Wednesday, August 1, 2018

Poster Session: 9:30 AM - 5:00 PM

Technology/Design Development

B-445

Analytical evaluation and clinical performance of an enzyme-linked immunosorbent assay for measurement of afamin in human urine

L. Pang, N. Duan, D. Xu, L. Jiao, C. Huang, J. Du, Q. Guo, H. Li. *Peking University First Hospital, Beijing, China*

Background: Afamin is the fourth member of the albumin superfamily, which comprises albumin, α-fetoprotein and vitamin D-binding protein. Although the presence of urine afamin has been verified by mass spectrometry and western blot, there is still a lack of a robust enzyme-linked immunosorbent assay (ELISA) method for determination of urine afamin (uAFM). The aim of this study was to evaluate the analytical characterization and clinical value of an ELISA for measurement of uAFM. ***Methods:*** We determined uAFM and calculated afamin-creatinine ratio (AfCR) of 136 healthy volunteers and 129 biopsy-proven glomerulonephritis patients. We evaluated precision, linearity and detection limit of the assay and determined reference intervals according to the Clinical and Laboratory Standards Institute (CLSI) guideline. ***Results:*** The percentage coefficients of variation of repeatability and within-laboratory precision were 12.2% and 12.5% at a mean concentration of 38.85 ng/mL, and 5.4% and 14.0% at a mean concentration of 12.47 ng/mL. Linear range of the method is 1.95-76.41 ng/mL. The limit of blank and the limit of detection were 2.31 ng/mL and 3.21 ng/mL. uAFM and AfCR values were different significantly between males and females. For uAFM, the reference intervals were < 65.60 ng/mL (males) and < 37.20 ng/mL (females). For AfCR, the reference intervals were < 75.26 μg/g (males) and < 47.75 μg/g (females). In the clinical performance evaluation, uAFM and AfCR levels were significantly increased in patients with PMN, IgAN and MCD. uAFM and AfCR were positively correlated with urine albumin and albumin-creatinine ratio, respectively, rather than eGFR. ***Conclusion:*** Our study provided supports that the assay is a reliable and robust test for measuring uAFM. uAFM and AfCR may be attractive biomarkers for glomerular barrier function.

B-446

Comparing operational performance of available immunochemistry systems using six different workloads

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Objective:
Laboratories face the pressure to deliver quality results as fast as possible, thus time to result is a key performance indicator of today's laboratories. In our study we compared the operational performance of five commercially available immunochemistry systems while processing different types of workloads under standardized conditions:
Methods:
Two workloads with request patterns reflecting a commercial laboratory and a hospital laboratory setting (each for 100 samples), were processed in the same manner on the following five immunochemistry systems: ADVIA Centaur XPT, ARCHITECT i2000SR, **cobas** 8000 e 801, Immulite 2000XP*i* and UniCel DxI 800. The 100 samples of the commercial laboratory workload resulted in 176 test requests. The 100 samples of the hospital laboratory workload resulted in 135 requests including 8 emergency samples. Furthermore, four infectious disease panels were measured with 50 samples (using negative control material) on the following four immunochemistry systems: ADVIA Centaur XPT, ARCHITECT i2000SR, **cobas** 8000 **e** 801 and Liaison XL. Panel 1 (pregnancy) consisted of HBsAg, HIV, Toxoplasmosis IgG, Toxoplasmosis IgM, Rubella and Syphilis; Panel 2 (Hepatitis/HIV) consisted of anti-HAV, anti-HAV IgM, anti-HBc, anti-HCV, HBsAg and HIV; Panel 3 (blood screening 1) consisted of HIV, HBsAg, anti-HCV, Syphilis and anti-HBc. Panel 4 (blood screening 2) consisted of HIV, HBsAg, anti-HCV and Syphilis. Time to first result and time to last result were reported. Starting point was the loading of the samples onto the analyser.
Results:
The hospital workload was processed within ~1h on two (**cobas e** 801 and ADVIA Centaur XPT) of the five applied analysers, maximum duration was 2:52h on Immulite 2000XP*i*. Similarly, processing time for the commercial pattern workload ranged from ~1h to ~2.5h. Across the four varying serology panels processed on four different analysers, time to first result was ≤21 min on the **cobas e** 801 module, ≤30 min on the ARCHITECT i2000SR, ≤32 min on the ADVIA Centaur and ≤53 min on the Liaison XL. Time to last result ranged from ≤02:38 h (**cobas e** 801 module) to 04:25 h (Liaison XL).
Conclusions:
During this study, the time to result differed considerably between the included immunochemistry systems. Differences up to more than 100% in operational performance, depending on the panel, may be seen when comparing identical workloads on the six tested immunochemistry systems.

B-447

A Comparison Study of qPCR and ddPCR Assays in Measuring Plasma Epstein-Barr Virus DNA Levels

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Background: Plasma Epstein-Barr Virus (EBV) DNA is a routine test in molecular diagnosis laboratory, for confirming EBV infection, to evaluate therapeutic efficacy in patients taking immunosuppressant with autoimmune disease or after transplantation, and to aid in lymphoma and nasopharynx cancer diagnosis and prognosis. However, hyposensitivity has been always criticized in clinical application. Development of digital PCR exerts enormous potential in molecular diagnosis owing to its high sensitivity and its ability of absolute quantification. This study was conducted to compare the droplet digital PCR(ddPCR) and routine qPCR method for detecting EB viral load. **Methods:** A total of 510 patients (immunocompromised:201; lymphoma:128; untreated nasopharyngeal carcinoma:39; treated nasopharyngeal carcinoma:142) who were highly suspected with EB infection were enrolled in the study, DNA was extracted from Plasma, BamHI-W fragment was amplified by qPCR using EB viral load quantification kit (Sansure Biotech), while ddPCR was performed by BIO-RAD QX200. **Results:** Based on ddPCR method, 369 patients were EBV positive, the median of viral load was 360 copies/mL(P_{25}-P_{75}:67-2905copies/mL). Among the four patients groups, EB viral load of untreated nasopharyngeal carcinoma patients was highest, the median viral load was 4590 copies/mL, followed by lymphoma (840copies/mL) and treated nasopharyngeal carcinoma patients (430 copies/mL), and the immunocompromised patients (130copies/mL) was the lowest. Since the cutoff point of qPCR was 400 copies/mL(designated by the kit), most EBV positive patients (252/369) were missed by qPCR. Hence we reviewed all of the amplification curves of qPCR, 231 of 252 false-negative EBV by qPCR had typical amplification curves, if we designated those with typical curves as positive, qPCR sensitivity would improve greatly, nevertheless the false-positive EBV also raised (53 patients). We attempted to perform ROC analysis, setting the cutoff value as 10.6 copies/mL showed the best diagnostic efficacy in our data. On the other hand, when EBV were detected positive by both the two methods, the quantitative values of EB viral load were moderately accordant(R^2=0.533). **Conclusion:** EBV viral load is mostly very low in clinical practice, routine qPCR method is difficult to satisfy clinical demand, optimize qPCR to increase its sensitivity or replace it with ddPCR is a considerable choice.

B-448

Evaluation of methods for the collection and enrichment of mRNA in liquid biopsy samples

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Background: Liquid biopsy is a low invasive procedure which can be repeatedly conducted, making it suitable for long-term monitoring of disease. Circulating RNAs in liquid biopsy are protected by extracellular vesicles (EVs). Among them, messenger RNAs (mRNAs) are promising markers in EVs. However, most previous studies focused on exosomes and their containing microRNA markers. Only few reports addressed the issues of mRNA collection. The aim of this study is to compare different methods for the collection and

enrichment of EVs and containing mRNA markers in liquid biopsy samples. **Methods:** Conditioned medium from *in vitro* culture, EDTA-plasma, and serum were used as liquid samples. We first applied sequential centrifugation and ultra-centrifugation to separate larger particles (mainly microvesicles and apoptotic bodies) from smaller particles (mainly exosomes), and examined the distribution of mRNA. Then we tested the efficiency of two methods to enrich EVs, including ExoEasy kit which bound all membrane vesicles, and magnetic beads which captured vesicles having phophatidylserine. The collected EVs were subjected to RNA extraction and the purified mRNAs were quantitated by real-time reverse-transcription PCR for housekeeping gene transcripts. **Results:** We could purify and amplify mRNA from these liquid samples. In the centrifugation experiments, we found that the majority of mRNAs were associated with larger particles. In the enrichment experiments, magnetic beads that bound to phophatidylserine had a higher efficiency of enriching and collecting mRNA than the ExoEasy kit. In addition, the amount of mRNA recovered from serum was slightly higher than that from plasma. **Conclusion:** This study demonstrates that circulating mRNA is mostly encapsulated in larger particles, probably microvesicles or apoptotic bodies. To collect these particles, centrifugation at high speed should be avoided, and magnetic beads is helpful for enrichment. The optimized procedures will be applied to analyze cancer-specific mRNA markers in our future studies.

B-449

The diagnostic accuracy of Xpert MTB/RIF for pulmonary tuberculosis: A systematic review and meta-analysis.

M. Lyu, J. Zhou, K. Wu, T. Fu, B. Ying. *West China Hospital, Chengdu, China*

Background: Pulmonary tuberculosis accounts for 80% of all kinds of tuberculosis which is the ninth leading cause of death in the world. Xpert MTB/RIF is a novel diagnostic tool for pulmonary tuberculosis, however, its diagnostic performance has not yet been reached consensus. The objectives of this study were to evaluate the diagnostic accuracy of Xpert MTB/RIF referenced to culture and provide some reliable advice for clinical practice. **Methods:** A comprehensive literature search of Web of Science, PubMed and Embase was conducted from their reception to October 9, 2017. Data from included studies were pooled to yield summary sensitivity, specificity, positive likelihood ratio (LR+), negative likelihood ratio (LR-) and area under the curve (AUC) with a 95% confidence interval (95% CI) to determine the diagnostic performance of Xpert MTB/RIF. The bivariate random-effects model was carried out in quantitative synthesis. Quality assessment was performed according to each question of the Revised Tool for Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2). All statistical analysis was performed by Meta-DiSc software v.1.4 and Review Manager V.5.3. **Results:** Thirty-three studies were included in our analysis with a total of 19768 participants. The pooled sensitivity and specificity were 87.2% (95% CI: 0.861-0.882) and 96.6% (95% CI: 0.962-0.969), respectively. The AUC of Xpert MTB/RIF was 0.973. The heterogeneity of all articles could be accepted. **Conclusion:** According to our research based on a more strict definition of pulmonary tuberculosis, it is a better choice to apply Xpert MTB/RIF to diagnose this disease regarding its high specificity. Although its sensitivity may be lower than culture, it can provide a result within a shorter time and is more suitable for rapid diagnosis and prompt treatment.

B-450

BAMEditor : a benchmarking toolkit for somatic variant detection

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Background:
The molecular diagnostics of cancer by the adoption of next-generation sequencing (NGS) are transforming to match identified genetic alterations with clinical actionable strategies. However, the complexity of the human genome sequence and NGS methods makes the variant detection challenging, especially for somatic variants detection of the target sequencing of cancer. Several studies have indicated that NGS bioinformatics pipelines with different variant calling algorithms and parameters exhibit substantial discrepancies among variant calls. Without a uniform gold standard, clinical laboratories may generate hidden, and/or inaccurate results due to improperly developed, validated, and monitored bioinformatics pipelines. Although many cancer genome simulation tools have been made available, they often cannot fully model the sequencing errors and other sources of error introduced during target capture and library preparation or exist some limitations. Therefore, to create a comprehensive gold standard for somatic mutation detection, we developed a cancer genome simulator for somatic mutation detection. **Methods and Results:** Here we present the BAMEditor tool, a newly developed tool for mutation simulation by editing the existing reads. BAMEditor can add a comprehensive of mutations, including single-nucleotide variant (SNV), small insertion and deletion (Indel), copy number variation (CNV) and large structural rearrangement to any alignment stored in BAM format, such as whole genome, whole exome and targeted sequence data. In addition, BAMEditor also supports flow space data in Ion Torrent sequencing reads. As a demonstration of the utility of BAMEditor, we invited 125 clinical laboratories and academic medical centers performing NGS routinely to process our synthetic tumor-normal pairs by applying BAMEditor to an already sequenced cell line. In this survey, our analysis reveals the contributions of individual pipeline components and parameters on the accuracy of variant detection. We found that variant calling algorithms and variant filter strategy are the key point of variant detection.
Conclusion:
In summary, our BAMEditor tool provides a comprehensive resource for somatic variant detection benchmarking. In addition, our survey provides a comprehensive assessment of bioinformatics pipelines for variant detection of target sequencing which may propose useful guidelines for the benchmarking of bioinformatics pipelines.

B-451

Increased Resilience of Aspiration Monitoring, Incorporation of Commodity Dilution and Interface with Existing Laboratory Infrastructure in the Alinity i System

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Background: Advanced technologies have enabled improvements in instrument autonomy, reliability and electromagnetic compatibility.
• Wash monitoring [WM] can detect errors such as loose connections, decreased vacuum and improperly prepared samples real-time as well as predict future malfunction. Optimized instrument functionality and algorithm enhancements also contribute to decreased false sample/reagent detection.
• Wash buffer is a common medium for immunoassay analyzers in the field and is typically reconstituted in a stand-alone system, with carboys being transported to the immunoassay analyzer by laboratory personnel. Incorporating the reconstitution of wash buffer into the instrument would allow onboard dilution on demand.
• High volume laboratories often rely on a track system to distribute patient samples to the different instruments available in their labs. Incorporating next generation analyzers with these track systems and lab informatics software will allow progression of detection technologies via adoption by established laboratories.
Methods:
• Inaccurate liquid detections from agents such as bubbles or unintended contact between the probe and the vessel wall were mitigated by adding enhancements to the WM algorithms to detect and filter out such events. Using embedded capacitance and ferrites tuned to be resistive at certain frequencies, WM has reduced its noise footprint and become less susceptible to external electromagnetic interference.
• Technological development of an embedded system to supply the instrument with wash buffer on demand was incorporated into immunoassay analyzers. Sensors monitor functions to ensure the dilution is being made as intended and careful material selection promotes the same quality offered by a stand-alone system.
• Design considerations focused on interfacing both software and hardware with the existing track systems to encourage integration while still allowing operators to override the track scheduling with STAT samples. Automation and Informatics reduce the human interaction with the samples and information to improve turn-around time, reduce human error and distribute the patient load across instruments while ensuring optimum handling conditions.
Results:
• The enhancements and optimizations to WM increased the resilience and robustness of the measurements by improving the reliability of accurate liquid detection to 99.8%. Incorporation of the onboard reconstitution station has resulted in a reduction of footprint and increased walk-away time for laboratory personnel.
• Technicians spent up to 57 minutes replenishing supplies on previous systems, but with the system generating wash buffer on demand, that replenishing time is cut to zero. Previous generations required the use of a separate reconstitution station at a cost of 2.2 square meters of laboratory real estate. The incorporation of the station into the instrument means that separate footage cost is cut to zero and due to other technological advances, the instrument is still half the size of the average previous generation system.
• Next generation analyzers interface with the track while still allowing access to the processing bay for operators to run STAT samples. Software is universal across the family of instruments and interfaces with existing lab informatics software to ensure continuity despite adoption of new technology.

Conclusion: Improvements in autonomy, reliability and electromagnetic compatibility have been realized in the next generation analyzer technologies.

B-452

2018 AACC Carryover Reduction

D. Kuffel, T. Mizutani. *Beckman Coulter, Chaska, MN*

Introduction
Sample carryover is an inherent risk and can cause erroneously high patient results for immunoassay tests. In the IVD industry, sample to sample carryover has been the main focus and has been tested over various systems but there are other areas in which carryover can occur. Sample to sample (= external carryover) and total system carryover (= internal carryover) must be assessed to secure patient results.
Method
As part of new system development, three areas were evaluated to minimize carryover; a) sample pipettor for aliquots, b) sample pipetter for tests, and c) first aspiration probe in bound/free separation process. 1. To eliminate sample carryover, the new system uses a single-use pipetting tip for a) each aliquot sampling and for b) each test sampling. 2. Amount of probe carryover is tiny and below assay detection limit, then direct Alkaline-phosphatase (= ALP) reaction with substrate was tested to evaluate first aspiration probe carryover. Add 500µL of high concentrated Alkaline-phosphatase (4.2 µg/mL) into a reaction vessel and measure relative light unit (= RLU) of following known negative buffer. 3. To evaluate total system carryover, three known high positive HBsAg samples and known negative samples were tested together using Access HBsAg test kit.
Results
1. The engineering design solution of single-use pipetting tips provide 0 carryover 2. Observed RLU was 7,959 which corresponds to 2.865 ppm. The protocol uses 21 times high ALP concentration and 3.333 times larger reaction volume than Access2 HBsAg test kit (25µL of 1.0 µg/mL conc. of ALP into 150µL of total reaction volume), therefore possible maximum HBsAg carryover can be 0.0409 ppm (= 2.865/21/3.3) 3. Observed HBsAg carryover results on negative samples were 0.0*** ppm, 0.0*** ppm, below the assay detection limit.
Conclusion
The new system with single-use pipetting tip eliminates sample to sample carryover and demonstrates <0.1 ppm or below the assay detection limit for total system carryover to prevent false positive results.

