

Tuesday, July 28, 2015

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

Factors Affecting Test Results

A-127

**Evaluation of Hemoglobin A1c Immunoassay and Capillary Electrophoresis Methods**R. R. Henriquez, H. F. Mohammad, C. Bishop, C. Hammett-Stabler.  
*University of North Carolina, Chapel Hill, NC***Background:**

Hemoglobin A1c (HbA1c) results have a major impact on the diagnosis of diabetes, as well as patient management. Two methods reported to have minimal interferences from variant hemoglobins are the Vitros5600 HbA1c immunoassay (Ortho Clinical Diagnostics) and the CAPILLARYS 2 Flex Piercing HbA1c capillary electrophoresis (Sebia, Lisses, France.). Our laboratory uses both technologies with the 5600 configured in an open work-cell to facilitate testing across all shifts, while the capillary electrophoresis system is used within special chemistry on one shift. We sought to compare the analytical performance of these methods, as well as the impact of each on workflow within our Core Laboratory, and to characterize the incidence of Hb variants in the patient population undergoing HbA1c testing. Our laboratory receives ~100 samples for this analysis daily from across the hospital system.

**Methods:**

The study protocol (IRB# 14-2344) received exemption from IRB review. Over a 28-day period, all patient samples (n=943) submitted for HbA1c testing using the Vitros methodology were anonymized and reanalyzed using CAPILLARYS HbA1c. Discordant samples (>0.6% difference) were frozen (-80°C) and analyzed by an NGSP reference laboratory using ion-exchange HPLC (Tosoh G8). Discordant samples containing Hb variants were verified using boronate-affinity HPLC (Trinity Ultra 2). Additionally, known homozygous or double heterozygous hemoglobin variants (HbSS, HbEE, HbSC, HbDD, provided by Sebia, Inc.) were analyzed using each method. A 7-Cycle timing analysis was performed to determine the impact of each system on workflow. Data were analyzed using EP Evaluator software (Data Innovations, LLC).

**Results:**

Method comparison showed good correlation ( $y = 1.03x - 0.05$ ,  $R = 0.991$ ) between the two methods. Seventeen discordant samples were sent to the NGSP laboratory. Four original Vitros results were >0.6% from the NGSP result of which 2 were found to have hemoglobin AS and AC variants. One Sebia result was >0.6% from the NGSP value; no Hb variant was detected. Prevalence of hemoglobin variants was 4.4% of the total population tested. One sample reported 4.6% HbA1c by the Vitros but showed an elevated hemoglobin F (27.5%) with no reportable HbA1c by CAPILLARYS HbA1c. NGSP laboratory reported 5.9% HbA1c for this sample. HbA1c values were not reported on known homozygous and double heterozygous samples using the CAPILLARYS HbA1c, but the Vitros method generated results (4.03-5.1%) for some glycated hemoglobin on several of these specimens.

The workflow study showed preparation time for the Vitros averaged  $4.2 \pm 0.8$  minutes compared to an average of  $1.4 \pm 0.16$  minutes for the CAPILLARYS2 Flex Piercing. The estimated run time for 40 samples was 60 minutes on the CAPILLARYS2 compared to 23 minutes using four Vitros5600's as the current configuration permits.

**Conclusion:**

This study reports high agreement for HbA1c measurement between the Vitros5600 immunoassay and the CAPILLARYS2 Flex Piercing electrophoresis methods in normal patient samples. The methodology of each system provides reliable results, with little interference from hemoglobin variants in the Vitros method. Thus, discrepant values observed are most likely due to the presence of a variant hemoglobin or random error. However, our current workflow design favors the use of the Vitros5600.

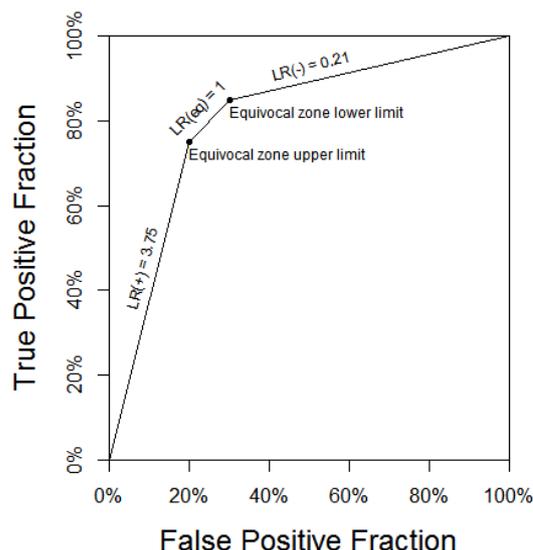
A-128

**Assessing Performance for Assays Reporting Equivocal Results**V. H. Petrides<sup>1</sup>, H. S. El Mubarak<sup>2</sup>, M. V. Kondratovich<sup>2</sup>, K. L. Meier<sup>3</sup>, J. Ye<sup>2</sup>, S. H. Gawel<sup>1</sup>, S. Akseirod<sup>2</sup>, A. Charnot-Katsikas<sup>4</sup>, K. Simon<sup>5</sup>. <sup>1</sup>Abbott Laboratories, Abbott Park, IL, <sup>2</sup>U.S. Food and Drug Administration, Silver Spring, MD, <sup>3</sup>Illumina, Inc., San Diego, CA, <sup>4</sup>The University of Chicago, Chicago, IL, <sup>5</sup>Biologics Consulting Group, Alexandria, VA

**Background:** Assessing the performance of qualitative tests reporting one of two possible results (positive or negative) relative to a gold standard is typically straightforward. Common performance measures include positive and negative predictive values, sensitivity and specificity, and likelihood ratios. However, what performance measures should be reported when a test includes equivocal results? Equivocal results are often simply ignored. The objective of this investigation is to demonstrate how the perception of assay performance can vary depending on the how equivocal results are treated and to provide recommendations for assessing performance in these situations.

**Methods:** An equivocal result here is defined for *in vitro* diagnostic tests with a continuous underlying signal, where one of two results is preferred but not always possible. Equivocal results are non-missing, non-erroneous results which are neither positive nor negative. Typically, the percent of equivocal results in a study is relatively small. We consider hypothetical data with three possible outcomes to show how various approaches described in literature affect performance estimates.

**Results:** The true positive fraction results ranged from 75% to 85%, which corresponds to the same values when calculating sensitivity for a dichotomous test. The false positive fraction results ranged from 20% to 30%, which corresponds to specificity values ranging from 70% to 80% for a dichotomous test. The likelihood ratio positive, equivocal, and negative values were 3.75, 1.00, and 0.21; the percentages of positive, equivocal, and negative results were 38%, 10%, and 52%, respectively; and the pre-test risk of the target condition was 33%.



**Conclusion:** Sensitivity and specificity calculations alone can be misleading in understanding the performance of assays with equivocal results. Likelihood ratios along with the corresponding percent for each outcome and the pre-test risk provide useful information about performance of tests with equivocal results.

A-129

**Serum concentrations of macro-AST relative to normal AST: limits consistent with IgG or IgA complexes and with normal rates of serum AST production**A. S. Rubin, L. J. McCloskey, B. M. Goldsmith, D. F. Stickler. *Jefferson University Hospitals, Philadelphia, PA*

**Background:** Our laboratory is occasionally asked to evaluate isolated enzyme elevations for the presence of macroenzymes. Evaluation is based on lability to polyethyleneglycol (PEG) precipitation. One recent case was consistent with macro-AST. The physician asked whether, despite this finding, one could still rule out a

circumstance of overproduction of AST. Our objective was to demonstrate our approach to answering this question for this specific case and for other instances of macro-AST reported in the literature.

**Methods:** Elevation of macroenzymes is assumed to be explained by the extended lifetime of enzyme in circulation. Normal concentration of enzyme (E) in serum/plasma is assumed to represent a steady-state ( $d[E]/dt = 0$ ) balancing the rate of appearance in plasma ( $k_i$ ) and the rate of elimination ( $k_e$ ). The rate of elimination is assumed to be first order with respect to enzyme concentration,  $k_e = k[E]$ . Thus, in steady-state,  $k_i - k[E] = 0$ , or  $k_i = k[E]$ . For macroenzyme E', altered  $k_e$  is assumed to reflect a substantial change in the rate constant  $k$  to a lesser value,  $k'$ . If  $k_i$  is unaltered, then  $[E']$  should reflect an otherwise normal enzyme concentration  $[E]$  according to the equality  $k[E] = k'[E']$ . Our answer to the physician's question is that  $[E']$  is consistent with altered circulation lifetime, rather than to an alteration in  $k_i$ , if a calculated  $[E] = k'[E']/k$  (Eqn.1) is within the reference range for AST. For macro-AST,  $k'$  was assumed to be consistent with that of IgG ( $t_{1/2} = 20$  days,  $k' = 0.035/\text{day}$ ) or IgA ( $t_{1/2} = 6$  days,  $k' = 0.116/\text{day}$ ), where  $k' = -\ln(0.5)/(t_{1/2})$ . For normal AST,  $t_{1/2} = 17$  hours (0.71 days);  $k = 0.98/\text{day}$ . Given patient values for  $[E']$ , we compared calculated values for  $[E]$  (according to Eqn.1) to the reference range for AST ( $[E] = 7-42$  U/L).

