IgG kappa + IgG lambda (g/L) (r = 0.93 (95% CI 0.91-0.94), 2 tailed P< 0.0001). Scatter plots of IgG kappa and IgG lambda were used to display the HLC ratios. In serum samples with quantifiable bands on SPE, 22 / 383 had normal heavy light / light chain band ratios. In serum samples whose banding was too small to quantify due to the polyclonal background, 36 / 81 had abnormal heavy light / light chain ratios.

Concusions: Measurements of IgG kappa / IgG lambda ratios provide a sensitive method for identifying monoclonal proteins when quantification was achievable by SPE measurements. Utility of this assay includes providing a quantitative result to IFE measurements that are SPE negative and IFE positive, and an increased sensitivity over SPE and IFE, in many cases.

E-144

Assessment Of Vegf And Endothelin -1 In Obstructive Sleep Apnea Associated With Hypertension And Dyslipidemia

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Background: Studies have shown that vascular endothelial growth factor (VEGF) concentrations are elevated in patients with severe obstructive sleep apnea (OSA) and are closely correlated with the degree of oxygen desaturation as a consequence of nightime hypoxia Changes of VEGF system in OSA can have serious impact on the development of cardiovascular abnormalities in these patients enhanced by

endotehelin-1 (ET-1) which is a strong vasoconstrictor released mainly by vascular endothelial cells under the influence of hypoxia and other stimuli. This study evaluated plasma levels of VEGF and ET-1 in subjects with OSA associated with comorbidities (hypertension and dyslipidemia)

Methods: We selected 100 patients from the Institute of sleep with a mean age of 51.17 ± 7.98 years and BMI 28.16 ± 3.62 kg/m^2 (53 women and 47 men). Divided into seven groups: GI (OSA + DLP + HYP), GII (OSA + DLP-HYP), GIII (DLP), GIV (control), GV (OSA) and GVI (HYP) and GVII (OSA-DLP + HYP). Clinical, polysomnographic and laboratory measurements including Endothelin -1 and VEGF by enzyme immunoassay (R & D Systems, Inc - Minneapolis, MN - USA) were performed in all patients. For comparison between groups used a one-way ANOVA with post hoc Tukey.

Results: There was no significant difference in BMI between 7 groups, with older GI than GII, GIII, GIV, GV, GVI and GVII (56.23 ± 5.54; 45.83 ± 4.06, 46.44 ± 4.41; 43.92 ± 6.90; 51.87 ± 7.46, 53.77 ± 7.50 and 52.45 ± 6.78 years) respectively. Averages of higher concentrations of VEGF for the GII, GIII and GIV compared with the control (Table 1). However, the ANOVA showed no significant difference in measurements of VEGF and ET-1 between groups.

Conclusion: Our data showed no significant differences in the concentrations of VEGF and ET-1. We recommend performing the study with severe OSA and standardizing the groups.

Table 1- Analysis of the results of the levels of VEGF and Endothelin -1 for each study group .

Parameters	groups	N	Mean	Std. Deviation	p*
VEGF pg/mL	OSA+DLP+HYP	12	25.22	23.95	0.963
	OSA+DLP-HYP	6	45.50	50.71	
	DLP	9	52.70	51.74	
	Control	14	27.50	18.32	
	OSA	23	55.86	93.25	
	HYP	28	30.43	46.02	
	OSA-DLP+HYP	8	22.38	15.50	
	Total	100	38.49	58.39	
Endothelin-1 pg/mL	OSA+DLP+HYP	12	1.91	1.19	0.491
	OSA+DLP-HYP	6	1.54	1.21	
	DLP	9	1.87	0.65	
	Control	14	1.97	0.93	
	OSA	23	1.94	0.88	
	HYP	28	1.84	0.98	
	OSA-DLP+HYP	8	1.60	1.05	
	Total	100	1.87	0.95	

* <0.05 sinifcant level

E-145

Reported Adverse and Serious adverse events after the administration of influenza A (H1N1) vaccine in Central Ghana

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Introduction: The emergence of influenza A (H1N1) virus prompted the development of influenza A (H1N1) monovalent vaccines (2009-H1N1). The use of the vaccine was recommended by the Centers for Disease Control and Prevention (CDC) Advisory Committee on Immunization Practices (ACIP). Adverse events after vaccinations occur but are generally rare. The study was carried out to identify reported adverse and serious adverse events associated with an influenza A (H1N1) vaccine.

Methods: This cross sectional study was carried out between mid of July 2010 to the 31st of August 2010 in Kintampo North Municipality and Offinso South Municipality in Ghana. Data were collected from consented participants using questionnaire.