B-453

Validation of high sensitive troponin T in Roche cobas 8000 system

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Background: Following the FDA approval, the long-awaited high sensitivity troponin is finally available in the US. The Roche Troponin T Gen 5 STAT assay is a highly sensitive and specific marker for myocardial damage. Cardiac troponin increases rapidly (as early as within 1 hour detected by high sensitivity assay) after acute myocardial infarction and may persist for up to 2 weeks. This new assay identifies and measures cardiac troponin at previously undetectable levels, enabling earlier diagnosis of acute coronary syndrome (ACS), faster rule-out of acute myocardial infarction (AMI), and improved prediction of adverse outcomes. The performance of the Troponin T assay meets the requirements of The Third Universal Definition of Myocardial Infarction (MI) and the IFCC recommendations. The assay performance was validated following the requirements of regulatory agencies and CLSI guidelines as part of the process of implementation. **Methods**: The within-run and between-run precision was assessed at five levels by measuring Bio-Rad, Roche PreciTroponin and Randox Cardiac quality control materials following CLSI guidelines EP15-A3 and EP6-A. Linearity and accuracy was assessed using Roche Calcheck material. Interference from known unconjugated bilirubin, triglycerides and hemoglobin materials (Sun Diagnostics, LLC, New Gloucester, ME) was assessed. Comparison between Roche Troponin Gen 5 STAT and TnI assay on Beckman DXI or cardiac troponin T (TnT Gen4) on Roche was conducted. **Results**: The Limit of Blank (LoB), Limit of Detection (LoD) and Limit of Quantitation (LoQ) of the hs-TnT assay were 2.5, 3, 6 ng/L, respectively. The linearity was in the range from 0 to 9850 ng/L. Linearity verification revealed slope=1.026, y-intercept=0.00. A five-day precision study assayed in duplicate at two separate times of the day demonstrated within-run precision of CV<7%, between-run of CV <10%. Interference studies revealed no significant interference of bilirubin, triglycerides and hemoglobin on troponin levels. The comparison study was analyzed using Deming regression. With the concentrations of TnI from 0 to 2.09 ng/mL, the slope, y-intercept and correlation coefficient (r) was 470, 3.58, 0.978, respectively. There is a good correlation with troponin T Gen 4 with the slope, y-intercept and correlation coefficient (r) was 1004, 3.14, 0.995, respectively. The reference intervals were verified and established with the cutoff at 19 ng/mL for both males and females. **Conclusion**: In conclusion, The Roche cobas 8000 system is a robust, high-throughput method for TnT. The performance of the Troponin Gen 5 assay is acceptable for patient testing in clinical laboratories.

B-454

The New Total Bile Acids Assay for the Architect cSystems Instrument

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OBJECTIVE: To present the performance and interference test results of the new Abbott ARCHITECT Total Bile Acids assay on the *c*Systems instruments. **RELEVANCE:** The new Total Bile Acids assay (list number [LN] 03R04) is now liquid, ready-to-use, with longer on-board and calibration intervals, which can be used to measure both plasma and serum bile acids. It utilizes a decreased sample volume relative to the predicate Bile Acids LN 06K90 assay. **METHODOLOGY:** The Total Bile Acids assay (LN 03R04) utilizes the enzymatic cycling colorimetric methodology. The enzyme, 3-α-hydroxysteroid dehydrogenase, reversibly oxidizes the bile acids in the sample to their respective 3-α-oxosteroids in the presence of excess nicotinamide adenine dinucleotide (NADH) and thionicotinamide adenine dinucleotide (thio-NAD^+). During this reaction, thio-NAD^+ is reduced to thio-NADH. The rate of production of thio-NADH is monitored at 404 nm and is proportional to the concentration of bile acids in the specimen. **VALIDATION:** The table below displays the performance characteristics of the new Total Bile Acids assays (LN 03R04) relative to the predicate assay LN 06K90. The interference results for the new Total Bile Acids 3R04 is displayed in the lower portion of the table. All interference data shown, represent the highest acceptable interference levels.

Characteristic	LN 06K90				LN 03R04			
Configuration	Lyophilized				Liquid, Ready-to-Use			
Sample Type	Serum				Serum and plasma			
Sample Volume (µL)	32.0				3.0			
Imprecision	N	Mean (µmol/L)	Total SD (µmol/L)	Total %CV	N	Mean (µmol/L)	Total SD (µmol/L)	Total %CV
	48	3.08	0.21	6.95	80	3.3	0.2	5.1
	48	12.69	0.30	2.40	80	9.7	0.2	1.9
	48	44.03	0.49	1.12	80	18.0	0.3	1.7
	-	-	-	-	80	48.0	0.9	1.8
	-	-	-	-	80	167.3	2.8	1.7
Method Comparison:	Abbott AEROSET vs. Comparative Method				03R04 vs. 06K90			
	N	62			N	136		
	R	0.9906			R	0.997		
	Equation	Y = 0.9659x – 0.4914			Equation	[03R04] = 1.08[06K90] – 1.44		
	Range (µmol/L)	3.3 – 49.7			Range (µmol/L)	1.4 – 148.9		
On-Board and Calibration Stability in hours	168 (7 days)				672 (28 days)			

Interferent	[Interferent]	[Bile Acid] (µmol/L)	Difference (µmol/L)	% Difference
Bilirubin (Conjugated)	22.4 mg/dL	4.9	0.1	2.8
	22.4 mg/dL	21.7	0.4	1.9
Bilirubin (Unconjugated)	14.9 mg/dL	5.0	0.6	11.2
	29.9 mg/dL	22.0	1.5	7.0
Hemoglobin	1000 mg/dL	2.9	-0.5	-15.9
	1000 mg/dL	23.1	-1.5	-6.4
Human Triglycerides	1739 mg/dL	4.7	0.2	4.7
	1703 mg/dL	20.7	-0.2	-0.8
Intralipid	750 mg/dL	4.9	0.5	10.4
	2000 mg/dL	19.7	1.5	7.7
Protein	14.0 g/dL	5.2	-0.5	-10.4
	12.2 g/dL	24.7	-2.5	-10.1

CONCLUSIONS: The new Total Bile Acids LN 03R04 assay displays enhanced performance characteristics and improved ease of use over the predicate Bile Acids LN 6K90 assay.

B-455

Kinetics Study of Hemolysis: Evaluation of the Hemolytic Strength of Lytic Reagents

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Background:
Cell lysing solutions are essential reagents used by hematology analyzers to categorize and enumerate cell types such as erythrocytes (RBC), thrombocytes (platelets), and leukocytes (WBC) in whole blood. Lysing reagents are not easily pre-assessed for strength before formulation and use in hematology assays. Lytic strength depends on the chemical properties and the concentration of the lytic agent. The relationship between the lytic strength and the hemolysis rate has been mostly an experimental practice in laboratories and hasn't been well characterized. We propose to study the lysis kinetics of red blood cell control samples by lytic agents or commercial lysing reagents for assessing their hemolytic strength, since it is directly related to the determination of the hemolysis rate constant. The study of the lysis kinetics of control samples by the lytic agent, sodium dodecyl sulfate, was to verify our hypothesis: whether the reaction followed first order kinetics and the possibility of using the lysis rate constant to assess the hemolytic strength of the lysing reagents.
Methods:
The kinetics study of the hemolysis was conducted with a control blood sample and a lytic agent: sodium dodecyl sulfate (SDS). The course of hemolysis was monitored on a UV-Vis spectrophotometer, which measured absorption. The absorption measure comes from RBC particles scattering incident light, which decreases as the number of particles diminishes. The lysate was monitored at a wavelength of 700 nm to avoid potential interference from hemoglobin absorption. Concentration of RBC was fixed and lytic agent concentrations were varied in the experiment.
Results:
Lysis of the blood control sample by the lytic agent yielded S-curves of the time course of absorption change due to light scattering by RBC particles. The A_{half} point was the point at which the absorbance value reached half of the initial absorbance + the background absorbance. The time at which this value was reached was recorded as T_{half}. Analysis of the data of variables $1/T_{half}$ and lyse concentration demonstrated a linear relationship, which confirmed the first order kinetics of hemolysis. The lysis rate constant is obtained by the slope of the linear regression equation.
Conclusion:
We have demonstrated that $1/T_{half}$ of the cell lysis reaction has a linear relationship with the concentration of the lytic agent, SDS. We confirmed the hypothesis that the RBC lysis followed a first order reaction kinetics given a constant and excessive concentration of SDS. Hemolytic potential can be extracted from the slope of $1/T_{half}$ vs [Lys], which is 1.44k, k is the pseudo first order rate constant. We have potentially found a better way to assess the lytic strength of the lysis reagent for hematology assays.

B-456

Performance on site evaluation of the new Abbott Alinity i system

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Background: The goal of our study was to evaluate the analytical performance of Alinity i, Abbott's next-generation immunoassay (IA) system in independent laboratory setting. **Methods:** Alinity i is based on chemiluminescent microparticle immunoassay (CMIA) detection technology for the quantitative determination of analytes in human serum, plasma, urine and cerebrospinal fluid. Performance profiles including precision, linearity, limit of quantification and method comparison with our routine analytic platforms (Vista 1500, Siemens / IDS-iSys, Immunodiagnostic Systems / Architect i2000, Abbott) for selected assays (TSH, fT3, fT4, high sensitive cardiac troponin I (hsTnI), vitamin D 25) were assessed following the CLSI guidelines. **Results:** The observed total imprecision (CV) ranged from 1.54% to 5.42%; the recovery calculation for the linearity experiment showed reasonable values between 91.8% and 116.4%, except for fT3 (<80%); the correlation slope values (Passing-Bablok) varied from 0.88 to 1.11. We additionally report the limit of quantification for TSH in serum (0.0081 IU/l, CV 3.7%) and hsTnI in plasma (5.35 ng/l, CV 7.7%) as representative examples. **Conclusion:** Precision, bias and correlation to the current methods have been satisfying and reflected the manufacturer's declared performance. The fT3 assay showed a disturbed linearity. LOB, LOQ, LOD of the TSH and hsTnI assays exceeded the manufacturer declared performance showing high precision in the lower measuring range.

B-457

Induction Heating: An Advanced Decontamination Technology to Preserve Sample Integrity on Abbott ARCHITECT i2000SR and Alinity i Analyzers

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Background: In automated diagnostic testing, sample integrity can be at risk during sample pipetting steps due to the potential for sample contamination from a previous sample (carryover). Common industry practice is to use replaceable pipette tips (increasing cost to the user and disposable waste) or fluidic cleaning processes whereby mechanical and chemical mechanisms are used to decontaminate a re-usable pipette. Abbott platforms have proven, robust sample car-

ryover mitigation by leveraging patented technologies and designs with re-usable pipettes. Recent breakthroughs in assay sensitivity, however, require a more advanced decontamination technology to minimize risk of sample carryover and preserve sample integrity. To meet this need, we present a novel application of induction heating on the ARCHITECT i2000SR and Alinity i systems. **Methods:** A recently published method (US 9,073,094B2) discloses a novel contact-free decontamination technology whereby a metal pipette is inductively heated. With this technology, the pipette warms from its own resistance to coil-induced electrical currents (Joule heating). By sweeping the pipette through an induction coil, temperatures on the pipette are elevated throughout its length. To demonstrate this technology, a next-generation, high sensitivity HBsAg immunoassay was used to quantify carryover using the ARCHITECT i2000SR and Alinity i platforms. These platforms were upgraded with the induction heating technology and performed tests organized to induce carryover from sample cups into reaction vessels and between sample cups. **Results:** Tests yielded a quantified carryover ($\mu \pm \sigma$) on the ARCHITECT i2000SR and Alinity i of 0.002 (± 0.001 ppm) and 0.002 (± 0.002 ppm), respectively. Induction heating reduced carryover well below requirements supportive of next-generation, high sensitivity assays and more than 10x below the current requirement of 0.1 ppm. **Conclusion:** Results presented here underscore the effectiveness of this technology in controlling sample integrity, thereby reinforcing the accuracy and continued confidence of results from Abbott ARCHITECT i2000SR and Alinity i platforms. Furthermore, induction heating enables the development of higher sensitivity detection assays without having to sacrifice the benefits of using re-usable pipettes.

B-458

markBTM: A Novel Point-of-Care Immunoassay Platform for Quantification of Blood Biomarkers

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Background: To examine analytical performance of BBB's markB™ point-of-care immunoassay platform for the quantification of analytes in human blood. markB™ is based on BBB's novel power-free plasma isolation technology and magnetic electrochemical sandwich immunoassay, so-called MESIA. Once a drop of finger-prick blood is loaded on the markB™ strip, pure plasma is spontaneously separated from the whole blood by a filter membrane and flows through a microchannel to a reaction chamber by capillary action. The plasma dissolves pre-spotted detection probes, which are magnetic nanoparticles coated with electrochemical labels and target-specific antibodies. In MESIA, magnetic field drives efficient reactions among the detection probes, target analytes, and capture antibodies on the electrochemical sensor to form sandwich complexes. After the active mixing process, magnetic field removes unbound detection probes from the electrochemical sensor. Finally, the amount of analytes in the sample is quantified by measuring electrochemical signals from the probes bound on the sensor surface. markB™ platform was validated for a blood tumor marker, prostate-specific antigen (PSA). **Methods:** markB™ strip consists of two modules for power-free plasma isolation and identification of target analytes based on MESIA, respectively. A filter membrane for the complete removal of blood cells was embedded in a thermoplastic device having hydrophilic microchannels. The detection probes, 500-nm-diameter gold-coated magnetic nanoparticles conjugated with anti-PSA antibodies, were pre-spotted and dried in the reaction chamber. Screen-printed carbon electrodes were utilized for the electrochemical detection based on cyclic voltammetry. The electrochemical sensor was composed of three electrodes - the working, counter, and reference electrodes, and anti-PSA capture antibodies were immobilized on the working electrode. For analysis, a strip was placed on a mobile markB™ reader system, and ~ 30 µL of whole blood sample was loaded. Once the reaction chamber was filled with the blood plasma, the MESIA procedure was automatically performed by two permanent magnets, and the test result was obtained within 10 min. For preliminary evaluation of analytical performance of markB™ immunoassay system, more than 10 strips from 2 distinct lots were tested. **Results:** Once the blood sample was loaded on the system, the whole processes of plasma separation and immunoassay for quantification of PSA were automatically performed. The plasma was successfully separated from the whole blood without any external forces. According to the preliminary evaluation of analytical performance of markB™ for PSA, the lower and upper limit of detection of were determined to be 0.1 and 50 ng/ml, respectively. The mobile features of markB™ reader system enabled to measure, store, transfer, and manage the analysis records. **Conclusion:** markB™ allows for the quantification of target analytes in a tiny amount of whole blood sample without any moving elements or buffer exchange steps as well as without any bulky and expensive detection components. This technology would provide a solution for the needs of point-of-care testing market, which have sought a method for simple, automated, rapid, and accurate detection of disease markers from a drop of biological fluids using a handheld device.

B-459

Evaluation of the ASI Evolution® Automated RPR Syphilis Analyzer and the Repeatability of the Interpretation of Serological Syphilis Screening.

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Background:
The evaluation of the ASI Evolution Automated RPR Syphilis Analyzer to determine its ability to identify persons with possible syphilis infection using the CDC recommended nontreponemal algorithm[1]. A manual RPR test is dependent on good procedural technique with adequate lighting and visual acuity to interpret the presence of flocculation. A manual RPR test is subject to a lack of consistency and standardization between personnel interpreting results. To bring standardization and consistency to the interpretation of the RPR test, the ASI Evolution fully automated RPR analyzers was developed using a camera and mathematical algorithms to analyze the test well images and differentiate between flocculation and non-flocculation. To determine the consistency of the analyzer, a repeatability study was also performed to determine if there were any variations in measurements taken by the instrument on the same item and under the same conditions.
Evaluation - Methods and procedures:
The interpretation of 3757 serum and plasma specimens using the ASI Evolution were evaluated with the results by the ASiManager-AT. The testing requirements were as follows:
1. All samples were qualitative tested using the ASiManager-AT with cards manually prepared.

2. All samples were qualitative tested using the ASI Evolution automated syphilis analyzer.

3. The results of the two methods were evaluated for agreement.

Evaluation Results:
A total of 3757 serum and plasma specimens were evaluated to determine reactivity. Of the 3757 specimens, 1629 were reactive and 2128 specimens were nonreactive. The ASI Evolution identified 1629 of the 1629 reactive specimens as reactive and 2128 of the 2128 nonreactive specimens as nonreactive. A sensitivity of 100% and a specificity of 100% were determined. The reactive samples ranged in reactivity from minimal 1:1 titers to 1:64 titers.
Repeatability - Methods and procedures:
The interpretation of 10 specimens using the ASiManager-AT and the ASI Evolution were evaluated for reactivity. The testing requirements were as follows:
1. All qualitative testing was conducted using the procedure in the package insert.
2. Each qualitative sample was tested 192 times.
Repeatability Results:
A total of 10 specimens were evaluated to determine repeatability of reactivity. Of the 10 specimens, 7 were reactive and 3 were nonreactive. The reactive samples had titers of 1:1 (4 samples), 1:2 (1 sample), 1:8 (1 sample), and 1:256 (1 sample). Each of the 10 specimens were repeated 192 times to evaluate the reactivity. Results showed 100% concordance for each sample.
Conclusion:
The data above shows that the ASI Evolution gives an objective and standardized interpretation of the test results with a high degree of accuracy and repeatability.
References:
1. CDC, 2011. "Discordant Results from Reverse Sequence Syphilis Screening - Five Laboratories, Untied States, 2006-2010", *Morbidity and Mortality Weekly*, 60(05);133-137.