**Results:** For our physician's macro-AST patient,  $[E'] = 428$  U/L. By calculations per Methods, this value was consistent with normal AST  $[E]$  of 15 U/L, assuming IgG complexes, which was at the 14th percentile of the reference range for  $[E]$ . From literature, 49 examples of  $[E']$  formed a bimodal distribution (a. 68% of total, 60-300 U/L, median 124 U/L; b. 32% of total, 300-1150 U/L, median 434 U/L). Among all  $[E']$ , 71% were compatible with normal  $[E]$  based on IgA (100% from (a)); 41% were compatible based on IgG (100% from (b)); 10% were compatible based on either IgA or IgG; 0% had elevated  $[E]$  based on IgG. Based on IgG,  $[E'] = 1180$  U/L is the calculated upper limit for  $[E']$  compatible with normal  $[E]$ .

**Conclusions:** Calculations described can be used to assess whether values for macro-AST are consistent with normal rates of appearance of AST into circulation, assuming the lifetime of macro-AST is consistent with the lifetime of IgG or IgA. Values for macro-AST reported in literature were all found to be consistent with otherwise normal AST. Caveats to the analysis include selection bias and potential effects of comorbidities on AST lifetimes in cases where macro-AST was an incidental finding.

### A-130

#### Monoclonal Protein Interference with Chemistry Measurements Performed on the Siemens Advia® 1800 Chemistry Analyzer

C. Schein, R. Carey-Balough, Y. Posey, E. Sykes, K. Simkowski. *Beaumont Health System, Royal Oak, MI*

**BACKGROUND:** Previous articles have reported monoclonal protein (MP) interference with routine automated chemistry and immunoassay tests on a number of testing platforms. Assays affected included inorganic phosphorus, glucose, and HDL, possibly due to precipitate formed by MP during reactions. We are unaware of any previous study that has examined MP interferences on Siemens Advia® 1800 chemistry analyzers.

**OBJECTIVE:** With a large elderly and oncology population, we were interested in determining what routine chemistries performed on the Siemens Advia® 1800 are impacted by the presence of MP. In addition, we wanted to investigate how the type and quantity of the MP affect the amount of interference observed.

**STUDY DESIGN:** Remnant sera from 15 patients with serum protein electrophoresis showing a MP greater than 1.5 g/dL were run on the Siemens Advia 1800 for albumin, alkaline phosphatase, ALT, AST, total bilirubin, blood urea nitrogen (BUN), total calcium, CO<sub>2</sub>, creatinine, glucose, magnesium, uric acid, total cholesterol, HDL, triglycerides, total protein, and inorganic phosphorus (IP). Reaction curves were analyzed to determine presence or absence of interference. Where quantity allowed, two-fold dilutions (5 samples) and protein-free filtrates (5 samples, using CentriFree® Ultrafiltration Devices, Millipore) were prepared and re-tested for inorganic phosphorus to determine the magnitude of the interference.

**RESULTS:** Patient characteristics were as follows: average age: 63 years (36-80, 9 male, 6 female); diagnoses: 3 B-cell lymphoma, 2 lymphoplasmacytic lymphoma, 9 multiple myeloma, 1 unknown; average total protein: 9.0 g/dL (7.2-14.7 g/dL); and average MP concentration: 3.08 g/dL (1.84-8.66 g/dL); 9 IgG kappa, 2 IgG lambda, 4 IgM kappa).

The majority of samples (12/15) showed a positive interference for IP. The average value obtained for IP in specimens with interference was 5.71 mg/dL (3.62-15.78 mg/dL), versus 3.38 mg/dL (3.13-3.55 mg/dL) in specimens without interference. Two-fold dilutions were not sufficient to abrogate the interference. Protein-free filtrate testing on 5 samples with IP interference (average IP 4.08 mg/dL, 3.62-4.63

showed an average reduction in IP of 0.66 mg/dL (0.37-0.77 mg/dL). Preparation of a protein-free filtrate did not significantly alter IP values in patients without MP. The lowest level of monoclonal protein at which interference was observed was 1.93 g/dL; however, one of the specimens not showing interference had a monoclonal protein level of 3.6 g/dL; both samples were IgG kappa.

**CONCLUSIONS:**

No interference due to MP was observed for albumin, alkaline phosphatase, ALT, AST, bilirubin, BUN, calcium, CO<sub>2</sub>, creatinine, glucose, magnesium, uric acid, cholesterol, HDL, triglycerides, or total protein.

The presence of MP >1.5 g/dL is very likely to cause positive interference with IP measurement on the Siemens Advia 1800. Preparation of a protein-free filtrate is a simple way to obtain an accurate IP value for these patients, and in studies of patients without a monoclonal protein does not significantly alter the IP values obtained.

### A-131

#### Potassium vs. hematocrit in filter paper bloodspots: effect of large differences in potassium per unit area between center circular punch samples vs. outer annular remainder samples

M. L. Rufail, L. J. McCloskey, D. F. Stickle. *Jefferson University Hospitals, Philadelphia, PA*

**Background:** Certain quantitative assays using filter paper bloodspots (FPB) are adversely affected by hematocrit (HCT) as an unknown preanalytical variable. Capiou et al. [Anal Chem 2013;85:404] recently demonstrated use of potassium (K) from FPB punches to estimate HCT. Because the majority of K in blood is from red blood cells (RBCs), and because there is significant accretion of RBCs at the perimeter of FPB, our objective was to compare correlation of K with HCT for center punch specimens vs. remainder (outer annular) specimens. For our study, a remainder specimen was defined as the outer, annular specimen (a specimen with a middle hole) that remains after a single center punch has been removed from an intact bloodspot.

**Methods:** Primary samples were Li-heparin whole blood submitted to the lab for ionized calcium, on patients for whom simultaneously-drawn K-EDTA samples were submitted for complete blood count including HCT (Sysmex). FPB were formed by bolus addition of 40 µL whole blood to Whatman 903 cards (Perkin-Elmer). After drying (24 h), total bloodspot area was determined by image analysis. A standard office hole punch (approximately 6 mm diameter) was then used to produce center punch (P) and remainder (R) samples. Punch area measured by image analysis was treated as constant (0.314 cm<sup>2</sup>). R sample areas were taken as the difference between total area and punch area. P and R samples were extracted in 400 µL 2.5 mM K-EDTA solution (>1 hour) with periodic vortexing. The resulting solution was measured for K (Roche Cobas c501). After correction for extraction solution K, values of K/area (α, µmol K/cm<sup>2</sup>), and correlations of α to HCT, were compared between results for P and R samples.

**Results:** A total of 43 patient samples were utilized. Bloodspot areas (A, cm<sup>2</sup>) were normally distributed ( $A = 0.91 \pm 0.046$  cm<sup>2</sup>) and were in aggregate negatively correlated with HCT ( $A = -0.0042$  HCT%) + 1.06 cm<sup>2</sup>;  $r = -0.687$ ). Combined P+R recovery of K from 40 µL whole blood was  $88 \pm 4.5\%$ . K/area (α) was normally distributed:  $\alpha(P) = 1.23 \pm 0.26$  µmol/cm<sup>2</sup>;  $\alpha(R) = 1.86 \pm 0.41$  µmol/cm<sup>2</sup>. Note that  $\alpha(R) \gg \alpha(P)$ ; the overall ratio  $\alpha(R)/\alpha(P)$  was  $1.51 \pm 0.15$ . For both P and R, α was highly correlated with HCT:  $\alpha(P) = 0.030$  HCT(%) + 0.015 µmol/cm<sup>2</sup> ( $r^2 = 0.795$ );  $\alpha(R) = 0.052$  HCT(%) + 0.010 ( $r^2 = 0.912$ ). Slopes of correlations showed higher resolution for α(R) vs. HCT. Errors (E, calculated HCT - measured HCT) for calculated HCT were normally distributed:  $E(P) = -0.042 \pm 3.8\%$ ;  $E(R) = 0.059 \pm 2.4\%$ . The standard deviation for E(R) was reduced by 37% compared to that for E(P).

**Conclusions:** K per area (α) was significantly higher in R samples compared to P samples, with approximately a 60:40 ratio in representation of total K per total FPB area ( $\alpha(R)/\alpha(P) = 1.5$ ). Correspondingly, whereas α for both punch (P) and remainder (R) samples were reasonably well correlated with HCT, there was higher resolution for α(R) vs. HCT. Both the correlation coefficient for α(R) vs. HCT, and standard deviation of errors E(R) in predicted HCT, were numerically superior compared to those for P samples.