Results: Of the 420 forms that were given out to consented participants in the two regions, 379 (90.2%) were returned with completed information related to the Influenza A (H1N1) vaccine.

Participants who took the vaccine reported of adverse events such as fever, headache, chills, stomach ache, diarrhoea, pain in the heart and fast heartbeat.

4.4% (16/366) of those who received the vaccine were hospitalized for the adverse event they reported to have experienced after vaccination.

Of the 4.4% of the vaccinated participants that were hospitalized, 43.8% (7/16) were males and 56.3% (9/16) were females. There was no difference between the proportions (p=0.97) of males and females that were hospitalized after vaccination.

Conclusion and Recommendations: Ghana started use of Pandemrix- Influenza A (H1N1) vaccine in June 2010. Symptoms reported ranged from expected (CDC website) to reported death case (GNA July 2010).

This survey of 379 people recorded sixteen (16) hospitalizations due to symptoms reported after vaccination. The study was not controlled and therefore could not make claims of whether the serious adverse events were associated with the vaccine.

We would want to recommend post-marketing monitoring of adverse events after vaccinations.

E-146

Evaluation of an Albumin assay for use on the Binding Site SPA PLUS turbidimetric analyser

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Albumin measurement is routinely carried out in Immunology laboratories to diagnose kidney disease and is recommended in staging multiple myeloma patients. Here we describe the evaluation of an Albumin assay for use on the SPA PLUS analyser, a small bench-top turbidimeter available from The Binding Site Group Ltd. The instrument is an automated, random-access analyser with host interface capability, primary sample ID and reagent management systems. Precision is promoted through a combination of air pressure reagent mixing and acid /alkali cuvette washing. The analyser was programmed to construct a calibration curve from a six point, serum-based calibration set which was then validated by assay of control fluids. All dilutions were made with the instrument's on-board pipetting system, which was able to make dilutions between neat and 1/100. The main assay characteristics are summarised in the table below:

Assay	Albumin
Measuring range (g/L)	2.9 - 92.4
Sample dilution	1/30
Min sample dilution	1/1
Sensitivity (g/L)	0.1
Intra-assay precision %CV	3.4% (79.2g/L)
(mean)	2.7% (54.1g/L)
(n=84)	2.4% (5.1g/L)
Inter-assay precision %CV	1.6% (79.2 g/L)
(mean)	0.0% (54.1 g/L)
(n=84)	3.2% (5.1g/L)
Between-day precision %CV (mean) (n=84)	2.6% (79.2g/L) 4.0% (54.1g/L) 5.0% (5.1g/L)
Total assay precision %CV (mean) (n=84)	4.6% (79.2g/L) 4.8% (54.1g/L) 6.4% (5.1g/L)

No significant interference (within ±10%) was observed upon addition of bilirubin (200mg/L), haemoglobin (5g/L) or chyle (1430 FTU = formazine turbidity units) to samples with known Albumin concentrations. Linear regression analysis demonstrated a high degree of linearity of the assay when expected values were compared to measured values of a serially-diluted pool of high samples using the standard 1/30 sample dilution: $y = 0.9906x + 0.0925$g/L, $R^2 = 0.9984$. Albumin concentrations were measured in 51 normal blood donor sera with the following result: median Albumin 44.8g/L (range 37.9 - 51.9g/L). Comparison was made to the Siemens Albumin assay for the BN™II. Good agreement was demonstrated by Passing Bablok plot: $y = 0.99x - 1.45$g/L, $r = 0.977$ (n=53). We conclude that the Albumin assay for the SPA PLUS is rapid and precise, and may be of use in laboratories where a large instrument may not be appropriate.

E-147

Specific immunoglobulin heavy chain/light chain pairs: Normal ranges and assay utility in patients with monoclonal gammopathies

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Background: The detection and quantification of monoclonal proteins by serum protein electrophoresis is the most used technique for the screening of monoclonal gammopathies. However this can often be difficult, especially in cases where the paraprotein is of low amount and in cases where the band is masked. Immunofixation (IFE) improves sensitivity to the detection protocol but is not quantitative. A new assay (HevyliteTM - The Binding Site) is now available that allows the quantification of specific heavy chain/light chain pairs (HLC) (IgAk, IgAL, IgGk, IgGL, IgMk, IgML and it is our aim to determine normal ranges in healthy individuals considering

that the use of HLC ratios (HLCr) will help us improve the diagnostic and follow-up of monoclonal gammopahties.

Methods: We measured HLC and immunoglobulin's (Igs) G and A concentrations in blood donor sera by turbidimetry (SPA+ - The Binding Site). 35 myeloma samples of treated and untreated patients (16 IgA MM + 19 IgG MM), 4 MGUS, 1 Hodgking Lymphoma and 1 Mielodisplasic syndrome were also analyzed.