B-460

A basic performance of novel automated coagulation analyzer

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Background: We developed a new automated coagulation analyzer as successor instrument to STACIA™, that allows for high performance measurements despite the compact size. The features of this instrument are as follows. 1) A maximum throughput is achieved up to 240 test per hour for full random access. 2) A high on-board reagent stability is accomplished by using an automatic openable bottle top. 3) A risk of cross-contamination is avoided by employing a non-contact-stirring system. Here, we will report a basic performance of the new automated coagulation analyzer in detail. **Methods:** The new coagulation analyzer we developed demonstrates coagula-

tion time, latex photometric immunoassay (LPIA) and chromogenic assay as an assay principle. At first, we investigated a basic performance of the following assays: prothrombin time (PT), activated partial thromboplastin time (APTT), antithrombin III (AT III), fibrinogen/fibrin degradation products (FDP) and D-dimer on the new analyzer. Next, we also evaluated the other applicable reagents, fibrinogen, thrombotest, hepaplastintest, coagulation factor activity, thrombin-antithrombin III complex (TAT), soluble fibrin (SF), protein C (PC), factor XIII, plasminogen (PLG), α2-plasmin inhibitor activity (α2pI), plasmin-α2-plasimin inhibitor complex (PPI) and total plasminogen activator inhibitor-1 (t-PAI). All assay was performed by using the reagent manufactured by LSI Medience Corporation. **Results:** The assays using PT, APTT, AT III, FDP and D-dimer showed that the coefficient of variation (C.V.) of within-run repeatability was less than 3.0%, while that of between-day was less than 5.0% in each reagent. The linearity ranges of AT III, FDP and D-dimer were 10% to 170%, 2.5 to 80 μg/mL and 0.3 to 60 μg(D-dimer)/mL, respectively, which were obtained a good recovery rate within ±10% for all sample. Further basic examinations including the other applicable reagents such as TAT are currently under investigation. **Conclusion:** A novel automated coagulation analyzer we developed possess excellent properties for coagulation and fibrinolysis tests, which indicates that this instrument is quite helpful for clinical laboratories.

B-461

Evaluation of Select Assays on the Mindray BA-800M Chemistry Analyzer

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Background: The Mindray™ BA-800M Chemistry Analyzer is a fully automated, discrete, random access chemistry analyzer designed for mid- to large-volume laboratories with a throughput of 800 photometric tests per hour, and up to 1200 tests per hour including ISEs. The analyzer is capable of performing routine and non-routine analysis simultaneously or independently, including chemistry, toxicology, and specialty assays. The sample delivery module has a capacity of 300 samples and the independent sample carousel contains 140 positions for barcoded primary collection tubes or sample cups and offers STAT testing capability. The refrigerated reagent carousel contains 120 reagent positions and can accommodate testing methodologies up to 4 reagents. The reaction carousel consists of a dry bath heating system utilizing glass cuvettes coupled with an 8-step washing/rinsing/drying process. The analyzer offers many features: intuitive software interaction; touch screen monitor; on-board operator's manual with intelligent indexing; intelligent probe management system offering bubble detection, collision protection with auto-recovery, liquid level sensing and clot detection (Sample Probe Only); and remote access diagnostic capability. **Objectives**: The study evaluated the performance of a selection of assays on the Mindray BA-800M Analyzer, using another validated method, the Mindray BS-480 analyzer for most assays, as a reference testing analyzer. The general chemistry reagents are manufactured at the MedTest corporate headquarters located in Canton, Michigan. **Methods**: Analysis was performed based on modified versions of applicable CLSI Protocols. Within Run and Total Precision were determined by running three levels of control material. Within Run Precision was determined by running 20 replicates of controls in a single day. Total Precision was determined by running materials in duplicate across 20 shifts. Accuracy assessment through a correlation of patient samples on the Mindray BA-800M and the comparative analyzers is in process. Limit of Detection for calibrated assays was determined by statistical analysis of response values from five replicates of a low sample and ten replicates of a negative sample. For factored enzymes the limit of detection will be determined by observation of the lowest concentration sample yielding nonzero results. Limit of Quantitation will be determined by assaying a minimum of 40 replicates over at least 5 runs for each sample, using an acceptable precision of CV ≤ 20%. **Results**: Evaluated assays yielded within run precision CVs below 3.9%. Evaluated assays yielded total precision CVs below 7.0%, and most assays had CVs ranging between 0.7% to 5.3%. Limits of detection for evaluated assays were comparable to other similar test systems. Correlation and limit of quantitation studies are currently in process; but preliminary data suggest comparable performance to the Mindray BS-480. **Conclusions**: The performance characteristics of the assays on the Mindray BA-800M Analyzer were comparable to the Mindray BS-480 (or other validated test system). Based upon data generated to date, it can be concluded that the Mindray BA-800M Analyzer is a suitable instrument for use in mid- to large-volume laboratories based upon throughput capabilities and performance.

B-462

UniCel DxI 800 Access Immunoassay System Reliability

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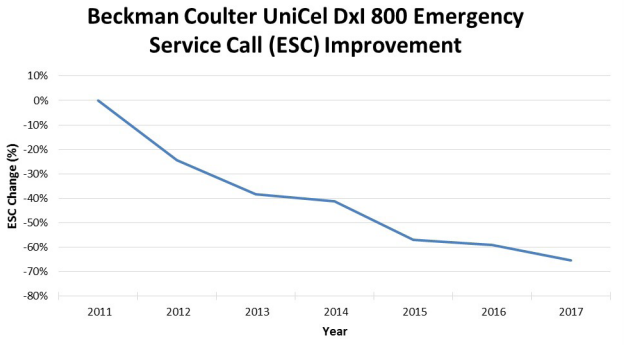
Background: The Beckman Coulter UniCel DxI 800 Access Immunoassay Systems are designed to meet the throughput needs of high volume laboratories. The analyzers offer a broad menu of assays using proven chemiluminescent detection and magnetic particle-separation technology. The reliability of the analyzers is important to customers to minimize downtime. Beckman Coulter has a reliability program in place which drives continuous improvement for better customer experiences. **Methods:** The Beckman Coulter reliability program uses multiple sources for driving improvements. These sources include feedback from both customers and internal personnel including Design, Service, Manufacturing and Supplier Quality. A key input source is customer Emergency Service Calls (ESCs). These are calls which cannot be resolved over the phone and result in dispatching Field Service Engineers to customer sites to resolve issues. ESC information such as service date, analyzer serial number, issue and resolution is recorded in a database which can be used by the reliability team. Reliability engineers analyze all ESCs and group calls into categories based on issue. Categories are investigated to determine root causes of the issues and action plans are developed to address them. Actions are implemented and ESC rates are monitored for the expected impact. **Results:** The reliability program has resulted in a significant decrease in ESCs. The below graph shows the DxI 800 reliability performance from 2011 to 2017. The number of ESCs has decreased every year, with a total decrease of over 65% from 2011 to 2017.



Conclusion: The Beckman Coulter reliability program monitors customer call information, performs data analysis on this information, and then drives improvements into the system and processes. The reliability program has driven a significant improvement in the ESC volume for the DxI 800 from 2011 to 2017. ESC rates during this period have decreased by over 65%. This improved system reliability leads to increased uptime and higher customer satisfaction.

B-463

Specimen Stability and Integrity: Essential factors to quantification of Donor-Derived cfDNA in Transplant recipients

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Background: The release of circulating cell-free DNA (cfDNA) into plasma can serve as an important biomarker for transplant rejection. We developed and validated a next-generation sequencing test (AlloSure®) for quantifying donor-derived cell-free DNA (ddcfDNA). Specimen integrity and stability are essential because total cfDNA is present in low concentrations in the plasma and the dd-cfDNA is a small fraction of the total. Donor and recipient levels must remain stable regardless of storage or handling conditions. Additionally, contamination of the plasma cfDNA by genomic DNA released from the recipient's nucleated blood cells can interfere with the measurement of the dd-cfDNA. For this reason, a hemolysis score based on visual comparision to a hemolysis chart is used as an indicator of potential lysis of nucleated cells. Specimens with more than a trace amount of hemolysis are excluded from testing. The studies reported here evaluated 1) the impact of hemolysis on dd-cfDNA measured in specimens from kidney transplant recipients that expands on studies using simulated hemolysis in plasma from normal healthly volunteers and 2) the stability of cfDNA in plasma stored frozen at -70C. **Methods:** Blood was collected into Streck Cell-Free DNA BCT® collection tubes

from kidney transplant patients and tested using AlloSure. Two tubes were collected from each patient and a hemolysis score was assigned upon receipt of specimens. Extraction was performed using Qiagen's Circulating Nucleic Acid kit. A subset of samples were quantified for total cfDNA using a qPCR method while %dd-cfDNA was calculated using the AlloSure workflow. In the plasma stability study, panels were created from normal healthy volunteers using plasma from one individual spiked into the plasma from another in levels that are consistent with those found in kidney transplant patients. Replicate tubes were stored at -70C for up to 10 months to determine if there were changes to the total amount of cfDNA and donor fraction over time. **Results:** Changes to the level of dd-cfDNA measured were detected when using simulated hemolysis samples with the %dd-cfDNA lower in some hemolyzed samples compared to non-hemolyzed controls. However, our preliminary results using plasma pairs from kidney patients do not show the same effect on the measured dd-cfDNA levels and the %dd-cfDNA levels were similar with differing hemolysis scores. cfDNA in plasma was stable when stored at -70C for up to 10 months after draw. **Conclusion:** Genomic DNA released using simulated hemolysis may not be reflective of the DNA released when hemolysis occurs during routine blood collection and handling. Further understanding of the impact of hemolysis to dd-cfDNA measurement in patient specimens may allow a more relaxed criteria for acceptance of samples showing low levels of hemolysis. The stability of cfDNA in stored plasma allows for use of archived specimens for alternative assessment testing programs and other quality control testing to monitor accuracy and consistency of the test system over time.

B-464

Novel XNA Molecular Clamp Application in NGS Diagnostic Platform OptiSeqTM Cancer Panels

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Background: Next-generation sequencing (NGS) technologies, also known as massively parallel sequencing (MPS), is widely used to detect sequence variations and an array of genetic markers including common and rare variants in cancer genes. NGS and bioinformatics are increasingly used to analyze multiple biomarkers and have been applied in clinical cancer samples. However, one of the challenges in detecting cancer variants with routine NGS analysis is the low frequency in which these mutations are present amongst a background of normal cells. Xenonucleic Acid (XNA) Molecular Clamp Technology is an innovative nucleic acid molecular oligomers that hybridize by Watson-Crick base pairing to target DNA sequences. The xenonucleic acid oligomers are used as molecular clamps during polymerase chain reaction (PCR) to selectively suppress wild-type DNA and amplify mutant DNA. Here, we introduce a highly sensitive NGS Diagnostic Platform Optiseq™ Cancer Panel that combines with the proprietary XNA technology to detect low-frequency somatic variants in cancer samples. **Methods:** Herein we describe a highly sensitive cancer hotspots detection assay to investigate the effects of XNAs on the detected frequency of hotspot variants, particularly variants with low frequency (< 1%), by spiking in a pool of 16 different XNAs in combination or respectively, at varying ratios to the OptiSeq™ Pan-Cancer Panel (DiaCarta) and the QIASeq v3 Human Actionable Solid Tumor Panel (Qiagen). Real patient samples were also tested by spiking in six XNAs individually with XNA Nano Panel (DiaCarta). The assay can be performed directly on patient plasma and FFPE samples and can be readily automated. **Results:** For the OptiSeq™ Pan-Cancer Panel, XNA was found to enrich the cancer hotspot 'driver' and 'drug resistance' mutations (Horizon Discovery) when spiked into a healthy control FFPE DNA. XNA also efficiently enhanced cancer hotspot variants detected by XNA Nano Panel for known positive reference samples. **Conclusion:** XNA technology has the capability to become a powerful NGS diagnostic tool by suppressing abundant wild-type background to detect low-frequency variants of interest in conjunction with targeted cancer panels and allows rapid, efficient and cost-effective determination of the genetic landscape of patient's cancer. Significant progress has been made in characterizing and optimizing the use of XNA in conjunction with oncology NGS and bioinformatics.

B-465

Application of an overall equipment effectiveness indicator in an automated production line for clinical analyses.

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Background: Overall Equipment Effectiveness (OEE) is a measurement of continuous improvement for equipment and productive processes applied in industry to allow for comparison between distinct productive units and the continuous improvement of a manufacturing plant. This methodology is seldom used in the health field, and with the changes that are taking place in clinical analysis laboratories, which are turning into mega-laboratories with automated production lines resembling those in industry, its application will be able to help in the understanding of these transformations, reducing costs and guaranteeing market competitiveness. The present study proposes to apply and make viable the use of the OEE indicator installed in a clinical analysis laboratory.**Methods:**Data collection strategies were designed that would make it possible to faithfully calculate indicators that contribute to the OEE, Availability, Performance and Quality. The following were calculated: total time available, time programmed, time spent producing, time of setup, time spent reloading, mean time stopped, time of production cycle, theoretical production, real production, and good and bad products. For the calculation of the OEE, the contributing factors were considered in multiplied percentage. The data were calculated for two pieces of equipment of the production line, which were the first and last pieces of equipment in the Immunochemical (hormones) line, ADVIA Centaur XP 1 and XP 13, respectively; these, together with the equally automatized sample distribution equipment, are known as Aptio®.**Results:** The results showed Availability of 65.96 % and 64.91 % for the two pieces of equipment analyzed, which means that the laboratory could increase its demand for tests and produce for longer periods, but this also means idle periods and costs for the laboratory. The Performance calculated was 33.64 % (34.4 tests/hour) and 27.55 % (18.1 tests/hour), which is well below the expected value when compared to the manufacturer's description of 100 % performance, which corresponds to 240 tests/hour. In relation to the indicator Quality, the results revealed 96.64 % and 98.02 %, reflecting small margins of error, which means that the quality control and calibrations carried out in both pieces of equipment show a good performance and guarantee a correct final result, which is reflected in the clinical analysis report and the safety of the patient. These multiplied indicators generated an OEE of 21.44 % and 17.53 %, far below the values proposed for industry by Nakajima, who considered the ideal values to be 90.00 % for Availability, 95.00 % for Performance and 99.00 % for Quality, with an OEE of 85.00 %. **Conclusion:**The calculations of the contributing indicators and of the OEE allowed the inefficiencies and strong points present in the laboratory's production line to be analyzed and identified. Just as in industry, the applied OEE was seen to be an exceptional tool for the diagnosis of problems and inefficiencies present in automated laboratory lines. These problems would be difficult to quantify by means of simple strategies, such as the use of internal quality control, which would not detect all the faults that OEE did.

B-466

Evaluation of a New Version Safety Subculture Unit 2

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Background:
Current methods and tools used to subculture blood culture bottles present significant risk for exposure to biohazards and sharps injury. Safety SubCulture Unit 2 (SCU2) is a new subculture device consisting of a needleless cap that fits on blood culture bottles with an internal plastic piercing tip and an external dropper intended for dispensing sample drops from blood culture bottles for subculture and slide preparation.**Methods:** To evaluate ease of use, two (2) studies were performed by experienced laboratory technologists using blood culture bottles positive for bacteria or yeast. Study metrics included:

- device stayed on the bottle,
- initial drop dispensed in ≤10 seconds,
- drop size similar to the original SCU,
- number of drops dispensed

Supplies included forty three (43) SCU2 devices, positive blood cultures in Becton Dickinson and bioMerieux aerobic and anaerobic bottles with and without resins or beads, bioMerieux MP bottle and petri dishes. Positive cultures included categories of Gram Positive and Negative Bacilli (GPB, GNB), Gram Positive Cocci (GPC), Mycobacterium and yeast.
Results:
Data from the two studies were combined and are summarized below;

- device stayed on the bottle: 43 of 43
- initial drop dispensed in ≤10 seconds: 38 of 43

o 5 of 43 produced an initial drop in >10 seconds*, in these 5 cases, bottle type/organism included;

§ 3 aerobic bottles with resins containing GNB

§ 1 aerobic bottle with resins containing GPC

§ 1 aerobic bottle without resins containing GNB

- drop size similar to the original SCU: 42 of 43

• number of drops dispensed: 39 of 43 dispensed 5 or more drops

o 4 dispensed less than 5 drops*, in these 4 cases bottle type/organism included;

§ 4 aerobic bottles with resins containing GNB

Overall performance: SCU2 safely dispensed drops from blood culture bottles *Bottles containing resin and low gas producing organisms (GNB) may increase dispense time and in some cases clog the dispensing tip. Adherence to sampling technique described in the Instructions for Use (IFU) is recommended.
Conclusion:
Safety SubCulture Unit 2 provides an easy to use, efficient method for dispensing samples from blood culture bottles and improves safety by eliminating use of a needle.

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New automated chemiluminescence immunoassays for CSF beta-amyloid determination

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Background: With approved treatments for Alzheimer's disease (AD) on the horizon, diagnostics based on the determination of beta-amyloid 1-42 and beta-amyloid 1-40 in cerebrospinal fluid (CSF) are becoming increasingly important. However, programs like the Alzheimer's Association Quality Control (AAQC) have shown in the past that the field is struggling with high inter-lab variances for these parameters. This makes the comparison of values and determination of global cut points impossible between centers. New, closed test systems could possibly rectify technical issues leading to the observed variances. **Methods:** Magnetic bead-based chemiluminescence immunoassays (CLIA) for beta-amyloid 1-42 and beta-amyloid 1-40 have been developed and validated following the Clinical and Laboratory Standard Institute (CLSI) guidelines on the closed benchtop random access RA Analyzer 15 (Euroimmun). **Results:** The newly developed Euroimmun Beta-Amyloid 1-42 and Beta-Amyloid 1-40 CLIAs with a time-to-first-result protocol of 20 min show inter-assay coefficiants of variation (CV) of 1.5-9.6%. Correlation between the manual Euroimmun Beta-Amyloid 1-42 and Beta-Amyloid 1-40 ELISAs and the new Beta-Amyloid 1-42 and Beta-Amyloid 1-40 CLIAs, respectively, is excellent (99%). **Conclusion:** The newly developed Euroimmun CLIAs for beta-amyloid 1-42 and beta-amyloid 1-40 can be run on a closed random access system (RA Analzyer 15) and show excellent analytical validation data. Significantly reduced hands-on time will further contribute to low variances between labs and a better robustness when including the ratio of beta-amyloid (1-42)/(1-40). However, even though these assays are excellent and accurate diagnostic tools, the field needs to improve and implement global guidelines for pre-analytical sample handling in order to overcome variance that is linked to the sample itself.