**A-132**

**Impact of Sample Handling on Intact Parathyroid Hormone (PTH) Concentrations in Specimens**

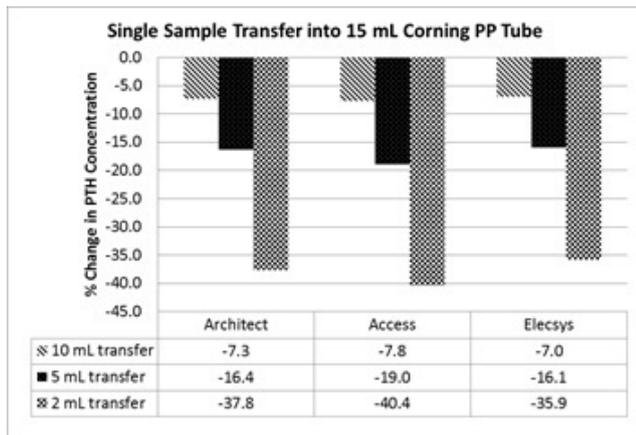
G. S. Beligere<sup>1</sup>, E. M. Brate<sup>1</sup>, D. Elliott<sup>2</sup>, K. M. Gignac<sup>1</sup>, F. C. Grenier<sup>1</sup>, J. W. Jaraczewski<sup>1</sup>, M. S. Matias<sup>1</sup>, L. Sokoll<sup>2</sup>. <sup>1</sup>Abbott Laboratories, North Chicago, IL, <sup>2</sup>Johns Hopkins Medical Institutions, Baltimore, MD

**Background:** Parathyroid Hormone (PTH) is an 84 amino acid peptide hormone important in the regulation of calcium homeostasis. During recent studies with Intact PTH assays, we observed the PTH concentration in fresh plasma samples decreased approximately 4-8% after each transfer into a new tube. Because it is a common practice to aliquot samples for bio-banking, clinical studies and routine lab work, it is critical to be aware of the effect of pre-analytical factors on the PTH concentration. The loss of PTH due to sample handling was investigated using various tube types.

**Method:** Normal samples were freshly drawn into EDTA tubes and centrifuged to collect plasma. A portion of each specimen was spiked with PTH and the neat and spiked specimens were transferred sequentially to different tubes. All the specimens were tested on the Abbott Architect PTH STAT, Beckman Access 2 Intraoperative PTH and Roche Elecsys 2010 PTH STAT assays.

**Results:** Analysis of different tube types showed the PTH concentration decreased after each transfer by 6-15%. Nalgene cryovials and standard Eppendorf polypropylene tubes showed 10% and 50% decreases after one and three transfers, respectively. Eppendorf Protein LoBind tubes showed the smallest decrease. A similar effect was observed irrespective of the assays used with both endogenous PTH as well as spiked PTH specimens and in both fresh and frozen specimens. Increasing the surface area relative to volume also increased the magnitude of the effect (see Figure).

**Conclusion:** Pre-analytical factors can have a significant effect on PTH concentrations. In order to minimize changes in PTH concentration, it is necessary to select the appropriate tube type and size and avoid multiple tube-to-tube transfers.



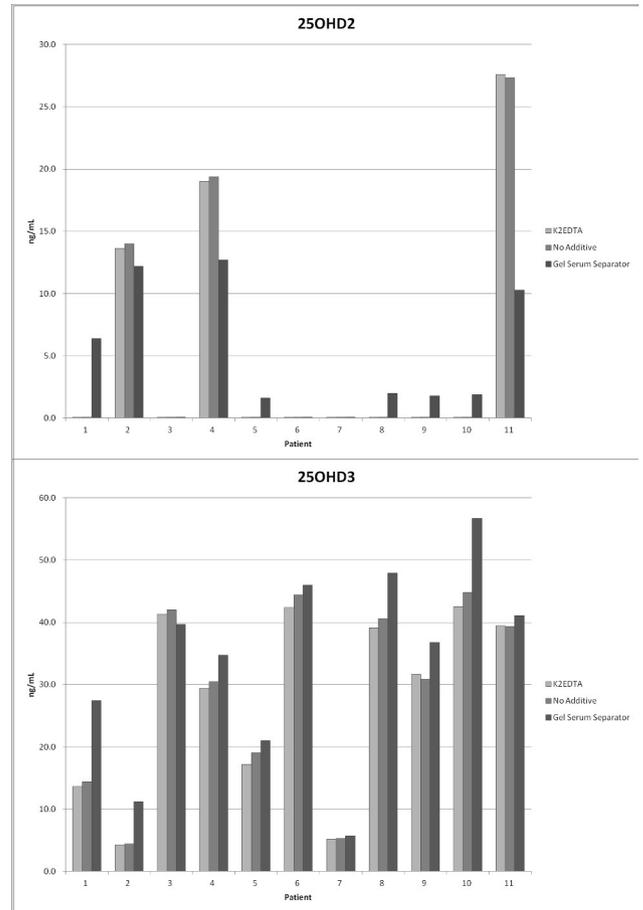
**A-133**

**Gel Separator in Blood Collection Tubes may Cause Interference with 25-Hydroxyvitamin D Measurement by Liquid Chromatography-Tandem Mass Spectrometry Methods**

J. Gabler<sup>1</sup>, K. Lembricht<sup>2</sup>, D. Payto<sup>2</sup>, S. Wang<sup>2</sup>. <sup>1</sup>ThermoFisher Scientific, West Palm Beach, FL, <sup>2</sup>Cleveland Clinic, Cleveland, OH

**Background:** Vitamin D nutritional status indicated by blood levels of 25-hydroxyvitamin D (25OHD) is important to bone health and is also linked to other aspects of human health. In 2009 we reported a liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for quantitation of serum 25OHD2 and 25OHD3. Later on, unusual chromatography – especially for 25OHD2 – was noted for some patient samples. An investigation prompted the hypothesis that gel separator in the blood collection tubes may cause interference. **Objective:** To determine if gel separator tubes cause chromatographic interference with 25OHD2 and 25OHD3 by the LC-MS/MS method. **Method:** Three BD tube types were compared, gel separator serum tubes, K<sub>2</sub>EDTA plasma tubes, and serum tubes with no gel separator. Specimens from each tube type, all from the same phlebotomy, were obtained from 11 patients. Each matched patient set was analyzed by the published LC-MS/MS method.

**Results:** Patient samples collected in BD gel separator serum tubes exhibited different chromatography than the same patient samples collected in K<sub>2</sub>EDTA or serum tubes with no gel separator. The altered chromatography from the gel separator serum tubes significantly affected the quantitation of both 25OHD2 and 25OHD3 for some patients. The degree of the interference varied across the patients. **Conclusion:** Gel separator serum collection tubes caused chromatographic interference with 25OHD2 and 25OHD3 quantitation by the LC-MS/MS method compared to K<sub>2</sub>EDTA plasma tubes or serum collection tubes with no gel separator.



**A-134**

**Using Urine Results Produced By Flow Cytometry On The Sysmex UF-1000i To Rationalise Urine Culture Orders, Increase Clinical Efficiency And Conserve Healthcare Spending.**

S. T. Abdul Latiff, D. P. Bernal, C. H. Yeoh, A. Omar, M. S. Wong. *Kho Teck Puat Hospital, Singapore, Singapore*

**Background:** The objective of this study is to see if the number of urine cultures could be reduced by using results from routine urinary analysis performed on the Sysmex UF1000i (Sysmex Corporation, Kobe, Japan) fluorescence flow cytometer. If this routine screening process is able to reliably predict the outcome of urine cultures, the laboratory will move away from physician based ordering of urine cultures to a reflex algorithm based on flow cytometry parameters. In this way, we intend to decrease the number of unnecessary cultures which do not manifest significant bacteria or urinary tract infection. By instituting the appropriate algorithm, we hope to automate this process, with an aim to increase analytical productivity and ensure consistency in our reporting.

**Methodology:** We reviewed 322 anonymised random urine results that had been analysed on the \ UF1000 and had subsequent urine cultures performed. Outcomes were analysed at five cutoffs of 30, 40, 50, 125 and 200 bacteria cells per microliter. Additionally, the presence of white blood cells (WBC) was used as an indicator of pyuria to see if the decision making could be made more sensitive or specific.

Results were analyzed using frequency analysis, quantitative and semi-quantitative comparisons.

Results: We found that a cutoff of 50 bacteria cells/uL provided the best specificity and sensitivity of 85.1% and 81.5% respectively. The negative and positive predictive values were 0.8086 and 0.8563. When a WBC count above 12 cells/uL was included as an additional indicator of infection, the specificity and sensitivity were 42.2% and 92.9% respectively. For the range of cutoffs from 30 to 200 bacteria cells/uL, the sensitivities ranged from 86.3% to 62.5% while the specificities ranged from 79.2% to 92.2% respectively.