Results: Normal ranges were calculated (Table 1) and are in agreement with the other ranges previously published. The correlation obtained for the summation of HLC K + L was in good agreement with the results of total Igs (tIgs) (tIgA vs HLC IgA, r2 =0,91; tIgG vs HLC, IgG r2 =0,91). 13/16 IgA and 15/19 IgG patients presented altered HLCr. Patients who presented normal HLCr for both IgA and IgG were in complete response with negative IFE.

Conclusion: HLC assays allow the determination (typing and quantification) of individual immunoglobulin k and L concentrations and ratios. It presents an enormous potential for the identification and the follow-up of patients with very low monoclonal components or in cases where it is difficult to identify a monoclonal protein hidden by other proteins.

Normal ranges for specific immunoglobulin heavy/light chains pairs.

HLC IgA			
	IgAk g/L	IgAL g/L	IgAk/IgAL
N	83	83	83
Mean	1,37	0,92	1,47
Median(95% Range)	1,34(0,38-3,12)	0,92(0,32-2,01)	1,44(0,66-2,47)
Median(95% Range)1	1,27(0,42-2,36)	0,87(0,40-1,73)	1,40(0,58-2,52)
Median(95% Range)2	1,19(0,48-2,82)	1,00(0,36-1,98)	1,27(0,80-2,04)
HLC IgG			
	IgGk g/L	IgGL g/L	IgGk/IgGL
N	75	75	75
Mean	7,10	4,10	1,81
Median(95% Range)	7,00(4,08-9,56)	4,10(1,76-6,24)	1,75(0,98-3,69)
Median(95% Range)1	7,76(4,23-12,08)	4,00(2,37-5,91)	1,96(1,26-3,20)
Median(95% Range)2	6,85(4,03-9,78)	4,81(1,97-5,71)	1,87(0,98-2,75)

1 - Clinical Chemistry 55:9, 1646-1655 (2009)
2 - Serum free light chain analysis (plus Hevylite), 6th edition, 301-320 (2010)

E-148

Biomarkers Of Oxidative Damage And Their Diagnostic Utility In Patient With Recent Onset Rheumatoid Arthritis

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Background: Oxidative stress is an imbalance between oxidant/antioxidant mechanisms, and can be measured by biomarkers that reflect lipid or protein damage. Some studies have established association between rheumatoid arthritis (RA) and oxidative stress, but these have included patients with antioxidant therapies and established disease. The objective is to determine if patients with early RA without treatment have more oxidative stress compared to healthy controls, and to evaluate the diagnostic utility.

Methods: A case-control study was performed. A total of 55 patients with recently diagnosed RA (ACR, 1987 criteria) without DMARDs or steroids treatment from the Early Arthritis Clinic and 55 healthy controls were included in this study. Population was selected from the Rheumatology Service of the 3rd Level Teaching Hospital. Whole blood was obtained from every individual and colected in tubes containing K3-EDTA. Samples were centrifuged at 0º C to obtain fresh plasma. An antioxidant cocktail was added to every sample. Samples were stored at - 80 °C until analysis. Fatty peracids were extracted with chloroform before testing and subsequent lipid hidroperoxides (LOOHs) were measured by chemical oxidation of ferrous thiocyanate to ferric thiocyanate and measurement of absorbance at 500 nm. Carbonyl protein (PC) concentration was calculated on the basis of the selective reaction of these proteins with 2,4 dinitrophenylhydrazine, the subsequent precipitation in trichloroacetic acid and the measurement of its absorbance at 370 nm. Malonyldialdehyde (MDA) was measured by high performance liquid chromatography (HPLC). Statistical analysis was performed using the SPSS statistical software 15.0 and was done with non-parametric tests and receiver-operator curves were calculated.

Results: Patient and control group were similar regarding age (p= 0.67) and sex (p = 0.97), but there are differences variables associated to the RA (RF, anti-CCP) and the pack/years index, higher in RA patients. Serum levels of CRP, LOOHs, MDA and PC were different in the AR patients compared with the controls. The higher diagnostic utility was for PCO ≥36.6 µM/L with ROC area of 0.73, sensibility 0.94 and specificity of 0.44 (Figure1). LOOHs ≥17.6 µM/Lhad a ROC area 0,68, sensibility of 82% and specificity of 60%; and MDA ≥5.3 µM/Lhad an ROC area of 0,24, sensibility of 100% but specificity 0%.

Conclusion: Early RA patients without previous treatment had shown higher lipid and protein oxidative damage compared to healthy controls and had increased levels of smoking measured by smoking index. PC may be a biomarker of oxidative damage in disease.