B-468

Performance of the Apolipoprotein B assay for use on the Binding Site Optilite® turbidimetric analyser

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The Optilite Apolipoprotein B (Apo B) assay is intended for the quantitative in vitro measurement of Apo B in serum using the Binding Site Optilite analyser to aid in the assessment of cardiovascular disease and lipoprotein metabolism disorders. Apo B is synthesised in the intestine and liver. It is the principal protein component of low density lipoprotein (LDL) and is required for binding LDL particles to the LDL receptor which transports cholesterol to the cells. This may contribute to atherosclerotic plaque build-up in the arteries. There is one Apo B molecule present on each particle of very-low-density lipoprotein, intermediate-density lipoprotein and LDL. Therefore, Apo B concentration provides an effective indication of the total number of potentially atherogenic particles. Apo B determination can be useful in identifying patients who have an increased number of small dense LDL but present with normal LDL cholesterol concentration and could therefore be at increased risk of Coronary Heart Disease. Typically, total cholesterol and triglycerides testing are used for screening coronary risk, but measurement of Apo B, along with other lipoproteins such as lipoprotein (a) and apolipoprotein A-1, can provide further useful information. Here we describe the evaluation of The Binding Site Apolipoprotein B assay for the Optilite analyser. The limit of quantitation (LoQ) for this assay is defined as the bottom of the measuring range, 0.065 g/L. The LoQ verification study was based on CLSI EP17-A. The precision study was based on CLSI EP5-A2. The study was carried out over 5 days using one reagent lot on one analyser. The between run coefficients of variation (CVs) were as follows: 1.27% at 0.69 g/L, 1.43% at 1.25 g/L, 0.89% at 1.67 g/L and 1.93% at 1.99 g/L. A comparison study was performed by analysing 187 samples (including 97 samples with analyte levels within the reference interval) using the Optilite Apolipoprotein B Kit and an alternative commercially available assay. Passing Bablok regression analysis generated the following results: $y = 0.96x + 0.03$ (g/L) (y = Optilite; x = predicate analyser) and correlation coefficient $r = 0.997$ (calculated by linear regression). A linearity study was performed following CLSI EP6-A. The linearity of this assay has been confirmed using a serially diluted serum sample over the range of 0.24 - 3.98 g/L at the standard 1+2 sample dilution with deviation from linearity <10%. An interference study was performed following CLSI EP7-A2. No significant assay interference effects were observed when tested at the standard 1+2 sample dilution with Intralipid (1759 mg/dL), unconjugated bilirubin (61.85 mg/dL), conjugated bilirubin (54.4 mg/dL), haemoglobin (500 mg/dL) or rheumatoid factor (537 IU/ml). No antigen excess was observed up to a level of 3.6 times the top of the calibration curve at the standard 1+2 sample dilution. This is equivalent to 10g/L. In conclusion, the Apolipoprotein B serum assay for the Optilite analyser provides a reliable and precise method for quantifying Apolipoprotein B content in human serum and correlates well with existing methods.

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Human Factor Testing on the Low Level IgG Assay for the Binding Site Optilite® Analyser

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The Binding Site Optilite Low Level IgG Kit is intended for the quantitative in vitro measurement of IgG in urine, cerebrospinal fluid (CSF) and paired CSF and serum samples using the Optilite analyser. Measurement of this immunoglobulin aids in the assessment of the body's lack of ability to resist infectious disease. As part of the validation of this assay, human factor testing was performed using CSF at Labor Berlin, Berlin, Germany and using serum at St Vincent's University Hospital, Dublin, Ireland. Testing was conducted over a 5 day period. Each day a calibration curve was generated and QC samples were assayed twice. Acceptance criteria were provided on the quality control certificate provided with the assay. 3 precision samples were assayed on each day with 5 replicates of each sample in a single run. 5 samples from healthy donors were assayed on each day in singlicate; a different set of 5 samples was used on each day of testing, giving a total of 25 healthy donor samples. 10 samples from patients representing the intended use population of the assay were assayed on each day on the Optilite and results were compared to those generated using the analyser used routinely to measure IgG at each site. 10 different samples were assayed on each day, giving a total of 50 samples from 50 individual patients. All QC results at both sites were within the limits stated on the QC certificate provided with the kit. At Labor Berlin, the 3 precision samples reported mean values of 30.14 mg/L, 77.66 mg/L and 9888.89 mg/L. Total precision CVs were 1.2%, 3.5% and 3.4% respectively. At St Vincent's, the 3 precision samples reported mean values of 33.04 mg/L, 85.88 mg/L and 10729.68 mg/L. Total precision CVs were 3.7%, 2.6% and 4.3% respectively. 22 out of 25 normal samples were within the reference interval when assayed at Labor Berlin, 25 out of 25 samples were within the reference interval when assayed at St Vincent's. The comparison study performed at Labor Berlin using 50 CSF samples gave an ordinary linear regression equation of $1.11x + 2.29$ with a correlation coefficient of $r^2 = 0.987$. The comparison study performed at St Vincent's using 50 serum samples gave an ordinary linear regression equation of $1.09x + 577$ with a correlation coefficient of $r^2 = 0.996$. This testing allowed the laboratories to generate feedback responses based on their experience with the assay in the intended use environment. These responses were returned to Binding Site in the form of a questionnaire. In conclusion, human factor testing of the Low Level IgG assay on the Binding Site Optilite analyser did not identify any issues with the safety or usability of the assay. Both St Vincent's University Hospital and Labor Berlin provided positive feedback on their experiences with the assay, generating positive data that validated the performance of the assay in a clinical laboratory.

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Comparison of Precision Performance across six immunochemistry analyzers

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Objectives: Here we summarize the outcome of a precision study performed at two European sites comparing 13 assays according to CLSI EP05-A3 (5 days) 1x5x5 model. Assays were selected from five indication areas and evaluated on two **cobas e** 801 analytical modules (Roche Diagnostics), ARCHITECT i2000SR (Abbott), UniCel® DxI 800 (Beckman Coulter), ADVIA Centaur XPT / Immulite 2000 XP*i* (Siemens Healthineers) and Liason® XL (DiaSorin). **Methods:** For the precision study, pooled quality control material from Biorad at three different concentration levels per analyte were distributed to both sites. The 13 assays included in this study covered the indication areas Anemia (Ferritin), Bone (PTH), Fertility (Estradiol, Progesterone, Testosterone), Oncology (CEA, CA 125, CA 15-3, CA 19-9, fPSA, tPSA) and Thyroid (FT4, TSH). Testing was done on five days in 5-fold determinations per assay and applied sample pool material. CVs were calculated per site as repeatability and within-lab precision. **Results:** The analyte concentration ranges covered per assay are shown in the table below as mean over all applied methods:

Indication Area	Assay	Unit	Concentration Range	Indication Area	Assay	Unit	Concentration Range
Anemia	Ferritin	µg/L	~ 47.2-641	Bone	PTH	pg/mL	~ 28.8-978
Oncology	CA 125	U/mL	~ 31.4-242	Fertility	Estradiol	pmol/L	~ 368-1762
	CA 15-3	U/mL	~ 21.4-95.1		Progesterone	nmol/L	~ 2.83-76.2
	CA 19-9	U/mL	~ 23.6-219.7		Testosterone	ng/mL	~ 1.93-10.3
	CEA	U/mL	~ 2.57-85.7	Thyroid	FT4	pmol/L	~ 10.7 - 80.6
	fPSA	ng/mL	~ 0.044-10.8		TSH	mIU/L	~ 0.477- 32.1
	tPSA	ng/mL	~ 0.089 - 12.5				

The median repeatability /within-lab CVs calculated over all 13 assays and concentration ranges were: **cobas e** 801 at 1.1% / 1.8%; ARCHITECT i2000SR at 2.9% / 3.0%, UniCel DxI 800 at 3.7% / 4.3%, ADVIA Centaur XPT at 3.1% / 5.0%; Immulite 2000 XP*i* at 4.2% / 5.0%; Liason XL at 2.6% / 3.8%. **Conclusion:** This comprehensive study gives a very good comparison of the precision performance across high throughput immunochemistry analyzers from different manufacturers under standardized conditions.

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Urban inclusion of primary care laboratory services with point of care testing

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Background: City-labs project aims to facilitate in Brussels the access to laboratory tests and allow rapid transmission of results to physicians (general practitioner or specialist) as part of an integrated outpatient care for the chronically ill. The project involves point of care testing and also includes development of digital application for remote monitoring of clinical and biological data. Metrolab Brussels is a transdisciplinary and inter-university laboratory for applied and critical urban research. The Brussels Capital Region through its ERDF program funds both City-labs and Metrolab projects. **Methods:** The team members and researchers of City-labs and Metrolab evaluated different scenarios for an efficient urban inclusion of city-labs structures. Multidisciplinary exchanges were conducted with laboratorians, physicians, urbanists, sociologists and suppliers to define the user's needs and potential features of the solution. **Results:** The metrolab and city-labs researchers identified several key points of attention for the efficient inclusion of city-labs such. The connectivity in public transport and the access by the road will be important, and an easy access by car would be one of the advantages of the City-Lab, including the possibility of parking his vehicle. Our evaluation also allowed to clear two criteria that can guide the choice of location for a City-lab: that of the connectivity of the area (its location at the intersection of axes of different modes of transport) on the one hand, and that of the density or insufficiency of the supply of proximity on the other hand. The ability to drain patients coming from a broad radius requires also attention and an audience with a high mobility potential, moving easily by car or public transport, would be the target audience in priority. Another important point to consider is the insurance of a communication with specialized hospital services to encourage patients concerned about consistency and ongoing monitoring of their medical records. The communication and coordination with general practitioners and ambulatory care services will also represent a priority for the project team members. **Conclusion:** In an era of transition care and interactions between hospital and ambulatory care, the evaluation of the urban inclusion of new care services is mandatory and has to consider mobility and motility as well as communication with healthcare care providers as important determinants.

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Uncertainty of Measurement and Total Analytical Error: Better Together?

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Background: There is an ongoing debate about using uncertainty of measurement versus total analytical error (TAE). Manufacturers aim to address the needs of all customers, some of whom consider measurement uncertainty (MU) and others who use TAE. Both approaches are methods for estimating various forms of error. The approaches could be viewed as complementary, with one being potentially useful to clinicians outside the lab and the other being useful within the testing lab in the context of total allowable error (TEa). An assessment was performed to evaluate the relationship between the two error estimation methods on three clinical chemistry assays - calcium, total cholesterol and glucose - tested on the Abbott Alinity c system. **Methods:** Measurement uncertainty values, TAE and sigma metrics were determined at multiple concentrations across each assay's analytical measuring interval. Total analytical error was estimated using the equation: TAE = 2 × precision + |bias|. The within-laboratory (*i.e.,* intermediate) precision estimates for panels and controls from a 20-day study tested on the Alinity c system were used as the precision values. Bias was estimated from a weighted Deming regression model using data generated from >100 serum samples tested on the Alinity c and ARCHITECT *c*8000 systems. Both studies were conducted at Abbott. Using CLIA TEa goals, sigma values were determined using the equation: sigma = (TEa - |bias|) /precision and plotted on a normalized method decision chart. Measurement uncertainty values were determined using coverage factor (k) of 2, as described in the Guide to Uncertainty of Measurement. Production history and studies performed at Abbott were used to estimate uncertainty associated with repeatability, days, runs, instruments, calibrations, calibrator lots and reagent lots. The relationship between MU and TAE was illustrated in a MU/TAE profile plot showing MU on the Y1-axis, TAE on the Y2-axis and the assay concentration on the X-axis. Additionally, the relationship between MU and sigma metrics was illustrated in a graph showing MU on the Y1-axis, sigma metrics on the Y2-axis and the assay concentration on the X-axis. **Results:** Measurement uncertainty and TAE values were comparable across concentration levels. The performance of >80% (13/16) of the concentration levels evaluated demonstrated >6 sigma performance. One sample for each assay had <6 sigma performance. **Conclusion:** Both methods for characterizing assay quality are valuable. Reporting the measurement uncertainty of a specimen result informs the clinician of the variability around the result, which can be especially important for results near medical decision limits. When assessed versus a TEa goal, total analytical error estimates can be used to categorize the performance of an assay by calculating a sigma metric. This sigma metric can be used in multiple ways, one of which is to help determine appropriate quality control rules for an assay. By reporting measurement uncertainty values with results in addition to using total analytical error and sigma metrics to maintain, monitor and improve quality control, laboratories can take advantage of both methods, thus providing a more comprehensive and higher quality service to patients than either method alone.

B-473

Can we reduce empiric and unnecessary antibiotic use with urinary flow cytometry systems?

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Background: Urinary tract infections (UTIs) are one of the most common infections and rapid diagnosis and treatment are very important. Urine culture is gold standard but time-consuming method to diagnose urinary tract infections. Because the culture results in the earliest 24 hours, physicians start empirical antibiotic treatment. While the empiric treatment can be useful for patients who have UTIs, it causes unnecessary antibiotic use and resistance to bacteria in patients who have no UTIs. In this study, it was aimed to evaluate how much unnecessary antibiotic usage can be avoided by early prediction of negative urine cultures by 'flow cytometry' that is the reference method for cell counting in liquid samples. **Methods:** In this study 1886 urine samples (881 female, 812 male and 143 children) that are accepted in the Central Laboratory were included. At the first stage of study, samples were processed according to standard culture method and then were analysed by UF-1000i (Sysmex, Japan). The cut-off that providing 100% negative predictive values were determined for female, male and child patients. At the second stage, prescription of all patients in the study were investigated with antibiotic prescribing time that were obtained from hospital information system. The antibiotic started in the first 24 hours was accepted as empirically. Unnecessary antibiotic use was accepted in case of antibiotic prescription despite negative or contaminated culture result. **Results:** According to UF-1000i results, 45% of the total samples were not required to culture. All samples that were estimated as negative by UF-1000i were confirmed as negative by culture method. After urine culture request, in first six days, 244 patients were found prescribed antibiotic. Only 17% of 244 patients were observed as positive in urine culture results. When the prescription time and culture reporting time were evaluated together, it was observed that antibiotics were given 44% empirically, 46% unnecessary and 10% relevant to culture results. According to results of UF-1000i urinanalysis, antibiotic prescription could be prevented in 29,6% of patients with empiric therapy and 38,3% of patients with unnecessary antibiotic use. **Conclusion:** As a result, provided that unnecessary antibiotic usage decreases and depending on this cost reduces at least one third rate by usage of UF-1000i that giving rapid results in terms of UTI. We recommend that automated systems like flow cytometric method that provide rapid and accurate diagnosis to patients could be used in together with standart culture methods.

B-474

A global multi-site Sigma-metric assessment of 18 measurands on the Abbott Alinity ci system

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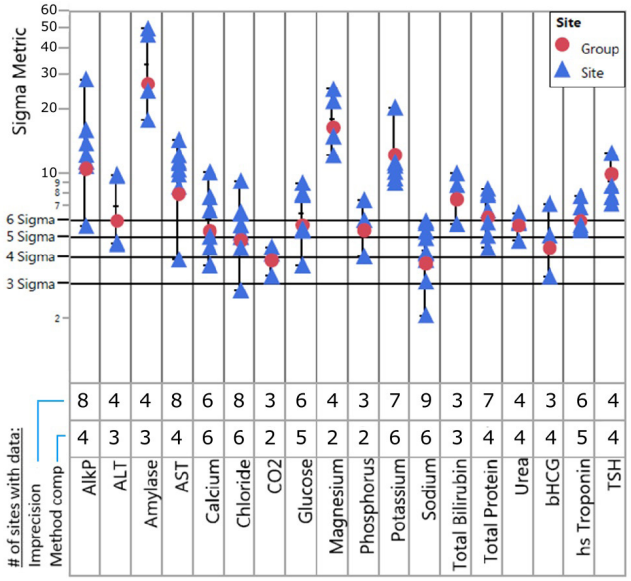
Background: The Abbott Alinity ci system is a next generation, integrated chemistry and immunoassay platform that has been launched in Europe and the Americas, providing an opportunity for evaluation under real-world conditions. The Sigma-metric offers a composite value of assay analytical performance, which was extended to assess performance across multiple sites. **Objective:** To assess multi-site Sigma performance of 3 ISEs, 3 immunoassays and 12 photometric assays on the Alinity ci system across 11 global sites including Austria, Belgium, Canada, France, Germany, Italy, Poland, Spain and UK. **Methods:** Total allowable error (TEa) goals followed the previously defined hierarchy (Westgard et al. Clin Biochem. 2017;50:1216-1221). Precision and method comparison studies were performed based on CLSI guidelines. At the medical decision point for each analyte, bias was calculated from available pooled method comparison data against the ARCHITECT system using Passing-Bablok regression analysis (minimum of two sites). Within-site Sigma-metrics were calculated as (%TEa - |%pooled bias|)/%CV and combined Sigma-metrics were calculated as (%TEa - |%pooled bias|)/%pooled total CV (minimum of 3 sites). **Results:** A total of 97 site-specific and 18 combined Sigma-metrics were calculated. Site-specific Sigma-metrics varied across sites, with 90% of assays performing at least 4 Sigma or higher. Similarly, 16 of 18 combined Sigma-metrics performed greater than 4 Sigma at or near the medical decision point, with Na and CO_2 having a performance just below at 3.7 and 3.9 Sigma, respectively. **Conclusion:** This multi-center study reveals realistic analytical performance of greater than 4 Sigma for majority of assays. Favorable Sigma-metrics were a result of both low imprecision and bias, indicating good comparability between the Alinity and ARCHITECT systems across independent sites but under a single manufacturer. Networked laboratories can use the combined Sigma-metric as a valuable tool to assess between-instrument analytic performance and consistency across multiple sites.