Conclusion: Forty percent of urine cultures were eliminated by instituting an algorithm utilizing a single variable of a bacterial cell count cutoff of 50 cells/uL. This equated to a monetary saving of US\$1400 per day of urine testing. Thirty-one out of 322 (9.6%) of patients tested would have been incorrectly diagnosed as having no urinary tract infection. Of these 31 patients, 17 of them were reported as mixed bacterial growth or having no significant growth and were therefore not clinically significant.

**A-135**

**Blood Donation Temporarily Improves Glycated Hemoglobin (HbA1c) Status In Healthy Men**

A. A. Borai<sup>1</sup>, A. Farzal<sup>1</sup>, C. Livignstone<sup>2</sup>, D. Balgoon<sup>1</sup>, A. Al Sufyani<sup>1</sup>, S. Bahijri<sup>3</sup>, I. Kadam<sup>1</sup>, K. Hafiz<sup>1</sup>, M. Abdelaal<sup>1</sup>, G. Ferns<sup>4</sup>. <sup>1</sup>King Abdullah International Medical Research Center, King Saud bin Abdulaziz University for Health Sciences, Pathology, King Abdulaziz Medical City, Saudi Arabia, Jeddah, Saudi Arabia, <sup>2</sup>Faculty of Health & Medical Sciences, University of Surrey, UK GU2 7XH., Guildford, United Kingdom, <sup>3</sup>Department of Clinical Biochemistry, Faculty of Medicine, King Abdulaziz University, Saudi Arabia, Jeddah, Saudi Arabia, <sup>4</sup>Division of Medical Education, Brighton and Sussex Medical School, Mayfield House, Falmer, Brighton, United Kingdom

Background: A significant change in glycemic status has previously been observed in patients with type 2 diabetes who underwent blood donation when compared to a control group. Our aim was to study the effect of whole blood donation on glycemic and metabolic markers in normal individuals at different time intervals.

Methods: 42 subjects with normal glucose tolerance (NGT) were recruited to the study. Glucose tolerance was assessed by oral glucose tolerance test before (visit A) and after the blood donation (1 day, visit B; 1 week, visit C; 3 weeks, visit D; and 3 months, visit E) on each subject. Fasting glucose, glycated hemoglobin (HbA1c), insulin, lipids, uric acid, C-reactive protein, homeostasis model assessment for insulin resistance (HOMA-IR) and Complete Blood Count were measured. A repeated measure ANOVA was used for comparisons of quantitative variables between different visits.

Results: After the blood donation, at visit C, both RBC count and total Hb concentration were decreased by ~ 9% from the baseline levels (visit A). At visit D, HbA1c ± SD (5.3 ± 0.4%) was significantly lower compared to visit A (HbA1c: 5.5 ± 0.4%, p<0.05) (Figure). Cholesterol at visit A (5.3 ± 1.2 mmol/L) decreased significantly following donation and remained decreased except in visit E. HOMA-IR did not change significantly following donation.

Conclusions: After blood donation, the reduction in RBC count and total Hb contribute to a temporary improvement in glycated hemoglobin but with no significant change in insulin resistance.

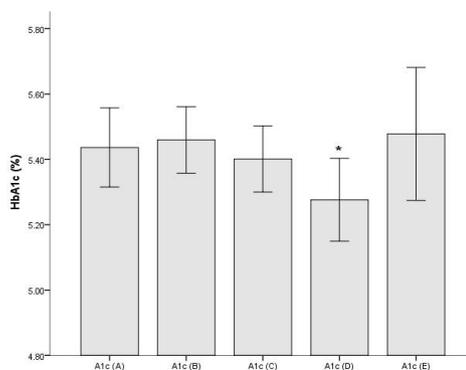


Figure: Levels of HbA1c before and at different time-points after blood donation (Error bars 95%CI). \*p<0.05

**A-136**

**Estimated Uncertainty using Routine Internal Quality Control Results for the Validation of Delta Check Method**

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Background: Laboratory test results are valuable medical information used for management of patients. Among the utilities of laboratory tests, patient monitoring is one of the main purposes of laboratory testing. Therefore, the criteria for significant change in test results are of practical importance in the interpretation of test results. Also, the use of these criteria could be expanded to delta check. In metrological community, measurement uncertainty has been regarded as a key principle in the robustness of measurement results and the concept has been recently introduced in the clinical laboratory area. We assessed the criteria for determining significant difference in clinical laboratory testing using the estimated uncertainty with the routine internal quality control (QC) data from one laboratory.

Methods: The uncertainty was estimated directly by the CLSI EP29-A guideline and Guide to the Expression of Uncertainty in Measurement (GUM) using the top-down approach. We used one-year's internal QC data to estimate uncertainty of 6 analytes including albumin, total cholesterol, triglyceride, glucose, protein and creatinine. We identified the sources of uncertainty, including pipetting, calibration errors, and lot variations as the components of type B evaluation. We collected inpatient data for six test items, and then current results and results upto 60 days prior were collected as paired test results. We calculated the relative combined uncertainty including biological variation and reference change value (RCV) for each analyte and determined the criteria for significant difference. And RCV was compared with delta check method.

Results: Combined uncertainty were 5.83% and 5.11% for albumin, 7.90% and 7.43% for total cholesterol, 21.06% and 20.49% for triglyceride, 6.78% and 6.78% for glucose, 5.66% and 4.93% for protein, 13.84% and 11.08% for creatinine at level 1 and level 2, respectively. With the uncertainty, we could estimate the minimal difference to claim the difference between test results. And the results of comparison between RCV and delta check method showed that detection rate of significant difference in RCV was higher about 2 times to 12 times than delta check method for all test items. Delta check method underestimated the differences in albumin, glucose and protein. In case of total cholesterol, triglyceride and creatinine, over-detections by current delta criteria were showed.

Conclusions: Uncertainty based change assessment can reflect laboratory performance characteristics without collecting the large volume of patient data. And straightforward estimation of significant change was possible. Based on our findings, current delta criteria needs to be more or less stringent. We conclude that our assessment model can be the efficient tool for verifying delta check method with the biological variations considered and applicable.

## A-137

**Estimation of biological variation and quality specifications for plasma ammonia concentrations in healthy subjects**

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<sup>1</sup>Diskapi Yildirim Beyazit Training and Research Hospital, Department of Clinical Biochemistry, Ankara, Turkey, <sup>2</sup>Hacettepe University Medical Faculty, Department of Biochemistry, Ankara, Turkey

**Background:** Most of the factors causing preanalytical and analytical variations in ammonia measurement are known. However, data on the biological variability of ammonia is still uncertain. The present study for the first time investigated the biological variation of ammonia in a group of healthy individuals by applying a recommended and strictly designed study protocol using fresh and frozen samples.

**Methods:** Twenty voluntary healthy individuals [12 female, 8 male; 21-55 years (min-max)] were recruited for this study. Blood samples were collected daily over a period of four consecutive days from each subject. Blood collections were performed in standardized conditions in order to minimize sources of pre-analytical variation. Blood samples for ammonia measurement were collected in K2EDTA (2.0 mL, K2EDTA 3.6 mg, BD Vacutainer®, UK) tubes. Immediately after sampling, blood samples were placed on ice bath, separated within 15 minutes of collection. All samples were immediately centrifuged (4°C, 10 min, at 2000xg). Also, after centrifugation each plasma sample was split into two aliquots; one was immediately analyzed as the samples were collected and the other was stored -80°C and analyzed at once in one analytical run at the end of the fourth day. All samples were assayed in duplicate for both fresh and frozen samples. Data were analyzed by SPSS 15.0 and estimations were calculated according to the formulas described by Fraser and Harris.

**Results:** All of the estimations were performed for fresh and frozen samples. The intra-individual or within-subject (CVI) and inter-individual or between-subject (CVG) biological variation were 13.78% and 16.91% for fresh samples, respectively. The calculated CVI and CVG were 18.91% and 18.43% for frozen samples, respectively. Reference change values (RCV) for fresh and frozen samples were 43.37% and 56.85%, respectively and individuality indexes (II) of ammonia were 0.92 and 1.11, respectively. Minimum, desirable and optimal analytical goals for imprecision, bias, total error were also estimated by using obtained data for fresh and frozen samples. Derived desirable analytical goals for imprecision, bias, and total error resulted 6.89%, 4.61%, and 15.98%, by using obtained data for fresh samples, respectively. Derived desirable analytical goals for imprecision, bias, and total error resulted 9.45%, 5.01%, and 20.61%, by using obtained data for frozen samples, respectively.

**Conclusion:** This study for the first time described the components of biological variation for ammonia in healthy individuals. These data regarding biological variation of ammonia could be useful for a better evaluation of ammonia test results in clinical interpretation and for determining quality specifications based on biological variation.