B-475

Multiple mutation detection for risk assessment in patients with breast cancer by using next-generation sequencing

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Background: Breast cancer is recognized as the most common cause of malignancy and cancer death worldwide, the most widely recognized causative genes for breast cancer are BRCA1 and BRCA2, mutations in which lead to hereditary breast cancer and ovarian cancer (HBOC) syndrome, and pose a lifetime risk of cancer between 40% and 80%. However, mutations in the cancer-related BRCA genes are detected in only 2%-3% of the patients with breast cancer. Moreover, risk-assessment studies have identified >90 genes associated with breast cancer incidence, although at least >1000 genes associated with increased risk of breast cancer remain to be identified. Because next-generation sequencing technology allows concurrent sequencing of numerous target genes, diverse cancer-susceptibility genes are now being evaluated, although their significance in clinical practice remains unclear. **Methods:**In this study, we enrolled 60 patients with sporadic breast cancer by using Ion Torrent sequencing technology. The BRCA-plus Panel was designed to target the entire CDS (100% covered) in these 6 key genes: BRCA1, BRCA2, TP53, PIK3CA, ERBB2 (Her2), and PTEN. We focused only on the germline mutations that could help us interpret the genetic phenomena of breast cancer. **Results:** Germline mutations were found to be carried by 9 patients (15%): 3 in BRCA1, 5 in BRCA2, and 1 in TP53. The overall mutation frequency of BRCA1/2 was 13.3%, and the mutation prevalence of non-BRCA genes was 1.6%. By comparison, the tendency of genetic-mutation occurrence in early breast cancer was clearer: before 40 years of age, 26.3% (5/19). The mutation frequency of the Luminal A samples was the highest (21.9%, 7/32), whereas that of the HER2-over-expressing samples was the lowest (0%); mutation frequencies of the Luminal B and triple-negative samples were 8.3% (1/12) and 12.5% (1/8), respectively. **Conclusion:** This study demonstrates the feasibility of using Ion Torrent sequencing technology for reliably detecting gene mutations in clinical practice for guiding individualized drug therapy or combination therapies for breast cancer.

B-476

Performance Evaluation of the Dimension Vista Hemoglobin A1c Assay

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Background: A revised Dimension Vista® Hemoglobin A1c (A1C) assay has been developed* for the measurement of HbA1c in venous whole blood. **Method:** The Dimension Vista A1C assay measures both HbA1c and total hemoglobin. The %HbA1c of the total hemoglobin is calculated and reported. **Total Hemoglobin Measurement:** Whole blood is added to a reaction vessel, which contains a reagent that lyses the red blood cells and converts the released hemoglobin to a derivative with a characteristic absorbance spectrum. An aliquot of the lysed whole blood is then transferred from the reaction vessel to a cuvette, where total hemoglobin concentration is measured photometrically. **Hemoglobin A1c Measurement (turbidimetric inhibition immunoassay):** The same aliquot of the lysed whole blood is used for the measurement of HbA1c. The cuvette contains an anti-HbA1c antibody that reacts with hemoglobin A1c in the sample to form a soluble antigen-antibody complex. A polyhapten reagent is then added, which reacts with excess (free) anti-HbA1c antibodies to form an insoluble antibody-polyhapten complex. The rate of this reaction is measured turbidimetrically and is inversely proportional to the concentration of HbA1c. Time to first result is <20 minutes. **Results:** Method comparison of the revised Dimension Vista A1C assay to NGSP yielded the following results: 1.000 (NGSP) using Passing-Bablok regression and 1.010 (NGSP) - 0.068 for Deming regression, n = 126. The assay's performance meets the current requirements for NGSP certification (≤ ±6.0% recovery bias) and would also meet the upcoming NGSP requirement of ≤±5.0% recovery bias. Within-lab reproducibility was ≤2.0% CV at NGSP target values of 5.0, 6.5, 8.0, and 12.0% HbA1c across three kit lots tested on three instruments each for each type of Dimension Vista System. The maximum total error at these same levels was found to be 5.4%. The assay is linear across the assay range of 3.8-14.0% HbA1c. No significant interference bias (less than or equal to ±5.0%) was observed for hemoglobin variants C, D, E, S, or A2. **Conclusion:** The Dimension Vista A1C assay from Siemens Healthineers demonstrates acceptable precision and correlation with the NGSP reference method. *Under development. The performance characteristics of this device have not been established. Not available for sale. Product availability will vary from country to country and will be subject to varying regulatory requirements.

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Performance Evaluation of the Dimension Hemoglobin A1c Assay

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Background: A revised Hemoglobin A1c (A1C) Assay for the Dimension® clinical chemistry system has been developed* for the measurement of HbA1c in venous whole blood. **Method:** The Dimension A1C Assay measures both HbA1c and total hemoglobin. The %HbA1c of the total hemoglobin is calculated and reported. **Total Hemoglobin Measurement**: Whole blood is added to the first cuvette, which contains a reagent that lyses the red blood cells and converts the released hemoglobin to a derivative with a characteristic absorbance spectrum. An aliquot of the lysed whole blood is then transferred to the second cuvette, where total hemoglobin concentration is measured photometrically. **Hemoglobin A1c measurement (turbidimetric inhibition immunoassay):** The same aliquot of the lysed whole blood is used for the measurement of HbA1c. The second cuvette contains an anti-HbA1c antibody that reacts with hemoglobin A1c in the sample to form a soluble antigen-antibody complex. A polyhapten reagent is then added, which reacts with excess (free) anti-HbA1c antibodies to form an insoluble antibody-polyhapten complex. The rate of this reaction is measured turbidimetrically and is inversely proportional to the concentration of HbA1c. Time to first result is <10 minutes. **Results:** Method comparison of the revised Dimension A1C Assay to NGSP yielded the following results: 0.991 (NGSP) - 0.034 (Passing-Bablok) and 0.979 (NGSP) + 0.046 (Deming), n = 121. The assay's performance meets the current requirements for NGSP certification (≤ ±6.0% recovery bias) and would also meet the upcoming NGSP requirement of ≤ ±5.0% recovery bias.. Within-lab reproducibility was ≤1.9% CV at target values of 5.0, 6.5, 8.0, and 12.0% HbA1c across three kit lots, each tested on three instruments. Maximum total error at these same levels was found to be 4.1%. The assay is linear across the assay range of 3.8-14.0% HbA1c. No significant interference bias (≤±5.0%) was observed for hemoglobin variants HbC, HbD, HbE, HbS, or HbA2. **Conclusion:** The Hemoglobin A1c (A1C) Assay for the Dimension® clinical chemistry system from Siemens Healthineers demonstrates acceptable precision and correlation with the NGSP reference method. *Under development. The performance characteristics of this device have not been established. Not available for sale. Product availability will vary from country to country and will be subject to varying regulatory requirements.

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EVALUATION OF NON INFERIORITY EFFECTIVENESS OF HYDROGEN PEROXIDE AND SILVER CATIONS DISINFECTION SYSTEM VS ACTIVE CHLORIDE FOR ERADICATION OF MULTIDRUG RESISTANT ORGANISMS

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Background: The objective of this study was to evaluate the non inferiority effectiveness of a disinfection system based on a micro-nebulization of hydrogen peroxide and silver cations vs. active chloride , by monitoring the reduction of microbial contamination of room surfaces. **Methods:** Active chloride (5.000 ppm) and saturated steam vapor (180 C°), vs. decontamination system based on a solution of 5-8%hydrogen peroxide and 60 ppm active silver ions (1mL/m^3 intensity of treatment) were compared. Two beds 26 rooms located in different wards were previously occupied by patients infected by: methicillin-resistant *Staphylococcus aureus* (MRSA) (4 cases); vancomycin-resistant *Enterococcus faecium* (VRE) (2 cases); extensively drug-resistant (XDR) *Acinetobacter baumannii* (2 cases); metallo-beta-lactamase-producing (MBL) *Pseudomonas aeruginosa* (2 cases)*; Kleibsiella* carbapenemase) producing (KPC) (4 cases); extended-spectrum *beta*-lactamase producing (ESBL) *Klebsiella pneumoniae* (2 cases), ESBL-*Escherichia coli* (2 cases), *Stenotrophomonas maltophilia* with phenotype of resistance to trimethoprim-sulfamethoxazole (2 cases) respectively. Environment and medical equipment disinfection procedures were performed prior to a new bed occupancy in addition to routine cleaning activities by the cleaner staff. Surface samples were taken from 10 high touch environmental surfaces which included: room door handle, headboard, footboard, bed frame, bedside table top, bedside table handle, light switch, floor corner, sink taps, soap dispenser. Microbial colonisation was assessed at Time 0 (T0) before cleaning, T1 immediately after cleaning and T2 after disinfection procedures, by use of pre-moistened with sterile saline cotton tipped sterile swabs. The swabs were used to sample surface areas approximately 57 cm^2 by standardized swabbing in two directions at right angles. All swabs were inoculated on blood agar plates and in broth and incubated for 48 hours at 37°C. Cleaning effectiveness of surfaces was evaluated by quantifying the total number of aerobic organisms from a sampled area (total aerobic colony count) and expressed in colony forming units (CFU) per cm^2. Organisms were identified by standard microbiological methods. **Results:** 780 surface samples were collected as follows: 600 from rooms treated with hydrogen peroxide and 180 with active chloride and steam vapor, respectively. Before cleaning the surfaces all samples collected in the rooms resulted colonised, with an average density of mesophile organisms up to 56 CFU/57 cm2 (range 0-400). MDROs were isolated from samples collected in 20/26 rooms respectively. After manual cleaning with detergent followed by active chloride disinfection, an average density of organisms of 15 CFU/57 cm2 (range 0-270) was recorded. MDROs were found from samples collected in 2 rooms but only after an enrichment step. After heated saturated steam vapor disinfection, an average density of organisms of of 15 CFU/57 cm2 (range 1-30) was observed. MRSA were found from samples collected in 2 rooms. After hydrogen peroxide disinfection, a density of bacteria in the range of 0 and 3 CFU/57 cm^2 was observed and no MDROs were found. **Conclusion:** . Our data indicate that the hydrogen peroxide and active silver ions disinfection system, together with the manual cleaning procedures, is non inferior vs. active chloride based procedure. Hydrogen peroxide resulted effective in minimizing the overall microbial load on the hospital room surfaces and in eradicating MDROs

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Evaluation of the Freelite Mx™ Kappa Free and Lambda Free assays on The Binding Site Optilite® turbidimetric analyser using CSF samples

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Inflammation of the central nervous system caused by infections or autoimmune disorders can lead to the synthesis of intrathecal immunoglobulins. Immunoglobulin free light chains (FLCs) are typically secreted along with intact immunoglobulins from plasma cells and if produced intrathecally accumulate locally in CSF. The measurement of FLCs in CSF is a potentially sensitive marker of intrathecal immunoglobulin synthesis and elevated levels of free light chains in CSF may be indicative of central nervous system diseases. Performance characteristics of serum and urine Freelite assays have previously been described. Here we discuss the performance of Free-

lite Mx Kappa Free and Freelite Mx Lambda Free for the detection and quantification of FLCs in CSF on The Binding Site Optilite analyser. A linearity study performed according to CLSI-EP06 guidelines verified that the κ-FLC assay was linear over the measuring range of 0.33 - 12.7 mg/L at the 1+0 analyser dilution recommended for CSF analysis. The λ-FLC assay was also demonstrated to be linear using a serially diluted CSF sample over the measuring range of 0.74 - 17.4 mg/L at the 1+0 sample dilution; with all results being within 10% of expected values. Reference range analysis using 24 oligoclonal banding (OCB) negative CSF samples deemed the κ reference interval to be <0.1 - 1.96 mg/L whereas the reference interval for λ-FLC was not quantifiable. Precision studies were performed based on the CLSI approved guideline EP5-A2, testing two CSF samples on a single kit lot over three analysers and five days. The total precision coefficients of variation (CVs) were 9.4% at 0.51 mg/L and 6.8% at 10.79 mg/L for κ-FLC assessment, and 12.2% at 1.06 mg/L and 5.9% at 15.35 mg/L for λ-FLC assessment. A comparison study was performed by analysing 81 CSF samples using the Optilite Freelite Mx Kappa Free kit and an alternative commercially available assay. Statistical analysis demonstrated good agreement between the assays using Passing and Bablok analysis (y = 0.98x - 0.00) and linear regression (r=0.974). A comparison study was performed by analysing 99 CSF samples using the Optilite Freelite Mx Lambda Free kit and an alternative commercially available assay. Statistical analysis demonstrated good agreement between the assays using Passing and Bablok analysis (y = 0.95x - 0.21) and linear regression (r=0.996). Interference testing was performed according to CLSI EP7-A2 guidelines using a CSF sample close to both Kappa and Lambda medical decision points. No significant assay interference was observed with acetaminophen (1324µmol/L), acetylsalicylic acid (3.62mmol/L), haemoglobin (2.5g/L), conjugated bilirubin (200mg/L) and unconjugated bilirubin (200mg/L). We conclude that the Optilite Freelite Mx Kappa Free and Freelite Mx Lambda Free kits provide a reliable, accurate and precise method for quantifying free light chains in CSF and would be useful in identifying patients with central nervous system diseases.

B-480

Performance of the Lipoprotein (a) assay for use on the Binding Site Optilite® turbidimetric analyser

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The Optilite Lipoprotein (a) [Lp(a)] Reagent is intended for the quantitative in vitro measurement of Lp(a) in serum using the Binding Site Optilite analyser to aid in the assessment of lipid disorders and risk of atherosclerotic cardiovascular disease. Lp(a) is a lipoprotein found in plasma. It consists of a cholesterol-rich low density lipoprotein (LDL) molecule bound to one molecule of apolipoprotein B100 with an additional protein, apolipoprotein (a) [apo(a)], attached via a disulphide bond. Apo(a) can vary in size depending upon the isoform. Apo(a) may inhibit fibrinolysis by competing with plasminogen due to its considerable structural homology; this effect is not observed with LDL free of apo(a). Lp(a) is considered an atherogenic risk factor marker, independent of other lipid parameter markers and exogenous factors such as diet. Increased Lp(a) levels have a high predictive value for coronary heart disease (CHD), especially in combination with elevated LDL cholesterol. Whilst the determination of total cholesterol and triglycerides is used for coronary risk screening, measurement of Lp(a), alongside LDL-cholesterol, HDL-cholesterol, apolipoprotein A-1 and apolipoprotein B, is a valuable tool in differential diagnosis of CHD. Here we describe the quantification and performance of the immunoassay used to detect Lp(a) on the Binding Site Optilite analyser. A linearity study was performed following CLSI Approved Guideline EP6-A. The linearity of this assay has been confirmed at the standard 1+3 using a serially diluted serum sample over the range of 9.63 - 322.73 nmol/L with deviation from linearity <10%. A comparison study was performed by analysing 138 samples (including 103 samples with analyte levels within the reference interval) using the Optilite Lipoprotein (a) assay and an alternative commercially available assay. A Passing and Bablok regression of Y=0.99x + 2.20 was obtained with a correlation coefficient of r=0.999 generated via linear regression. A precision study based on CLSI guideline EP05-A2 was performed over a 5 day period with 2 runs per day. Precision was assessed using 4 samples with different analyte concentrations. The between run coefficients of variation (CVs) were as follows: 1.84% at 39.725 nmol/L; 1.94% at 52.440 nmol/L; 1.85% at 109.195 nmol/L; 1.67% at 155.655 nmol/L. An interference study was performed according to CLSI guideline EP7-A2. Interferents tested include Intralipid (2000 mg/dL), conjugated bilirubin (55.0 mg/dL) unconjugated bilirubin (69.1 mg/dL), haemoglobin (575 mg/dL) and Rheumatoid Factor (520 IU/mL). No significant assay interference was observed when performed at the standard 1+3 sample dilution. In conclusion, the Lipoprotein (a) assay for the Optilite provides a reliable and precise method for quantifying Lp(a) content in human serum and correlates well with existing methods.

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Performance Evaluation of ARCHITECT HSV-1 and HSV-2 IgG Assays on the ARCHITECT Analyzer

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Background: Herpes infection can be caused by both herpes simplex type 1 (HSV-1) and 2 (HSV-2) viruses. HSV-1 infection causes recurrent skin blistering, mainly affecting the facial area, although it can occasionally cause genital herpes. HSV-2 infection is the main cause of genital herpes, a recurrent ulceration in the genital area. When vertically transmitted, HSV-2 may cause neonatal herpes that may result in neurological damage or death. Both, HSV-1 and HSV-2 infections are chronic infections, highly prevalent and widespread throughout the world. HSV diagnosis and subtype differentiation is essential for the disease management. ARCHITECT HSV-1 and HSV-2 IgG assays are chemiluminescent, two-step immunoassays for the qualitative detection of antibodies against HSV-1 and HSV-2 in human serum and plasma. The aim of this study was to evaluate the assays performance on the ARCHITECT Analyzer. **Methods:** Performance was assessed by comparing the new ARCHITECT assays to the BIO-FLASH® HSV-1 and HSV-2 IgG assays, using two subsets of serum samples (549 for HSV-1, and 529 for HSV-2). Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA) were calculated according to CLSI EP12-A2. For assessment of the assay standardization, sample subsets with samples mainly within the assays critical zone were selected (72 for HSV-1 and 63 for HSV-2) and tested with HerpeSelect® 1 and 2 Immunoblot IgG method. Assuming concordant results between assays in samples beyond the critical zone (0.50-5.00 S/CO in HSV-1, and 0.30-3.00 S/CO in HSV-2), ROC analysis was applied to estimate optimal PPA and NPA against Immunoblot assay. Precision was also evaluated following a 10 days x 2 run x 2 replicates design as per CLSI EP15-A3. **Results:** For ARCHITECT HSV-1 IgG assay, PPA and NPA compared to the BIO-FLASH assay were 99.7% and 98.3% respectively. After further testing of samples within the assays critical zone, PPA and NPA compared to Immunoblot were 97.8% and 91.9%, respectively. Using ROC curves, an optimal cut-off for re-standardization of the ARCHITECT HSV-1 IgG assay was calculated at 1.70 S/CO (PPA and NPA of 96.7% and 95.7% compared to Immunoblot, respectively). Total imprecision for ARCHITECT HSV-1 IgG assay was found to be 0.020 SD for the Negative Control (0.31 S/CO) and 5.5%CV for the Positive Control (2.88 S/CO). For ARCHITECT HSV-2 IgG assay, PPA and NPA compared to the BIO-FLASH assay were 98.1% and 96.6%, respectively. After testing of samples within the critical zone, PPA and NPA compared to Immunoblot were 96.0% and 97.4%, respectively. An optimal cut-off for re-standardization of the ARCHITECT HSV-2 IgG assay was calculated at 0.79 S/CO (PPA and NPA of 98.9% and 96.9% compared to Immunoblot, respectively). Total imprecision for ARCHITECT HSV-2 IgG assay was found to be 0.013 SD for the Negative Control (0.30 S/CO) and 5.0%CV for the Positive Control (3.13 S/CO). **Conclusion:** After re-standardization, the ARCHITECT HSV-1 and HSV-2 IgG assays show suitable performance in terms of agreement to other commercially available methods, which together with the excellent features of the ARCHITECT analyzer, make these assays a remarkable choice for routine use in clinical laboratories.

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Performance characteristics of new UIBC reagent on ARCHITECT cSystems

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OBJECTIVE: To assess the performance characteristics of the new Abbott liquid Unsaturated Iron Binding Capacity (UIBC) assay (LN 04R29) in comparison to the lyophilized Abbott UIBC assay (LN 04P79) on the ARCHITECT *c*System instruments. **RELEVANCE:** The new ready to use liquid UIBC assay (LN 04R29), offered as a replacement for the lyophilized predicate UIBC assay (LN 04P79), is expected to display an improved performance in the form of improved measuring interval, calibration interval and precision profile. **METHODOLOGY:** The UIBC assay (LN 04R29) uses the same methodology as the predicate UIBC assay (LN 04P79), which is Ferene methodology. This methodology comprises of addition of a known concentration of iron to a serum or plasma sample, followed by *3-(2-pyridyl)-5,6-bis-[2-(5-furylsulfonic*

acid)]-1,2,4-triazine (Ferene-S) and a reducing agent. The residual iron from the first step of transferrin saturation forms a stable, reduced, ferrous complex with Ferene-S in the second step. The color intensity of this complex, measurable at 604 nm, is directly proportional to the unbound excess iron-binding capacity. **VALIDATION:** The interference of hemoglobin, intralipid, conjugated bilirubin, unconjugated bilirubin, triglycerides, total protein and rheumatoid factor with UIBC assay was evaluated using a low and a high level of analyte concentration. Analyte recovery within ±10% for analyte concentration greater than or equal to 143µg/dL or within ±14µg/dL for concentration less than 143µg/dL was deemed acceptable. Passing interferent levels for the low level of analyte were found to be 62mg/dL of Hemoglobin, 1000mg/dL of Intralipid, 59mg/dL of conjugated bilirubin, 53mg/dL of unconjugated bilirubin, 901mg/dL of triglycerides, 13.2g/dL of total protein and 100IU/mL of rheumatoid factor respectively. The table below displays the comparison of critical performance characteristics of the new UIBC assay (LN 04R29) and the predicate assay UIBC (LN 04P79).

Comparison of UIBC (LN 04R29) against predicate UIBC (LN 04P79)		
Characteristic	**LN 04R29**	**LN 04P79**
Sample Type	Serum and plasma	Serum and plasma
Limit of Quantitation (µg/dL)	≤ 25	≤ 41
Linearity and Measuring Interval (µg/dL)	25 - 500	41 - 500
Precision	≤ 7% or ≤10µg/dL	≤ 13.3%
Calibration Interval (hours)	168	24
Method Comparison	**LN 04R29 vs. LN 4P79**	
	N	109
	R	0.995
	Equation	Y = 8.03 + 0.9736X
	Range (µg/dL)	31.5 to 458.8

CONCLUSIONS: The new ready to use liquid UIBC assay (LN 04R29) demonstrates better performance in comparison to the predicate method (LN 04P79).

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Performance Evaluation of Atellica IM 1600 Analyzer Assays in a Clinical Chemistry Laboratory

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Background: At our institution, studies were performed to assess the analytical performance of immunoassays (IM) for the Atellica IM Analyzer with respect to verification of precision and linearity, and method comparison with Siemens current assays on ADVIA Centaur® XP System. **Methods:** Precision verification was performed according to EP15-A3, method comparison per EP09-A3, and linearity per EP06-A. For precision verification, three or four concentration levels were used; each level of QC materials and sample pool were tested as one run per day with five replicates per run, for five days (25 total replicates per sample for each assay). Method comparison studies were performed using 40 serum samples that covered each assay range, from low to high; samples fell into four assay concentration ranges). The number of levels of linearity material ranged up to nine depending on the assay. For each assay, three replicates of each sample level were assayed. Siemens Healthineers supported the study by providing systems, reagents and protocols and contributed to data analysis. **Results:** Within-run and total imprecision agreed with the manufacturer's claims. Within-run (repeatability) IM CVs ranged from 1.0% to 7.5% and total (within lab) IM CVs from 0.5% to 7.5%. Linearity studies were performed for all assays. Precision and method comparison studies are summarized below.

		Precision			Method Comparison
Atellica IM Analyzer Assay	**Units**	**Mean concentrations (low, high)**	**Within run %CV(SD) (low, high)**	**Total %CV(SD) (low, high)**	**Atellica IM Analyzer vs. ADVIA Centaur XP System**
Fer	ng/mL	29.43, 304.26	2.6(0.77), 1.0(3.18	2.6(0.77), 1.1(3.37)	*
VitD	ng/mL	16.06 95.86	5.4(0.87) 1.7(1.62)	5.4(0.87) 1.7(1.62)	*
PSA†	ng/mL	0.13, 15.13	4.2(0.01), 2.2(0.34)	4.2(0.01), 2.2(0.34)	0.992x-0.010
eE2	pg/mL	33.84, 948.34	5.8(1.96), 2.6(25.02)	5.8(1.96), 2.6(25.02)	1.102x-10.095
ThCG†	mIU/mL	5.30, 380.48	3.7(0.19), 1.9(7.07)	3.7(0.20), 1.9(7.07)	1.017x+1.734
PRGE	ng/mL	20.51, 5853.80	1.9(0.39), 0.5(27.26)	1.9(0.39), 0.5(27.26)	*
TSTII	ng/dL	135.54, 1132.11	2.2(2.92), 2.7(30.36)	2.3(3.05), 3.5(39.28	*
TSH3-UL	mIU/mL	0.64, 27.29	1.4(0.01, 1.7(0.46)	1.4(0.01), 1.8(0.48)	1.019x-0.015

*Method comparison not done. † Not available for sale in the U.S. Future availability cannot be guaranteed. **Conclusions:** All assays tested on the Atellica IM 1600 Analyzer demonstrated good precision and correlation to the current ADVIA Centaur XP System assays. The precision results were consistent with manufacturer's claims.

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Performance Evaluation of the Atellica CH 930 Analyzer Assays in a Clinical Chemistry Laboratory

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Background: Studies were performed at our institution to assess the analytical performance of clinical chemistry (CH) assays for the Atellica® CH 930 Analyzer with respect to verification of precision and linearity, and method comparison with Siemens current assays on the ADVIA® 1800 System. **Methods:** Precision verification was performed according to EP15-A3, method comparison per EP09-A3, and linearity per EP06-A. For precision verification, three or four concentration levels were used; each level of QC materials and sample pool were tested as one run per day with five replicates per run, for five days (25 total replicates per sample for each assay). Method comparison studies were performed using 40 serum samples that covered each assay range, from low to high; samples fell into four assay concentration ranges). The number of levels of linearity material (LGC Maine Standards) ranged up to six depending on the assay. For each assay, three replicates of each sample level were assayed. Siemens Healthineers supported the study by providing systems, reagents and protocols and contributed to data analysis. **Results:** Within-run and total imprecision agreed with the manufacturer's claims. Within-run (repeatability) CH CVs ranged from 0.2% to 4.8% and total (within lab) CH CVs from 0.4% to 8.2%. Linearity studies were performed for all assays. Precision and method comparison studies are summarized below.

	Precision			Method Comparison		Precision			Method Comparison
Atellica CH 930 Analyzer Assay	**Mean concentrations (low, high)**	**Within run %CV (SD) (low, high)**	**Total %CV (SD) (low, high)**	**Atellica CH 930 Analyzer vs ADVIA 1800 System**	**Atellica CH 930 Analyzer Assay**	**Mean concentrations (low, high)**	**Within run %CV (SD) (low, high)**	**Total %CV (SD) (low, high)**	**Atellica CH 930 Analyzer vs ADVIA 1800 System**
GluH_3	59.04, 348.00 mg/dL	0.5(0.32), 0.3(1.17)	0.6(0.36), 0.5(1.59)	0.979x - 0.45 mg/dL	TP	4.05, 7.15 g/dL	1.1(0.04), 0.7(0.05)	1.3(0.05), 0.8(0.06)	0.958x + 0.022 g/dL
Chol_2	108.20, 279.12 mg/dL	0.7(0.72), 0.7(1.88)	1.0(1.05), 0.8(2.33)	0.935x - 1.488 mg/dL	Alb	2.59, 4.39 g/dL	1.3(0.03), 1.0(0.04)	1.6(0.04), 1.2(0.05)	0.970x + 0.147 g/dL
UN_c	13.88, 72.64 mg/dL	2.3(0.35), 0.7(0.51)	2.3(0.35), 1.3(0.91)	1.005x - 0.988 mg/dL	Trig	93.40, 212.32 mg/dL	0.8(0.75), 0.6(1.30)	1.3(1.18), 0.8(1.77)	1.090x - 6.853 mg/dL
Crea_2	0.78, 6.44 mg/dL	1.1(0.01), 0.4(0.02)	1.1(0.01) 0.7(0.05)	1.045x - 0.007 mg/dL	TBil_2	0.66, 7.61 mg/dL	4.8(0.03), 0.6(0.05)	8.2(0.05), 0.8(0.06)	1.036 + 0.046 mg/dL
Ca	5.51, 13.45 mg/dL	1.9(0.11), 1.1(0.14)	2.5(0.14), 1.2(0.15)	1.011x - 0.090 mg/dL	Na	113.88, 157.48 mEq/L	0.4(0.40), 0.3(0.45)	0.5(0.55), 0.4(0.61)	1.000x - 0.100 mmol/L
AST	42.52, 293.28 U/L	1.8(0.76), 0.4(1.03)	1.8(0.76), 1.1(3.25)	1.050x - 1.729 U/L	K	2.60, 7.33 mEq/L	0.3(0.01), 0.2(0.02)	0.5(0.01), 0.4(0.03)	0.973x - 0.029 mmol/L
ALT	32.20, 214.04 U/L	2.6(0.85), 0.5(1.02)	2.7(0.87), 0.7(1.39)	1.073x + 0.368 U/L	Cl	78.00, 120.72 mEq/L	0.4(0.30), 0.2(0.28)	0.4(0.30, 0.7(0.80)	0.990x + 1.404 mmol/L
D-HDL	29.42, 82.96 mg/dL	1.7(0.51), 0.6(0.46)	1.7(0.51), 0.6(0.53)	1.048x + 0.560 mg/dL	APO A1	93.8, 126.1 mg/dL	0.7(0.69), 1.0(1.25)	2.1(1.96), 1.0,(1.27)	1.035x + 1.746 mg/dL
DLDL	61.43, 160.11 mg/dL	0.6(0.37), 0.6(0.98)	0.7(0.45), 0.7(1.14)	1.067x - 0.284 mg/dL	APO B	39.52, 133.00 mg/dL	1.4(0.57), 0.7(0.94)	2.2(0.86), 1.0(1.31)	0.967x - 8.282 mg/dL

Conclusions: All assays tested on the Atellica CH 930 Analyzer demonstrated good precision and correlation to the current ADVIA 1800 System assays. The precision results were consistent with manufacturer's claims.

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Evaluation of on-board storage and method performance for 8 assays on the Abbott Alinity ci integrated analyzer

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Background The Alinity ci is a next generation integrated system providing a standalone platform for continuous testing of chemistry and immunoassay tests. The Alinity ci also possesses a novel temperature-controlled, on-board storage (OBS) functionality for quality control (QC) and calibrator materials for improved ease-of-use. **Objectives** (1) To evaluate the Alinity ci for precision on the Alinity ci system. (2) To evaluate method comparison against the Abbott ARCHITECT system. (3) To determine the performance of OBS of QC materials compared to conventional off-board storage. **Methods** Eight assays were evaluated using the serum application: 3 ISE (Sodium, Potassium, and Chloride), 3 chemistry (Magnesium, Glucose, Creatinine), and 2 immunoassays (TSH, 25-OH vitamin D). Precision and method comparison studies were performed according to CLSI guidelines. Method comparison was performed in duplicate for both ARCHITECT and Alinity systems over 3 days with a minimum of 100 samples per day. For on-board storage performance, Alinity Technopath Serum S Plus controls for Sodium, Potassium, Chloride, and Glucose, at 3 levels of QC were tested (n=20 per level) for up to 6 days and compared against typical off-board storage condition in a refrigerator. **Results** Precision and method comparison data is summarized in the table below. OBS of QC materials performed similarly to the materials placed under conventional storage. Within-day absolute percent difference was ≤1.6% (Range: -0.4% to 1.6%) for all materials, and absolute percent difference in measured levels from the initial day of off-board storage, difference was ≤1.6% (Range: -0.9% to 1.6%). **Conclusion** The Alinity ci shows excellent real-world performance that is comparable to the ARCHITECT platform with the added storage functionality. On-board QC material storage showed comparable stability and performance to offline storage, thus allowing for improved workflow and ease-of-use.

Summary of Alinity ci Precision and Method Comparison Studies

Analyte	5-day Total Precision						Method Comparison	
	Level 1		Level 2		Level 3		Passing-Bablok Equation	Correlation (R^2)
	Mean	%CV	Mean	%CV	Mean	%CV		
Sodium (mmol/L)	119.0	1.9%	141.7	1.8%	162.0	1.8%	Y=0.999x+1.787	0.989
Potassium (mmol/L)	2.42	1.6%	3.55	1.8%	6.18	2.0%	Y=1.033x-0.042	0.998
Chloride (mmol/L)	77.5	1.8%	92.4	1.8%	105.2	1.8%	Y=1.023x-0.983	0.994
Magnesium (mmol/L)	0.51	2.7%	1.04	0.6%	1.77	0.8%	Y=1.049x-0.002	0.990
Creatinine (mg/dL)	0.66	2.4%	1.95	1.9%	5.74	2.7%	Y=0.987x-0.116	1.000
Glucose (mmol/L)	2.58	2.0%	6.66	1.4%	15.27	0.7%	Y=0.963x+0.008	0.999
TSH (mIU/L)	0.11	1.2%	6.14	1.8%	30.99	2.3%	Y=1.011x+0.001	0.975
25-OH VitD (ng/mL)	19.38	4.0%	40.72	3.0%	78.37	2.8%	Y=0.900x+1.671	0.982

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Cross Reactivity and Interference Results for a Novel 5-plex Panel for the Detection of Acute Kidney Injury

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Background: Acute kidney injury (AKI) is currently classified by serum-based creatinine (e.g., KDIGO) guidelines. However, since creatinine is a lagging index of impending AKI, studies are now underway to qualify a set of biomarkers for detecting drug-induced human kidney injury (DIKI) in clinical trials. Assessment of renal injury using multiple biomarkers is more clinically discriminating than single biomarker analysis. To provide improved turn-around time we have developed a multiplex immunoassay for the urine biomarkers of AKI; KIM-1, NGAL, cystatin C, clusterin and osteopontin (OPN). This work describes the cross reactivity and interference results for our novel multiplex immunoassay. **Methods:** Randox Biochip Array technology was chosen for the development of the five-plex immunoassay using proprietary and commercially available antibodies. Cross reactivity was tested for each analyte from potentially related, but non-panel proteins. For OPN; non-panel biomarkers included enterokinase, thrombin, MMP-7 and MMP-3. For NGAL; α1-acid glycoprotein, α1-microglobulin, HGF, MMP-2, -8 & -9 were tested. For cystatin C; complement C4, MMP-9, cystatin D, HRPG, kininogen, fetuin A & B, cathepsin -L, -B, -D, & -S were tested. For clusterin; Apo-A1, -A2, -B, -B100, -C1, -C2, -D, -E2, -E3, -E4, -H, –M and CLUL-1 were tested. Interfering substances known to be present in urine were titred to determine a non-interfering concentration (within +/- 10%) for cystatin C, clusterin, NGAL and OPN. Interferents included human serum albumin, hemoglobin, bilirubin, pH, glucose, sodium chloride and creatinine. Interference and cross reactivity of non-panel proteins for KIM-1 are pending. **Results:** Cross reactivity was <1% for all tested non-panel, related proteins. Human serum albumin non-interfering concentration was <0.5 mg/mL for clusterin, <3 mg/mL for OPN and <5 mg/mL for cystatin C and NGAL. Hemoglobin non-interfering concentration was <0.5 mg/mL for cystatin C and NGAL, <1 mg/mL for OPN and KIM-1, and <62.5 µg/mL for clusterin. Bilirubin non-interfering concentration was <1 mg/mL for cystatin C and <2 mg/mL for remaining panel biomarkers. Glucose, sodium chloride and creatinine non-interfering concentration was <30 mg/mL, <60 mg/mL and <5 mg/mL, respectively, for all panel biomarkers. **Conclusion:** Interference for substances known to be present in urine are above physiological levels. Cross reactivity results for non-panel proteins indicate no significant cross reactivity. Previously reported data show this multiplex for urine biomarkers is sensitive, precise, linear and has a wide dynamic range. These results and those from the current study demonstrating good selectivity, document the development of an in vitro diagnostic that fills the need for an objective, robust and cost-effective solution to diagnosis and monitoring DIKI and AKI in other settings.