## A-138

**The influence of analytical interference introduced by separator gel blood collection tubes on steroid molecules analysis**

J. D. Buse, D. J. Orton, J. Boyd, H. Sadrzadeh. *Calgary Laboratory Services, Calgary, AB, Canada*

**Background:** The ubiquitous use of serum gel separator tubes within clinical laboratories is a result of the numerous advantages they provide; including rapid serum separation, enhanced sample stability and reduced aerosolization. These advantages, however, can be offset by the release of molecules from the tube's gel element that can interfere with the qualitative and quantitative analysis of various analytes. Steroid molecules are one analyte class that is susceptible to analytical interference from molecules that are found within the gel element of serum separator tubes because of their low biological concentration and non-polar nature. Investigating the source of ion suppression and interference that results from the use of serum gel separator tubes and their influence on the quantification of steroid molecules, specifically 17-hydroxyprogesterone and aldosterone.

**Methods:** Five different blood collection tubes from BD Biosciences (Mississauga, ON, Canada) were utilized for this study and included gold top tubes (gel separator + clot activator [367977]), light green (mint) top tubes (gel separator + lithium heparin [367962]), red top tubes (clot activator [367812]), dark green top tubes (sodium heparin [367884]) and lavender top tubes (potassium EDTA [367861]). The blood samples collected within these tubes subsequently underwent serum separation, followed by analysis of the serum for 17-hydroxyprogesterone and aldosterone. Sample preparation for both analytes relied upon a methyl tert-butyl ether liquid-

liquid extraction, while chromatographic separation of analytes was carried out using an Agilent 1290 liquid chromatographic separations module (Santa Clara, CA, USA) and Agilent Poroshell C18 column (2.1 x 100 mm, 2.7 µ) with a Methanol and 5 mM ammonium formate gradient. Identification of both analytes relied upon an Agilent 6460 triple quadrupole mass spectrometer using multiple reaction monitoring for quantification; 17-hydroxyprogesterone (331.0 <math>\diamond</math> 97.1/109.1 [H-085, Cerilliant, Round Rock, Texas, USA]) and 17-hydroxyprogesterone-d8 (339.2 <math>\diamond</math> 100.0/112.1 [H-096, Cerilliant]), aldosterone (359.4 <math>\diamond</math> 331.4 [A-096, Cerilliant]) and aldosterone-d4 (363.4 <math>\diamond</math> 335.4 [Isosciences, Trevose, PA, USA]).

**Results:** An inability to correlate the serum concentrations of 17-hydroxyprogesterone and aldosterone between LC-MS/MS and immunoassay led to the hypothesis that tubes containing separator gel interfere with the quantification of these analytes by LC-MS/MS. Subsequent analysis of 17-hydroxyprogesterone concentrations within three patients using the five different blood collection tubes (identified above) resulted in a 1.48 (±0.25) times increase in its observed concentration within tubes containing separator gel; a decrease in concentration was attained for aldosterone measured in tubes containing separator gel. These changes in the measured concentrations of both analytes is a result of the interference produced by separator gel that modifies the chromatographic baseline and peak area, resulting in changes in measured concentrations of each analyte as well as the ratio of their monitored product ions.

**Conclusion:** Blood collection tubes that contain separator gel (Gold top and light green [mint] top tubes) interfered with the quantification of steroid molecules 17-hydroxyprogesterone and aldosterone by introducing extraneous molecules that interfered with LC-MS/MS analysis.

## A-139

**The effect of microcentrifuge tube plastic leachates on laboratory sample analysis and the identification of leachates by LC-MS/MS and GC-MS**

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**Background:** The prevalence of disposable plastic items within clinical laboratories is a result of their easy disposal and inexpensive nature. These products, however, are produced at varying levels of purity and then often sold as universally applicable to sample preparation, with some being advertised as compatible with liquid-liquid extraction protocols using organic solvents. Difference in the manufacturing and/or cleaning process of each tube can lead to sample contamination from materials that leached from the tubes and jeopardize patient management through their impact on the identification and quantification of certain molecules. Investigate the influence of various solvents on the leaching of materials that comprise microcentrifuge plastic tubes used for the preparation and storage of samples undergoing LC-MS/MS drug and steroid analysis.

**Methods:** Four different plastic microcentrifuge tubes were evaluated for leachates and included tubes from Bioplastics (Cat# 4036, Landgraaf, The Netherlands), Eppendorf (Cat# 022364111, Hauppauge, NY, USA) Rainin (LTT-170-N, Mississauga, ON, Canada) and VWR (Cat# 20170-026, Radnor, PA, USA). The effect of agitation and incubation time (0-90 minute) were evaluated on each plastic tube using; Millipore water, verified blank urine, verified blank serum, saline solution, methanol, ethanol, acetonitrile, methyl-tert-butyl-ether, isopropylalcohol and chloroform. LC separation of analytes was carried out using an Agilent 1290 liquid chromatographic separations module (Santa Clara, CA, USA) and Restek ultra biphenyl column (2.1 x 100 mm, 5 µ) and gradient of Acetonitrile and 5 mM ammonium formate. Identification of analytes relied upon an Agilent 6460 MS/MS. GC separation of analytes was carried out using an Agilent 6890 GC separations module (Santa Clara, CA, USA) and DB-5MS low bleed 5%-Diphenyl-95%-Dimethylsiloxane Copolymer capillary column (thickness 0.25µm, 0.25 x 15000 mm) (Chromatographic Specialties, Brockville, ON, Canada) with nitrogen as the carrier gas. Identification of analytes relied upon an Agilent 5973 MSD using electron impact full scan analysis.

**Results:** Incubation of aqueous solutions within each tube type did not lead to a significant level of leaching occurring from any of the tested tubes. Incubation or organic solvent within the tubes did lead to significant levels of leaching by the 1.5 mL VWR and bioplastic tubes for all tested organic solvents; with peak areas greater than 1.0e6 or 100 times greater than blank solvents. Conversely, the 1.5 mL Rainin and Eppendorf tubes reported peak areas comparable to the blank solvents; leaching was not found to be time dependent. GC-MS/MS analysis of the VWR and Bioplastic tubes as well as the correlation of discovered peaks with the NIST library allowed for the identification of some of the contaminants as hexadecanal, pentadecanal, methyl stearate, octadecanoic acid and assorted fatty acids. These identified products are common by-products of poor quality or recycled plastics and were found to interfere with the LC-MS/MS analysis of 17-hydroxyprogesterone in serum samples; a

molecule of identical m/z was discovered in the VWR and bioplastic microcentrifuge tubes.

**Conclusion:** Microcentrifuge tubes from VWR and Bioplastics were not suitable for use with all 6 organic solvents evaluated because they leached molecules that interfered with LC-MS/MS and GC-MS analysis.

### A-140

#### Establishing Evidence-Based Thresholds for Pseudohyperkalemia Alerts

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**Background:** Pseudohyperkalemia is a common pre-analytical error that can lead to inappropriate patient treatment and true hypokalemia. Previous work has suggested that pseudohyperkalemia is most pronounced at platelet counts above  $500 \times 10^9/L$  for serum samples and white blood cell (WBC) counts greater than  $100 \times 10^3/\text{microL}$  for plasma samples, but the generalizability of these thresholds is unclear. In 2010, our institution created laboratory information system (LIS) rules to append a result comment indicating the risk of falsely elevated potassium for patients with platelet counts above  $500 \times 10^9/L$  or WBC counts greater than  $150 \times 10^3/\text{microL}$ , irrespective of sample type. To evaluate the efficacy of these thresholds we analyzed 4 years of historical results.

**Methods:** Potassium results from 2011-2014 for two hospital laboratories were extracted from the LIS in conjunction with the most recent platelet count, WBC count, and whole blood potassium. The difference between serum/plasma potassium and whole blood potassium values collected within a 2 hour span was plotted as a function of platelet and WBC count and fit to a linear model. The number of results and distinct patients with relevant result comments was tallied, and the impact of changing platelet and WBC count thresholds was evaluated.

**Results:** Of the approximately 2 million potassium results from more than 300,000 distinct patients, 10,869 plasma and 1,676 serum potassiums were coupled with a whole blood potassium collected within 2 hours. Analysis of this data showed that plasma potassium concentrations were unaffected by platelet count but serum potassium concentrations increased by 0.05 mEq/L per  $100 \times 10^9/L$  increment in platelet count. Over 4 years, 45,430 results included a comment warning of the risk of falsely elevated potassium due to high platelet counts. However, over 90% were plasma samples, for which the comment is not relevant. In serum samples meeting platelet count criteria, 20% demonstrated a  $>1$  mEq/L increase in serum potassium relative to whole blood. In contrast, high WBC counts had a minor impact on serum potassium but significantly impacted plasma potassium, with increases of 0.35 mEq/L per  $50 \times 10^3/\text{microL}$  increment in WBC count. The comment for interference due to high WBC count was appended to 443 results in 115 patients. Specimens with corresponding whole blood potassiums ( $n=83$ ) showed a  $>1$  mEq/L increase in over 70%, suggesting that the WBC count threshold should be lower. Lowering the threshold to a WBC count of  $50 \times 10^3/\text{microL}$  would have flagged 924 patients during this period, and 23% of samples above this threshold had a  $>1$  mEq/L increase in plasma potassium relative to whole blood.