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Universal automated site-specific antibody conjugation

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Background: We previously demonstrated a novel, site-specific, chemoenzymatic, antibody labeling technology that can be applied to essentially any existing antibody with Fc-domain N-linked glycans. We showed this antibody labeling technology has universal application and can be applied to multiple workflows from conjugating antibodies with small organic molecules to large fluorescent Qdot probes and magnetic beads. We have also demonstrated application of the technology to the production of site-specific radioimmune-PET imaging probes, antibody-drug conjugates (ADCs), and tetravalent bi-specific antibodies. The conjugation technology ensures preservation of antigen binding activity, it allows for easy characterization of the sites of labeling, and the reproducibility of labeling is unparalleled when compared to other conventional labeling methods. Three key aspects of the conjugation method, the targeted enzymatic approach, the unprecedented antibody-to-antibody reproducibility of labeling, and the highly-stable labeling reagents, lend the technology to automation. We present here a "load-and-go" automated antibody conjugation platform that yields site-specifically labeled antibodies with high antibody-to-antibody reproducibility. **Methods**: Antibody glycans were modified using a permissive beta-galactosyltransferase enzyme which azide-activates antibody Fc domain glycans using an azide-functionalized sugar substrate. The azide-activated antibodies were conjugated to a fluorescent dibenzocyclooctyne (DIBO) dye in a copperless click reaction. Automated enzymatic azide-activation and DIBO-dye conjugation of the antibodies was performed utilizing magnetic agarose beads. After conjugation, antibodies were eluted from the beads, neutralized, and the degree of labeling (DOL) of the antibodies was determined using fluorescent spectroscopic and fluorescent protein gel scanning methods. **Results**: The entire automation process was completed in a single overnight run. Antibodies were site-specifically labeled with a degree of labeling of 2 or 3 (depending on the sample preparation method used). The antibody-to-antibody reproducibility of labeling was greater than 90% when antibodies were labeled at the same time, during the same run, or between different runs. The preliminary yields, without any optimization, were within the range of 55-65%. **Conclusions**: We present here a "load-and-go" automated antibody labeling platform that site-specifically labels antibodies with high reproducibly while preserving antigen binding activity and antibody integrity. In a single overnight run, up to 5 antibodies can be labeled simultaneously with different payloads. The automated work flow should be compatible with multiple different payloads including, but not limited to, fluorescent dyes, biotin, PET chelators, ADC toxins, and Qdots.

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Development of the Novel and New Multi-Test VITROS® XT Chemistry Products Slides

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Background: A series of new VITROS XT Chemistry Products Slides^ with dual test capability have been developed that are intended to reduce sample size and enhance operational efficiency while maintaining analytical performance versus a conventional single assay test element. The six new XT Slide products are the VITROS XT Chemistry Products UREA-CREA Slides, ALTV-AST Slides, TRIG-CHOL Slides, ALB-TP Slides, GLU-Ca Slides, and TBIL-ALKP Slides. **Methods:** These new slide products are unique in that they allow two tests to be run in a single test element, something that is not currently done in any solution based analytical element in the clinical chemistry laboratory on an automated analyzer. One enhancement of the new Multi-Test Slides is the total sample volume required can be reduced with the smaller test element (0.675 cm^2). The total sample volume to run the twelve XT Slides is 45.6 uL ranging from 2.7 uL for GLU to a maximum of 5.0 uL for ALKP, and decreasing the sample volume by 49% from the previously reduced low sample volume required for these same VITROS Slides (89.5 uL). A second planned improvement is that two tests are run in a single analytical element, therefore increasing the number of tests per hour and increasing the analyzer throughput. For example, the simulated throughput running the comprehensive metabolic panel increases from 681 to 976 tests/hr, a 43% throughput increase. If only the XT Slides were run in the sample mix, a 100% increase in throughput would be realized. **Results:** The new XT Slide products are also planned to maintain the same analytical performance observed with the current VITROS Slide products. We evaluated the accuracy of patient serum samples (UREA: n=124, 2.6 - 106.0 mg/dL; CREA: n=134, 0.13 - 13.68 mg/dL) on the VITROS XT 3400 Chemistry System (in development) compared to the VITROS Chemistry Products CREA Slides. The VITROS XT UREA-CREA Slides showed excellent correlation with the VITROS BUN and CREA Slides. VITROS XT UREA-CREA = 0.999 * VITROS BUN + 0.68; (r) = 0.999 for UREA; VITROS XT UREA-CREA = 0.986 * VITROS CREA - 0.01; (r) = 1.000 for CREA. For the XT ALTV-AST Slide, we evaluated the accuracy of patient serum samples (ALTV: n=132, 5.2 - 744.9 U/L; AST: n=123, 9.5 - 739.4 U/L) on the VITROS XT 3400 Chemistry System (in development) compared to the VITROS Chemistry Products ALTV Slides. The VITROS XT ALTV-AST Slides showed excellent correlation with the VITROS ALTV and AST Slides. VITROS XT ALTV-AST = 0.995 * VITROS ALTV - 0.68; (r) = 0.999 for ALTV; VITROS XT ALTV-AST = 1.001 * VITROS AST + 0.29; (r) = 1.000 for AST. The other four XT Slides show similar accuracy versus their corresponding VITROS Chemistry Products Slides, and all six XT Slides also show precision similar to their corresponding VITROS Chemistry Products Slides. **Conclusion:** With these added features and performance, the new VITROS XT Slides will provide an enhancement to the operational efficiency in the clinical laboratory. ^in development

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Performance Evaluation for an Automated Assay for the Measurement of 17aHydroxyprogesterone on Diasorin's ETI-MAX 3000 Analyzer by ELISA Method

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Background: 17 α - Hydroxyprogesterone (17 OHP) is a steroid produced by the adrenal cortex and gonads. Measurement of 17 OHP is useful indirect indicator of 21-hydroxylase defiency, the most common variety of congenital Adrenal hyperplasia (CAH), as 17 OHP is secreted in abundant excess. Measurement of 17 OHP is therefore valuable in the initial diagnosis of CAH. **Objectives**: The objective of the study is to evaluate the performance of ETI-MAX 3000 using Diagnostics Biochem Canada Inc. (DBC) reagents for both adult and pediatric patients, for the analysis of 17OHP. **Method:** 17OHP method validation was performed using Diagnostics Biochem Canada (DBC) ELISA KIT on ETI-MAX 3000 analyzer from Diasorin. Method validation was done according to the hospital policy which follows CLSI guidelines (EP05-A3/ EP06-A/ EP09-A3/ EP17-A2). Precision study was performed using 40 quality control samples of 2 different concentration for a period of 20 days : Mean , SD and %CV was calculated and compared to the manufacturer recommendation. Sensitivity test was performed using 10 samples of zero 17OHP standard, Mean and +2SD was calculated and compared to analytical sensitivity claimed by manufacturer. Method comparison study was done comparing 20 sample proficiency testing samples to peer group using ELISA method. Slope intercept correlation coefficient was calculated to check the acceptability of the method. Linearity study was done using 7 different concentration standards (calibrator) samples spanning the analytical measurement range (AMR) from 0.11- 20.0 ng/ml. Reference range: 20 normal males and 20 normal females samples were analyzed to verify the manufacturer's recommended reference range. Acceptable criteria is 90% (18 samples must be acceptable out of 20 sample). Age range 21- 48 yrs old for male and range 19- 50 years for female. **Result:** Between days precision study for low QC and high QC % CV was 3.0- 4.7 % respectively. Both %CV were consistent with those claimed by manufacturer. The limit of quantitation was observed at < 0.11 ng/ml which agree with the manufacturer claim. Method comparison acceptable criteria slope 0.90- 1.10, intercept close to zero and r > 0.975. Data was plotted on scatter plot the yield slope was 1.1, Intercept 0.251 and correlation coefficient (r)= 0.998, all results were within acceptable criteria. Linearity: The method was found linear over the AMR of 0.11- 20.0 ng/ml with reportable range up to 160 ng/ml with manual dilution. Reference range study : 100 % of males and females samples result were within the manufacturer's claim for reference range. **Conclusion:**. Overall performance of 17 α – Hydroxyprogesterone on DiaSorin ETI-MAX 3000 was acceptable. It provides reliable results for the required test for both adult and pediatric patients.to help in the diagnosis or monitoring of 17OHP, as the distinguishing characteristics of 21-hydroxylase deficiency is a high serum concentration of 17 α – Hydroxyprogesterone

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Evaluation and comparison of the new Free Testosterone CLIA on the ids/iSYS system

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Background: The measurement of free testosterone (fT) is indicated when imbalance between synthesis of testosterone and its binding proteins is suspected. The goal of our study was to evaluate the analytical performance of a new fT assay. **Methods:** The new test is a competitive, heterogeneous immunoassay based on chemiluminescence detection technology for the quantification of fT in human serum on the ids/iSYS system. Evaluation included intra- and inter-assay precision assessment using control materials and patient sera and method comparison with a Beckman radioimmunoassay. The fT assay was additionally validated using 70 samples of hypogonadal obese men and 30 specimens collected from young athletes. **Results:** The observed imprecision (CV) ranged from 2.6% to 6.6% on replicates of quality control samples provided with the test kit (inter-assay) and from 2.7% to 6.5% on replicates of patient samples distributed over the measurement range (intra-assay). Method comparison resulted in a correlation coefficient (Pearson) of r^2 = 0.9689, slope (Passing-Bablok) of 0.76 (95% CI, 073 - 0.80) and intercept (Passing-Bablok) of 0.15 (95% CI, -0.28 - 0.60). The median of the relative bias observed amounted to -23.5%. The mean fT value in the hypogonadal obese group (28.2 pmol/L, CI95% 26.2 - 30.2) was significantly lower ($p << 0.05$) than in the athletes group (35.6 pmol/L, CI95%, 32.1 -39.1). Comparison between measured and estimated fT shows a median bias of -86.7% which is in accordance with the literature. **Conclusion:** Precision results have been fully satisfying and reflected the manufacturer's declared performance. The observed bias to the current method is stable over the whole measurement range and might be explained by the use of different assay antibodies and reference material. The newly provided reference ranges take this bias into account. The fT assay shows satisfying analytical performance and could be helpful as a complementary biomarker for the diagnostic work-up of hypogonadism in combination with total testosterone and clinical assessment.

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Development of Assays for Micro blood samples: CBC, Biomarker and hormonal assays

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Objective
To modify and perform various assays, Complete blood count (CBC) and Biomarkers/hormonal assays in 100 ul of whole blood, collected using commercial blood collection device.
Relevance
The process of blood collection is painful and involves collection of multiple tubes by venipuncture . This has difficulty in collecting blood in children and elderly patients. Finger pricking has been used to collect whole blood or blood spot in filter paper. This has challenges with red cells and tissue fluid contamination and development of normal reference ranges in capillary blood. Recently, several commercial companies have developed painless blood collection devices. We have investigated one of the devices in blood collection and to perform various routine assays.
Methods
The TAP blood collection device from Seventh sense Biosystems (Medford, MA) is applied to the upper arm region of the patient. It is virtually a painless process utilizing "microneedles" to puncture the skin and gentle vacuum for suction of blood into a chamber containing anticoagulants. The device will only provide 100 ul of sample. Because of the limited sample volume, assays are scaled down or dilution paradigms were utilized to maximize the utility of the sample. The study was done under IRB protocol. The CBC was determined using a Sysmex XS-1000i, Hemoglobin A1C using Beckman AU480 . The remainder of the whole blood was centrifuged and the plasma was used for LC/MS/MS based assays for Vitamin D & testosterone and immunoassays for TSH, PSA and sex hormone binding Globulins (SHBG). Quality control samples were treated similar to the blood samples. The micro samples tested were correlated with conventional venipuncture blood samples.
Results
Whole blood (100 ul) collected using the TAP device, was first used for CBC determination and a small volume is diluted and cells lysed for HbA1c analysis. The rest of the whole blood is centrifuged and an aliquot of plasma was diverted to LC/MS/MS assays for Vitamin D and Testosterone. The remaining plasma was diluted 1:10 in an immunoassay calibrator diluent and was tested for TSH, PSA and SHBG using sensitive manual immunoassays. All assays were validated and analytically correlated with a predicate method using previously tested proficiency samples and human specimens. The manual immunoassay compared against an automated immunoassay analyzer with a correlation of 0.92. The micro volume LC/MS/MS based methods were compared against comparative LC/MS/MS methods that required 10x the amount of sample. The CBC instrument using capillary mode resulted in micro volume requirement. Modified methodologies demonstrated good statistical correlation, good reproducibility (< 15 % CV) and similar reference ranges between the newly developed assays and the predicate methodologies.
Conclusion
Micro sampling of whole blood (100 ul / device) could be achieved using a painless blood collection device. To accommodate small blood sample volume, automated instruments, immunoassays and mass spectrometry assays were modified .These modifications could be used to quantitate various markers used routinely.

B-492

A novel ELISA for the quantification of tau phosphorylated at threonine 181 in cerebrospinal fluid

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Background: The prevalence of dementia constantly increases as the Western populations grow older. Biomarkers in cerebrospinal fluid (CSF) are a valuable diagnostic aid for the diagnosis of Alzheimer's disease (AD). We developed a novel phospho-tau (pTau(181) ELISA for use in routine diagnostic laboratories with good analytical performance, robustness, user-friendliness and the possibility of automation. **Methods:** A 4-hour protocol was developed for the Euroimmun pTau(181) ELISA. The protocol as well as the test kit components were adapted to allow parallel analysis of the complete CSF biomarker profile for AD: Euroimmun ELISAs for the determination of beta-Amyloid (1-42) and (1-40), total Tau and pTau(181). Clinically characterised samples from the University of Magdeburg, Germany, were used for comparison with a reference test, the INNOTEST Phospho-Tau (181P) (Fujirebio). **Results:** Evaluation of the Euroimmun pTau(181) ELISA revealed the following test characteristics: intra-assay coefficient of variation (CV) ranges from 1.3 % to 5.0 %, inter-assay CV from 3.1 % to 4.9 %, whole blood interference tolerated up to 1 % v/v, no hook effect observed up to 100 ng/ml analyte. Manual and automated (Euroimmun Analyzer I) protocols gave similar results. The assay is very specific for the phosphorylation site 181 and showed no cross reactivity against other sites (e. g. 175). INNOTEST Phospho-Tau (181P) and the new Euroimmun pTau(181) ELISA were compared using 110 clinically characterised samples (61 AD patients, 49 disease/healthy controls). The Euroimmun pTau(181) ELISA showed a sensitivity of 93.4 % and a specificity of 83.7 %, while the INNOTEST Phospho-Tau (181P) assay showed a sensitivity of 67.2 % and a specificity of 91.8 % using the same cut-off (61 pg/ml). According to ROC analysis, at a predefined specificty of 91.8 %, the sensitivity of the Euroimmun assay (86.9 %) even outperformed that of the INNOTEST (67.2 %) significantly. **Conclusion:** The novel pTau(181) ELISA was validated for manual as well as automated processing on open ELISA systems such as the Euroimmun Analyzer I. Analytical performance evaluation included intra-assay, inter-assay and interference studies. The assay meets all requirements of a routine diagnostic test and represents an even better alternative to the established INNOTEST ELISA. The 4-hour protocol allows the test to be run in parallel to tests for other Euroimmun neurodegenerative biomarkers, i. e. beta-Amyloid (1-42) and (1-40) as well as total Tau, and enables a shorter time to result compared to ELISAs from other manufacturers. The possibility of automated parallel processing of all classical CSF biomarkers for AD on an open ELISA system is a step towards more reliable and comparable results.

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Application of a Biochip Array to Simultaneously Measure Analytes Related to Metabolic Syndrome in Serum with the Use of the New Random Access, Fully Automated Evidence Evolution Analyser

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Background: Metabolic syndrome, a combination of several metabolic related disorders, is a precursor to many diseases such as cardiovascular disease, type

2 diabetes, stroke, and cancer. The simultaneous detection of analytes related to metabolic syndrome is beneficial in clinical settings. Biochip array technology enables the detection of multiple analytes from a single sample, which increases result output. The aim of this study was to evaluate the applicability of biochip array technology to the simultaneous quantitative measurement of analytes related to metabolic syndrome ferritin, insulin, leptin, Plasminogen Activator Inhibitor-1 (PAI-1) and resistin in serum through the use of a biochip array applied to the fully automated, random access with STAT capabilities Evidence Evolution analyser. This application will facilitate research into metabolic related disorders **Methods:** Simultaneous chemiluminescent sandwich immunoassays, defining discrete test regions on a biochip surface and applied to Evidence Evolution analyser, were employed. Analytical sensitivity, inter-assay precision and serum patient sample (n = 81 for ferritin, n = 90 for insulin and n = 55 for leptin) method comparison studies were conducted. **Results:** Analytical sensitivity values of 2.53 ng/mL for ferritin (assay range 2.53 - 941.74 ng/mL), 0.09 µIU/mL for insulin (assay range 0.09 - 282.18 µIU/mL), 0.68 ng/mL for leptin (assay range 0.68 - 105.02 ng/mL), 0.60 ng/mL for PAI-1 (assay range 0.6 - 152.27 ng/mL) and 0.20 ng/mL for resistin (assay range 0.20 - 68.6 ng/mL). Inter-assay precision for low, medium and high levels of precision material, expressed as CV (%) (n=20) was as follows: 4.0%, 5.6% and 5.9% for ferritin, 10.1%, 8.2% and 9.9% for insulin, 4.0%, 4.8% and 7.3% for leptin, 9.4%, 9.9% and 9.8% for PAI-1and 13.3%, 12.7% and 10.5% for resistin. Serum patient samples were assessed with the biochip array and another commercially available system and the correlation coefficients were r= 0.95 for ferritin, r=0.95 for insulin and r=0.96 for leptin. **Conclusion:** Data indicate that the developed biochip array for application to the new fully automated Evidence Evolution analyser detects simultaneously analytes related to metabolic syndrome (ferritin, insulin, leptin, PAI-1 and resistin) from a single serum sample. This platform presents optimal analytical performance, compares favourably with another system and represents a reliable new analytical tool in the research of metabolic related disorders.