**Conclusion:** Samples with extreme platelet and WBC counts are rare, but analysis of a large number of results provides data-driven thresholds at which high platelet and WBC counts are clinically significant. The suggestion of a  $500 \times 10^9/L$  pseudohyperkalemia alert threshold for platelet count in serum samples is likely appropriate, but our data suggests the WBC counts greater than  $50 \times 10^3/\text{microL}$  in plasma samples may lead to clinically significant false elevations.

### A-141

#### Therapeutic Concentrations of Hydroxocobalamin Interferes with Several Spectrophotometric Assays on Two Commonly Used Chemistry Analyzers

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**Background:** A 28 year-old female with cystic fibrosis underwent a bilateral orthotopic lung transplant. Her sample, drawn for a metabolic panel, had a distinct red discoloration, but a heme index of 0. A sample collected 6-h earlier appeared normal. Pharmacology review indicated that between specimen draws the patient had received a dose of hydroxocobalamin (5g IV infusion; OHCob). High-doses of OHCob are typically used to treat cyanide poisoning and more recently, cardiac complications (as in this patient). Since OHCob absorbs at multiple wavelengths (274, 351, 500 and 526 nm) that are often used in colorimetric assays, spurious laboratory results are likely to

occur. Furthermore, while these samples may appear hemolyzed, they are not flagged by automated analyzers due to differences in the absorbance spectrums of hemoglobin and OHCob. The extent of OHCob interference varies between platforms and had not been assessed using the specific instrumentation/assays used in our laboratory.

**Objective:** We aimed to examine interference caused by OHCob in colorimetric assays measured using the Beckman Coulter DxC800 and AU680.

**Methods:** OHCob concentrations were chosen based on observed therapeutic levels (0.4-1.3 mg/mL) after treatment for cyanide poisoning. OHCob (Sigma Aldrich, USA) was dissolved in water and spiked into pooled "healthy" and "unhealthy" patient samples at two different concentrations (0.15 and 1.5 mg/mL). For those analytes that showed a significant bias at 0.15 and 1.5 mg/mL, OHCob was titrated to the following concentrations: 0.2, 0.4, 0.8, 1, 1.2 and 1.5 mg/mL. Samples were run in duplicate or triplicate on the DxC and AU680 and compared to the unspiked pool. The following analytes were selected because their quantification relies on wavelengths near the wavelengths at which OHCob absorbs: Albumin, ALP, ALT, AST, DBil, TBil, Chol, CK, creatinine, Direct LDL, GGT, haptoglobin, HDL cholesterol, Fe, LDH, lipase, Mg, phosphate, total protein, transferrin, triglycerides and uric acid.

**Results:** Substantial interference was observed with low concentrations of OHCob (0.15 mg/mL) in TBil (0.6 mg/dL, 171% bias), Fe (5µg/dL, 31% bias) and triglyceride (77.3 µg/dL, 15% bias) results measured using the DxC; no interference was observed with the AU680. At high OHCob concentrations (1.5 mg/mL), significant bias was observed with a number of analytes on both the DxC and AU680: ALT, amylase, AST, TBil, Chol, creatinine, Mg, phosphate, and uric acid. DBil, Fe and triglyceride measurements were effected only on the DxC; direct LDL measurement was effected only on the AU680. Titration of OHCob revealed a  $>10\%$  bias in TBil, Dbil, cholesterol, triglycerides and iron even at the low end of the OHCob therapeutic range (0.4 mg/mL) using the DxC.

**Conclusions:** OHCob is a colored compound that is known to unpredictably interfere with several colorimetric chemistry measurements. Between the DxC and AU680, we found several analytes that were falsely increased or decreased at therapeutic OHCob concentrations. We recommend that laboratories understand how OHCob influences results on their platforms and that there is a workflow in place to notify the lab when these samples are expected.

### A-142

#### Assessing the impact of Enzymatic Creatinine on eGFR in an elderly outpatient population

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**Background:** The majority of laboratories in New Zealand use a rate blanked compensated Jaffe method for Creatinine measurement in serum. However it is widely accepted that enzymatic creatinine methods should be the preferred assay, especially in paediatric samples, due to limitations of the Jaffe method at low levels and the impact of various interfering substances. Literature search shows that enzymatic creatinine analysis in elderly outpatients does not appear to have been studied, particularly with regard to calculations of estimated glomerular filtration rate. The aim of this study was to establish the impact of enzymatic creatinine analysis versus Jaffe, particularly with regards to estimations of eGFR by the CKD-EPI equation, in a relatively consistently distributed for age and gender, outpatient population of patients greater than 65 years of age.

**Methods:** Cross-sectional study conducted in 100 elderly New Zealand outpatients, aged between 65 and 95 years of age. Mean age was  $80 \pm 9$  years, with equal distribution of men and women (50% each). Creatinine was measured by a traceable Jaffe method (Cobas 702, Roche Diagnostics NZ) and by an enzymatic method (CREA plus, Roche Cobas 502). The Jaffe results were compensated to Roche global Cfs at a value of -20, as per regional protocol, as well as to the compensation value of -26 quoted by Roche. eGFR was calculated using CKD-EPI GFR calculator by Stephen Z. Fadem (<http://touchcalc.com/calculators/epi>). Creatinine values were assessed for trueness using CLSI EP15-A2 on StatusPro, and for clinical comparability based on the RCPA Allowable limits of performance for Creatinine ( $\pm 8$  up to 100;  $\pm 8\%$   $>100$  µmol/L). The clinical impact of eGFR values was assessed based on the trigger point of  $<60$  mL/min/1.73m<sup>2</sup> indicating additional testing required. Change in CKD stage was also noted.

**Results:** The difference in creatinine values between the -20 Jaffe and enzymatic methods across all subjects met the bias claim of 0.5% at a significance level of 20%. In women, all results fell within the RCPA ALP, except for 1 patient with a very low creatinine level, however this difference did not alter the CKD stage of the patient. 5 of the CKD stages in women altered values around the trigger point of 60 mL/min/1.73m<sup>2</sup>, 2 removing the indication for further testing and 3 indicating further testing was required. There was more variation between the -20 Jaffe and enzymatic

methods in men, with 4 patients values falling outside the RCPA ALP, and while there were resulting changes in CKD stage in 3 of these patients, none of were at the trigger point of 60 mL/min/1.73m<sup>2</sup>. Use of the -26 Jaffe gave 22 % of values outside the RCPA ALP in men and 6% in women.

**Conclusion:** Based on this study, the use of enzymatic creatinine in the elderly appears comparable to that of the in-use -20 Jaffe method, and the impact of the change of technique for creatinine measurement is unlikely to have a major impact on referral rates based on CKD stage changes. A compensation value of -20 appears to be the most suitable in this population.

#### A-144

##### A Comparison Of Two Formulae Calculating Estimated Glomerula Filtration Rates In A Healthy South East Asian Population

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**Background:** It is common clinical practice to include an estimated glomerula filtration rate (eGFR) based on a serum Creatinine determination when a renal panel assessment is done. There are two western centric formulae, namely the Modified Diet in Renal Disease (MDRD) and the more contemporary Chronic Kidney Disease Epidemiology (CKD-Epi). Both utilize gender and American/African-American coefficient adjustments with the latter including consideration for the concentration of serum Creatinine.

In this study, we looked at healthy male and female individuals in an anonymised data set. They comprised of the four main races in Singapore, namely Chinese, Malays, Indians and Others (Caucasians, Eurasians and Sikhs) in a ratio of 7:1:1:1.

**Methods:** We reviewed 5470 serum Creatinine results over a period of 8 months from individuals attending health screening and non-disease related hospital visits. We applied both the MDRD and CKD-Epi formulae applying the American ethnic coefficient. We analysed the data using Statistical Package for the Social Sciences (IBM, United States) according to formula, gender and ethnicity.

**Results:** Overall, there was a shift in the eGFR mean from 88.15 [95% confidence interval (CI) 87.5-88.8] to 94.1 (CI 93.4-94.8) when we compared MDRD to CKD-Epi. The mean eGFR of males was 83.4 (CI 82.6-84.3) using MDRD compared to 93.2 (CI 92.1-94.3) using CKD-Epi. The difference for females was less significant at 93.2 (CI 92.1-94.2) for MDRD versus 95.0 (CI 94.1-95.9) for CKD-Epi. The standard deviation for males expanded from 23.46 to 28.90 while the calculation for females was reduced from 26.89 to 23.01 for MDRD and CKD-Epi respectively.

The eGFR means for the four races, Chinese, Malay, Indian and Others were 89.2, 82.2, 85.7, and 89.1 respectively using MDRD and 94.8, 88.0, 92.8, and 96.0 respectively using CKD-Epi. Additionally, the standard deviations (SD) were 25.00, 30.46, 24.32 and 25.38 for MDRD and 25.38, 31.76, 26.15 and 25.49 for CKD-Epi for the four races.

**Conclusion:** Our laboratory uses the cut-off of 90 mL/min/1.73 m<sup>2</sup> to demonstrate absence of kidney dysfunction when previously using MDRD and currently the CKD-Epi in assessing glomerula filtration rate. When we moved from an MDRD based calculation to a CKD-Epi based analysis, there was a clear reclassification of 14.3% of the population. This shift was predominantly in the male subjects where 18.1% were reclassified as having "normal" eGFR.

All races except Malays saw eGFR means being reclassified as "normal". The mean for Malays improved from 82.2 to 88.0 using the CKD-Epi formula. The Malays also had the largest standard deviation of 30.5 (MDRD) and 31.7 (CKD-Epi) compared to the all-race SD of 25.9. This may imply that this particular race may require an ethnic specific correction coefficient to be defined.

Our findings are consistent with current reports that there is clear improvement especially for eGFR values greater than 60 mL/min/1.73 m<sup>2</sup>, despite using a Caucasian derived correction coefficient on a predominantly Asian ethnic population. It is yet unclear if an Asian specific coefficient is necessary to improve eGFR specificity although an intra-ethnic coefficient for the Malay population may be indicated.

#### A-145

##### Interference analysis of PCT testing in bacterial infection diagnosis

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**Background:** Procalcitonin (PCT) is a biomarker for the clinical diagnosis of bacteria infection that is more specific and earlier than fever, changes in white blood cell count,

and blood cultures. The performance of instrument, conditions of sample processing, and underlying diseases in patients can affect PCT testing results that make clinical doctors confused. These factors should be confirmed, and the role of PCT in bacterial infection diagnosis should be re-evaluated when necessary.

**Methods:** In order to evaluate analytical performance, the precision and accuracy of detection, which was conducted using a Roche Cobas E601 Electrochemiluminescence Immunoassay Analyzer (Roche, Basel, Switzerland), were verified by following Document EP15-A2 from the Clinical and Laboratory Standards Institute (CLSI). In order to determine the impacts of sample processing, the PCT levels between heparin plasma, EDTA plasma, serum in separation gel coagulation promoting tubes, and serum in tubes without additives, were compared at various intervals (0h, 12h, 1d, 3d and 7d) and storage temperatures (room temperature, 4°C, -30°C and -70°C). Moreover, the interference of common endogenous substances, such as hemoglobin, direct bilirubin and chyle, were also analyzed using CLSI EP7-A2 Documents. In order to prove congestive heart failure could interfere with PCT levels in patients with or without bacterial infection, a total of 4698 cases, including those with different classes of congestive heart failure, bacteria infection, and bacteria infection complicated by congestive heart failure, and healthy individuals, were chosen for the diagnostic value analysis of PCT.

**Results:** The precision and accuracy were good for PCT detection using Cobas e601. The total coefficient of variation (CV) was below 3.59% and the deviation from definite value was below 3.38%, which were consistent with clinical requests. In all kinds of sample collection tube, PCT would degrade under room temperature after 24h. EDTA plasma was the most suitable sample type with lowest degradation rate, and could be stored at 4°C (or lower temperature) for more than 7d with at least twice freezing and thawing. The interference of common endogenous substances could not be observed when the levels up to Hb(2g/L), DB(428µmol/L), chyle(2000FIU). Patients with simple heart failure had significantly higher PCT levels than normal controls ( $P < 0.01$ ), whereas patients with bacteria infection complicated by congestive heart failure had significantly higher PCT levels than those with simple infection ( $P < 0.01$ ). Although it was useful for the diagnosis of infection (area under the ROC curve  $> 80\%$ ), the positive predictive value of PCT decreased significantly with increasing severity of heart failure ( $P < 0.05$ ), and the cutoff value of PCT concentrations for infection complicated by class-II, -III, and -IV heart failure were up to 0.086, 0.192 and 0.657 µg/L, respectively.

**Conclusions:** The analytical performance of PCT assay on Roche Cobas e601 meets clinical request, and it has strong anti-interference capacity against endogenous substances. However, sample type selection and patients underlying factors should be given consideration in PCT diagnostic value analysis.

#### A-147

##### Effect of specimen types and storage on the activity of Lactate dehydrogenase in Serum and Plasma

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**Objective:** The study objective was to evaluate the effect of pre-analytical variables due to and storage with various specimen tube types. For this purpose we measured Lactate dehydrogenase (LDH) activity in the serum and plasma samples stored separately in primary and aliquots tubes at 2-8 °C across 24 hours at various time points.

**Method:** The serum and plasma samples were collected from 15 apparently healthy volunteers using standard phlebotomy techniques. Each sample was collected into Serum tubes, Serum separator tubes (SST), Lithium heparin tubes (Li-heparin) and Lithium heparin tubes with gel (PST) and aliquoted. LDH (Abbott, LN 2P56) activity was measured on Abbott ARCHITECT c8000 systems. The % recovery of LDH measured using different sample types at various times points were compared with results obtained using SST aliquot samples at 2 hours post collection.

**Results:** The data demonstrate that the LDH activity measured using samples stored in either SST primary tubes or SST aliquots for various time points show very minimal or no change in the activity. In contrast, the plasma samples stored for 24 hours in the Li-heparin and PST primary collection tubes showed increased recovery of 20.8% and 33.0% respectively when compared to the SST aliquot tubes at 2 hours post collection. In addition, compared to SST aliquot tubes, the samples stored in the serum primary tubes recovered approximately 7% lower at 2 hours but demonstrated comparable recovery overtime.

**Conclusion:** The extended contact between blood cells and serum/plasma stored at 2-8 °C may produce spuriously high results for Lactate dehydrogenase. The increased LDH activity is likely related to the change in the cell permeability and to the

fragility of erythrocyte membrane during prolonged storage. Therefore, LDH activity measured in patient samples stored for extended period of time should be interpreted cautiously.

	Tube 1 Serum	Tube 2 Serum Aliquot	Tube 3 SST	Tube 4 SST Aliquot	Tube 5 Li- Heparin	Tube 6 Li-Heparin Aliquot	Tube 7 PST	Tube 8 PST Aliquot
2 hrs	92.9	93.1	99.1	100.0	109.6	104.3	111.3	103.5
6 hrs	97.9	94.0	101.5	100.1	116.0	104.2	120.0	103.6
12 hrs	98.3	92.8	101.7	99.4	117.9	103.5	122.3	101.8
24 hrs	101.8	93.6	103.2	101.1	120.8	104.1	133.0	103.0

**A-148**

**Data mining of serial blood gas data reveals that use of safePICO syringes significantly reduces preanalytic variation**

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**Background:**

In late 2010, Alberta Health Services replaced the Portex Pro Vent with safePICO syringes for virtually all arterial blood gas testing. Presumably, the electrolyte-balanced heparin in these syringes would reduce the variation in calcium measurement. We discovered the new syringes reduced the preanalytic variation of most of other analytes.

**Methods**

The Portex Pro-Vent arterial blood sample kits (Keene, NH, USA), contain 23.5 to 25 IU/mL of dry lithium heparin. The safePICO (Copenhagen, Denmark) uses 60 IU/mL of uniformly distributed dry electrolyte-balanced heparin. In 2010 we derived biologic variation (s<sub>b</sub>) of various blood gas parameters using the methodology described in *Clin Chem Lab Med*. 48: 1447–1454. We applied the same methodology to the last 2 years of safePICO arterial blood gas measurements from the identical general systems intensive care unit (approximately 40,000 Radiometer ABL800 FLEX pH, blood gas, electrolyte, and glucose measurements from 3079 patients). After exclusion of physiologic outliers we calculated the standard deviations of duplicates (SDD) of paired intra-patient results across intervals of 2 hours: 0-2h, 2-4h, 4-6h ... 20-22h, and 22-24h. Linear regression was applied to data points derived from more than 1000 paired results. s<sub>a</sub> was calculated from the formula where s<sub>a</sub> represents the analytic variation at the patient mean.

**Results:**

The Table compares the calculated s<sub>a</sub>, the corresponding patient means for both blood gas syringes and the reduction in the calculated s<sub>a</sub>.