B-494

Automated measurement of plasma free hemoglobin using hemolysis index check function

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Background: Plasma free hemoglobin is an important indicator of intravascular hemolysis. Most clinical laboratories measure plasma free hemoglobin spectrophotometrically using two or three wavelengths. A point-of-care testing device is also available to measure plasma free hemoglobin. The Roche Diagnostics Cobas chemistry analyzers have a function to check hemolysis index (HI) and equations have been developed to estimate plasma free hemoglobin concentrations based on HI. Since the Roche Diagnostics chemistry analyzers' HI check function can directly report hemoglobin concentrations, we aim to determine if the hemoglobin concentrations reported by the hemolysis check function can be used as a measurement of plasma free hemoglobin. **Methods:** The Roche Cobas chemistry analyzers took an aliquot of the lithium heparin plasma and dilute it in saline (0.9 % sodium chloride) to measure the absorbances for hemolysis at 570 nm (primary wavelength) and 600 nm (secondary wavelength) and hemoglobin concentrations were calculated. Two samples with low and high concentrations of free hemoglobin were measured 20 times to evaluate within-run and between-run imprecision. Two samples with hemoglobin concentrations of 6 and 11 mg/dL were repeated 20 times to determine the lower limit of quantification. Six samples with known concentrations between 5 and 506 mg/dL were measured induplicate to evaluate the analytical measurement range. Fifty two samples were analyzed with the present method and an existing reference spectrophotometric method to evaluate the correlation between them. Two samples with low and high free hemoglobin concentrations were measured repeatedly in various combinations to evaluate carryover. Bilirubin was added to samples with known free hemoglobin concentrations to evaluate the interference. To evaluate the interference from triglyceride, free hemoglobin concentrations in samples with different lipemic index were determined before and after removing triglyceride by high-speed centrifugation (21,380 g for 15 minutes). **Results:** Within-run and between-run CVs were 2.8-10.1% and 2.1-7.0%, respectively (n = 20). The lower limit of quantification was 11 mg/dL (CV = 8.1%) with the upper limit of analytical measurement range of 506 mg/dL. The results of the present method correlated well with the existing reference spectrophotometric assay: Y (present method) = 1.079X (reference method) - 3.9, r = 0.9996, n = 50). No significant carryover was observed. Bilirubin with a concentration up to 75 mg/dL and lipemic index up to 200 did not show significant interference. Since the present method and the existing method show an excellent correlation, the reference interval for the reference method (0-22 mg/dL) was transferred to the present method. **Conclusion:** The performance of the plasma free hemoglobin measurement directly by the hemolysis index check function on the Roche Cobas chemistry analyzers meets the analytical requirements of the clinical plasma free hemoglobin assays. It is simple, automated, convenient, and cost-effective.

B-495

Performance of the Apolipoprotein A-1 assay for use on the Binding Site Optilite® turbidimetric analyser

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The Optilite Apolipoprotein A-1 (Apo A-1) assay is intended for the quantitative in vitro measurement of Apo A-1 in serum using the Binding Site Optilite analyser to aid in the assessment of lipid disorders and risk of atherosclerotic cardiovascular disease. Apo A-1 is the principal protein component of high density lipoprotein (HDL). Expression of Apo A-1 may be largely responsible for determining the plasma level of HDL. Apo A-1 also functions as a cofactor for lecithin cholesterol acyltransferase, which is vital in removing excess cholesterol from tissues and incorporating it into HDL for reverse transport to the liver. Therefore Apo A-1 and HDL cholesterol (HDL-C) concentrations are thought to be inversely related to risk of coronary heart disease (CHD). Apo A-1 has been shown to be a strong predictor for CHD risk. Typically, total cholesterol and triglycerides testing are used for screening coronary risk, but measurement of Apo A-1, along with other lipoproteins such as lipoprotein (a) and apolipoprotein B, can provide further useful information. Here we describe the evaluation of an Apolipoprotein A-1 serum and plasma assay (manufactured by The Binding Site Ltd, UK) for the Binding Site Optilite analyser. The measuring range of the assay is 0.193 - 2.750g/L at the standard 1+3 dilution, with an overall sensitivity of 0.048g/L at the reflex low 1+0 dilution. A precision study was performed according to CLSI approved guideline EP05-A2 over a period of 5 days using one reagent lot on one analyser. The study was carried out using 4 samples with different analyte concentrations. The between run precision coefficients of variation (CVs) were as follows: 0.81% at 0.42g/L, 1.09% at 1.24g/L, 2.23% at 1.53g/L and 2.37% at 2.33g/L. The acceptance criteria was <4% CV for between run precision. A comparison study to the Hitachi 917 assay was performed using 150 samples ranging from 0.15g/L to 3.51 g/L (Passing and Bablok analysis slope y = 0.94x + 0.02). A Limit of Quantitation (LoQ) verification study was based on CLSI EP17-A. The LoQ for this assay is defined as the bottom of the overall measuring range, 0.048 g/L. A linearity study was performed following CLSI Approved Guideline EP06-A. The assay gave a linear response over the analyte range of 0.09 - 3.74g/L at the standard 1+3 analyser dilution using a serially diluted serum sample. Interference testing was performed following CLSI guideline EP07-A2 using 6 potential drug and metabolite interferents including Intralipid, triglyceride and haemoglobin at 2 testing levels; the medical decision point (1+3 analyser dilution) and a pathological level (1+0 analyser dilution). No significant assay interference effects were observed (all results <10% from a corresponding blank sample). In conclusion, the Apolipoprotein A-1 assay for the Optilite provides a reliable and precise method for quantifying Apo A-1 content in human serum and correlates well with existing methods.

B-496

Performance of the Complement C2 assay for use on the Binding Site SPAPLUS® turbidimetric analyser

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The Human Complement C2 Kit for use on SPAPLUS is intended for the quantitative *in vitro* measurement of human Complement C2 in EDTA plasma and serum using the SPAPLUS analyser. This test should be used in conjunction with other laboratory and clinical findings. C2 is a β1-glycoprotein which forms part of the classical complement pathway. It is cleaved by activated C1s into two fragments, C2a and C2b. The larger fragment of C2 then combines with C4b to produce C3 or C5 convertase. Reduced C2 plasma or serum concentrations may result from either a C2 deficiency or a complement-consumptive process. C2 deficiency is the most common inherited complement component deficiency, and is associated with systemic lupus erythematosis, glomerulonephritis, vasculitis and severe pyogenic infections. Here we describe the performance of an immunoassay for the detection and quantification of Human Complement C2 Kit for use on SPAPLUS. A linearity study was performed following CLSI Approved Guideline EP6-A. The assay was confirmed to be linear over the standard 1/10 measuring range of 4.0 - 45.0 mg/L using a serially diluted EDTA plasma sample. An interference study was performed according to CLSI guideline EP7-A2.

Interferents tested included bilirubin (200 mg/L), haemoglobin (5 g/L), intralipid (500 mg/dL) and triglyceride (1000 mg/dL). No significant assay interference was observed when performed at the standard 1/10 sample dilution. A limit of quantitation (LoQ) study based on CLSI EP17 confirmed a limit of 4.0 mg/L with the total error being <1.0 mg/L. A precision study based on CLSI guideline EP05-A2 was performed over a 21 day period with two runs per day, three reagent lots and three analysers. Precision was assessed using 5 EDTA plasma samples with different analyte concentrations. The total prevision coefficients of variation (CVs) were as follows: 10.9% at 7.509 mg/L, 8.0% at 9.268 mg/L, 6.7% at 11.226 mg/L, 5.5% at 23.631 mg/L and 7.8% at 34.364 mg/L. A comparison study was performed by analysing 119 paired EDTA plasma and serum samples using the SPAPLUS Complement C2 assay and the SPAPLUS CH50 assay. The two assays were observed to have a high degree of relative agreement (98.3%). In conclusion, the Complement C2 assay for the SPAPLUS provides a reliable and precise method for quantifying Complement C2 content in human EDTA plasma and serum. It correlates well with existing complement assays.

B-497

Association between the clusterin level in peripheral blood and its gene polymorphism and Alzheimer disease

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Background: To assess the association between the clusterin level and its gene polymorphism and Alzheimer disease among ethnic Han Chinese in southwest of China **Methods:** A total of 108 patients with AD and 115 healthy controls were enrolled in this study. SNaPshot SNP typing was used to genotype 11 SNP were selected. Serum levels of clusterin were detected by ELISA **Results:** The clustrin level of peripheral blood in AD group was significantly higher than Control group (P = 0.001). The rs3087554 locus of CLU gene was significantly different in the dominant model (P = 0.037, OR = 0.523, *95% CI* = 0.284-0.962), and the locus was significantly difference in the **APOE**ε4-carrying case group and control group (P = 0.036). The other eight loci genotypes and alleles in the case group and control group distribution was no difference. No significant association were found between genotypes and serum clusterin levels. **Conclusion:** The plasma levels of clusterin in AD patients are significantly higher than those in normal controls. This conclusion is consistent with the other reports that clusterin may play a role in the occurrence and development of AD. CC or CT genotype of rs3087554 in CLU gene may be a protective factor of AD and can be a biomarker. No associate was found between the other eight SNPs and AD disease.

B-498

Novel Predictive Biomarker for Monitoring Adverse Reactions to Radiation Therapy

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Background: Radiation treatment is required by 70% of cancer patients, however, there is currently no clinical method for determining the therapeutic response or radiation-induced toxicity that can be used during a course of radiation therapy to personalize the dose for individual patients. The only standard method is CT/PET and/or MRI. This is a major clinical concern for radiation oncologists with so many new agents being approved in combination with radiation therapy. **Methods:** Herein we describe a highly sensitive clinically validated assay that measures the extent of normal tissue damage induced by radiation by quantitation of circulating free DNA (cfDNA) derived from cellular apoptosis detected in plasma of patients undergoing radiation therapy. The assay employs DNA capture probes and SuperbDNA™ signal amplification technology with alkaline phosphatase labeled signaling probes coupled with dioxetane phosphate chemiluminescence detection. The assay can be performed directly on patient plasma samples and can be readily automated. **Results:** The lower limit of detection (LOD) for this assay was shown as 0.39 ng/ml, which equals 7.8 pg human genomic DNA given that 20 ul of samples were loaded. The assay has a wide range of linearity from 0.39 to 50 ng/ml that allows for quantitative measurements of circulating DNA at concentrations expected in cancer patients and healthy individuals. The inter- and intra-assay coefficients of variance were <21.4% and <12%, respectively. Freeze-thaw stability testing showed that the reagents of the assay were stable up to 9 freeze-thaw cycles. Plasma samples from 47 patients with prostate cancer were tested and the levels of circulating DNA in plasma pre-radiotherapy was 11.6 ~ 130.6 ng/ml with a median value of 32.2 ng/ml, significantly higher than the normal plasma which was 5.01 ng/ml. Within 5 days post-radiotherapy, 13 of 47 patients (27.6%) showed >2.0-fold peak increase of circulating DNA levels. In addition, the ratios of post-radiotherapy peak levels to pre- levels in patients receiving X-ray treatment were significantly higher than patients receiving proton treatment, suggesting X-ray caused more toxicity than proton. **Conclusion:** RadTox can be used both for research and clinical testing of plasma samples for patients undergoing radiation therapy for optimization and personalization of treatment.

B-499

Evaluation of a Standard Material Traceable Enzymatic Method Assay for Glycated Albumin: Analytical Performance and Establishment of Reference Values

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Backgound
Glycated Albumin (GA) is an intermediate-term marker useful for the monitoring of glycemic control (preceding 2-3 weeks) in diabetes mellitus patients. We developed the Lucica® Glycated Albumin-L assay (GA assay), a diagnostic reagent for the quantitative measurement of GA that is traceable to standard reference materials based on an enzymatic method. This study is designed to evaluate the performance of the GA assay and develop the reference range of GA in healthy subjects without diabetes.
Methods
The performance studies (Precision/Reproducibility, Linearity, Stability, Interference, Detection limit, and Reference range) were conducted in accordance with *CLSI* Guidelines. The traceability was studied using the Secondary Calibrators (Glycated Albumin Certified Material, JCCRM 611-1, M, H, HH: ReCCS, Japan). For the reference range study, a single-visit 2-sites study was designed. Subjects with HbA1c < 5.7%, and a fasting glucose < 100 mg/dL, and 2-h plasma glucose in 75g OGTT < 140 mg/dL were enrolled in the study as healthy subjects without diabetes. The reference range was constructed based on 2.5 and 97.5 percentiles for the GA data of healthy subjects.
Results
In the single-site precision/reproducibility study, five serum pools were tested two runs per day in duplicates for twenty days. The overall repeatability (%CV) and the overall within-laboratory precision (%CV) were not more than 3.7% and 4.2%, respectively. In the multi-site precision study, three serum pools were tested five replicates per run, one run per day, for five testing days at three different laboratories. The overall reproducibility (%CV) values among the laboratories were not more than 2.5%. The GA value showed good linearity from 173 - 979 mmol/mol across the assay range. The Calibrator and Control were traceable to the secondary calibrator. The shelf-life for the reagents was 12 months when refrigerated within a temperature range between 2 and 8 °C. The open reagent was stable for 1 month. The LoB was 6.9 µmol/L for GA concentration and 3.8 µmol/L for Albumin concentration. The LoD was 7.9 µmol/L for GA concentration and 7.0 µmol/L for Albumin concentration. The LoQ was 9.7 µmol/L for GA concentration and 21.8 µmol/L for ALB concentration. The following substances were found not to interfere at the concentrations indicated (bias < 10%): unconjugated bilirubin up to 20.0 mg/dL, conjugated bilirubin up to 20.0 mg/dL, glucose up to 1000 mg/dL, ascorbic acid up to 100 mg/dL, hemoglobin up to 288 mg/dL, triglycerides up to 1516 mg/dL, and uricacid up to 23.5 mg/dL. The reference range in 262 healthy subjects without diabetes ranged between 183 and 259 mmol/mol.
Conclusion
In conclusion, the results demonstrated that the Lucica Glycated albumin-L assay shows excellent performance and may be a useful diagnostic test for the intermediate-term monitoring of glycemic control in patients with diabetes.

B-500

Accurate and high-throughput,targeted quantification of CpG methylation without DNA extraction and bisulfite treatment

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Background: DNA methylations at specific CpG loci have been increasingly utilized as biomarkers for cancer diagnostics. Targeted quantification of CpG methylation levels currently requires tedious DNA extraction and complex multi-step procedures that prevented clinical applications involving a large number of specimens. In addition, accurate and reproducible quantification of CpG methylation is often difficult using bisulfite-based quantitative assays due to inconsistent C to U conversion and template DNA degradation. Methylation-sensitive endonuclease-based assays such as MS-MLPA bypassed problems with the bisulfite, but still requires DNA extraction, and the presence of unbound probes in reactions and the change of reaction tubes for different enzymatic steps reduces the assay specificity and sensitivity. Here we described a highly reproducible, endonuclease-based,

quantitative and high throughput CpG methylation assay that does not involve DNA extraction and bisulfite conversion, with an ELISA-like workflow using one 96-well plate, and with a multiplex capability of up to 20-30 CpG loci in each well. **Methods:** Blood or FFPE sample are lysed to release target DNAs, which are captured to the bottom of the 96-well plate via sandwich hybridization with multiple contiguous target-specific probes having defined 5'- or 3'-end tail sequences. After removal of unbound probes and the enzymatic ligation of the bound probes, the ligation products spanning each target CpG site are treated either with or without a methylation-sensitive restriction endonuclease, which will cleave at specific unmethylated-cytosine residues while leaving the methylated ones intact. After buffer change, PCR amplification is performed in the same well with a universal primer pair targeting the tail sequences. Quantification of the CpG methylation percentage levels is obtained by comparing between restriction-treated and untreated groups the amount of each amplified products. For single CpG measurement this is achieved via delta Cq with real-time PCR; and for multiple CpG determination this can be achieved via standard PCR and multiplexed quantitative single-base primer extension analysis of the amplified products with MOLDI-TOF mass spectrometry (MassARRAY platform). **Results:** We prepared a test series of SssI-methylated DNA samples with CpG methylation levels ranging from 0%-100%, and measured the degree of methylation in triplicate using our assay with the enzyme HpaII. A linear regression analysis revealed quantitative and reproducible recovery across the entire methylation range, with the root mean square deviation of less than 5.2%, and a slope of 1.016 with an R^2 of 0.993. With a significantly simplified procedure and a much higher throughput, our assay efficiently offered overall better accuracy and consistency of the measured methylation percentage values than obtained with the bisulfite-based, gold standard pyrosequencing of the same samples. The robustness and sensitivity of our assay and its application in quantitative CpG methylation assessment in cancer (both cell lines and clinical samples) as well as in human aging will be presented. **Conclusion:** With no need for nucleic acid purification and bisulfate conversion, our method provides a highly reproducible multiplex quantitative CpG methylation assay suitable for high-throughput clinical applications.