**Conclusions:**

This decrement in biologic variation can be classified as a decrease in preanalytic variation and results from the decreased variation in the sample milieu induced by the balanced heparin. Use of safePICO reduces the preanalytic variation of at least sodium, bicarbonate, calcium, chloride and glucose measurements, findings that have been intimated in a handful of small syringe evaluations.

Test	s <sub>a</sub> (mean) for non safePICO	s <sub>a</sub> (mean) for safePICO	Reduction in s <sub>a</sub>
Na <sup>+</sup>	0.77 (140.3)	0.51(140.0)	-39.1%
HCO <sub>3</sub> <sup>-</sup>	0.84(24.1)	0.66(24.5)	-26.2%
iCa <sup>++</sup>	0.026 (1.09)	0.020(1.10)	-25.4%
Cl <sup>-</sup>	0.81(107.8)	0.65(107.0)	-20.6%
Glucose	0.70(6.93)	0.65(7.40)	-13.4%
K <sup>+</sup>	0.19(3.89)	0.17(3.96)	-10.5%
pCO <sub>2</sub>	2.26(39.0)	2.08(39.0)	-8.3%
pH	0.022(7.41)	0.021(7.41)	-6.9%
pO <sub>2</sub>	11.48(85.0)	11.61 (85.1)	+1.0%

**A-149**

**Anomalous amylase activity on a diluted patient sample**

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**Background:** Amylase is a digestive enzyme produced in the exocrine pancreas and salivary glands. The reference range in human serum is 30 to 110 U/L. Elevated amylase can be indicative of pancreatitis, pancreatic cancer, cholecystitis, and mumps. Amylase is also monitored in surgical wound drainage as a predictor for postoperative pancreatic fistula. Hemoglobin is known to interfere in the Vitros amylase method and under circumstances where a sample is markedly hemolyzed (≥ 1 g/dL), standard procedure calls for dilution of the sample using a commercial diluent in order to reduce or eliminate the interferent. Here, we report a case where amylase activity was measured on a markedly hemolyzed surgical drainage sample (75 U/L). Due to high hemolysis, the sample was diluted 1:10 but unexpectedly had an activity of 50 U/L, which was ~7-fold higher activity over the undiluted sample after taking the dilution factor into account. We hypothesized that the commercial diluent contained amylase which contributed to this falsely elevated activity. **Method:** The surgical drain sample was centrifuged (1,100 x g for 3 min) to separate blood cells from the supernatant before analysis. Hemoglobin concentration was determined using the Sysmex XE-2100. Amylase activity was analyzed on the Vitros 5,1 FS and the diluent amylase activity was confirmed on a Beckman DxC. Both analyzers measure the volumetric activity of amylase based on a colorimetric assay, but use different substrates. **Results:** The surgical drain sample contained a hemoglobin concentration of 2.8 g/dL post centrifugation. Serial dilution of patient sample gave increasing amylase activity: 1:10 dilution, 500 U/L (10 x 50); 1:100 dilution, 5100 U/L (100 x 51). Analysis of the commercial diluent gave an amylase activity of 50 U/L (Vitros) and 55 U/L (Beckman). This was confirmed on a previously unopened vial of diluent. **Conclusion:** We conclude that the true amylase activity in our patient sample was <75 U/L and that the diluent contributed to the anomalous results. We recommend that if amylase is present in the diluent, then the amylase activity in the diluent should be analyzed along with patient samples. Results on the diluted sample need to be corrected for the amylase activity in the diluent. We also recommend that manufacturers of diluents that contain amylase should clearly state the concentration and activity on their package insert.

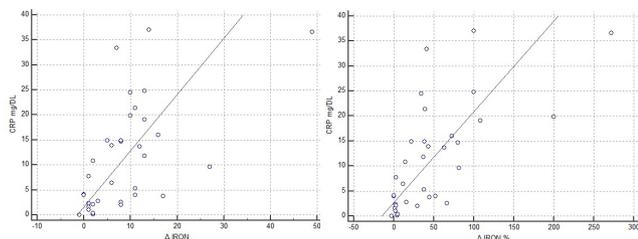
**A-150**

**Serum Iron assays during inflammation - What do they measure? An investigation triggered from the results of a proficiency testing scheme.**

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The role of Proficiency Testing schemes (ex. External Quality Control programs), with their interlaboratory comparisons, is of primary importance to the harmonization of laboratory results. Measurement of iron is considered an unreliable indicator of iron deficiency during inflammation and/or infection since low iron levels are a common finding, probably because iron is bound to the acute phase proteins (ferritin, NGAL etc.) and thus, cannot be reliably measured.

During last year's audit of the results from the Greek Proficiency Testing scheme (ESEAP), we observed that some iron measuring assays showed a persistent, but not constant, positive bias compared to the mean of the other methods. We tried to correlate the above bias with the value of CRP (as an inflammation marker) in the control samples, but the range of CRP in these samples was limited, inside the reference range, thus this study was inconclusive. To further investigate the influence of inflammation and/or infection on iron measurement, we measured iron levels in 34 patients with inflammation, infection and sepsis using two assays (Thermo-Scientific, Vantaa, Finland and Abbott Architect, Abbott Park II as reference) and their CRP levels, using an immunoturbidimetric assay (Sentinel Milano, Italy). We observed differences between the two assays, increasing with the increase of the inflammatory status. We found a strong positive correlation between the levels of CRP and the absolute as well as the percent difference between the two methods (Spearman's rank correlation coefficient: r=0.619, p<0.0001 and r=0.702, p<0.0001 respectively - Figure 1). These differences indicate that the degree of inflammation has an impact of the levels of measured iron depending on the used assay.



As normally assays are specified to measure the total serum iron, we suggest that an effort from IFCC and other competent authorities has to prepare guidelines for the iron measuring assays in order to eliminate the interference from inflammation.

**A-151**

**Level of cystatin C in patients with liver cirrhosis**

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**Introduction:** Cirrhosis of the liver is often accompanied by functional renal failure particularly in advanced stages of liver disease. For the evaluation of renal failure, commonly used in clinical practice, creatinine, which depend on gender, age and race, and is therefore unreliable for accurate assessment of GFR, particularly in the initial renal function impairment. (CysC) has been proposed as a specific marker of glomerular filtration rate (GFR) and an early indicator of impaired renal function.

**Objective** The aim of the study was to evaluate the level of CysC and its importance for the assessment of renal function, i.e. GFR in patients with liver cirrhosis.

**Methods:** The study included 63 patients (aged 50.8±13.5 years), 47 males and 16 females with alcoholic 41 (65.1%) and viral cirrhosis 22 (34.9%) treated at the Clinic for Gastroenterology and Hepatology, Clinical Center of Serbia, Belgrade. A healthy control group comprised of 30 age and gender-matched subjects. The study was conducted in accordance with Guidelines for Good Clinical Practice, the Declaration of Helsinki. The degree of liver insufficiency was assessed according to the Child-Pugh classification divided into three stages: A in 23 (36.5%), B in 21 (33.3%) and C in 19 (30.2%) patients. CysC serum concentration was determined by the PENIA method using commercial kits (Marburg, Germany), on a laser nephelometer (BN II SIEMENS). CysC referent value was 0.59-1.04 mg/L. Cr was determined according to the kinetic Jaffe's method, using commercial kits on analyzer Olympus AU 400 (Hamburg, Germany). Estimated GFR was calculated from serum Cr using the Modification of Diet in Renal Disease (MDRD) equation and from serum CysC using Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) Equation. Statistical analysis was performed using the *t* test, Mann-Whitney, ANOVA, Pearson's (*r*) or Spearman's and post-hoc multiple comparison procedures with the Statistical Package for Social Sciences version 15 (SPSS Inc., Chicago, IL, United States).

**Results:** The average value of CysC measured in patients with liver cirrhosis was 1.09 ± 0.42 mg/L, significantly higher (*P* = 0.036) than in the control group (0.88 ± 0.12 mg/L). Increased values of CysC observed in 23 (40%) patients. Increased Cr values detected in 7 (11.1%) patients. Post-hoc comparisons showed statistically significant differences in values of CysC between Child-Pugh A and B (*P* = 0.014) and between A and C (*P* = 0.007) stages, while there was no difference between B and C stages (*P* > 0.05). The mean GFR estimated using CysC (GFR<sub>CysC</sub>) and Cr (GFR<sub>Cr</sub>) was 77.6 mL/min/1.73 m<sup>2</sup>, respectively, 113.5 mL/min/1.73 m<sup>2</sup>. GFR < 90 mL/min/1.73 m<sup>2</sup> was obtained in 40 (63.5%) patients using the GFR<sub>CysC</sub> formula and in 13 (20.6%) patients using GFR<sub>Cr</sub> formula. GFR < 60 mL/min/1.73 m<sup>2</sup> observed in 16 (25.4%) patients using the GFR<sub>CysC</sub> formula and in 7 (11.1%) patients using GFR<sub>Cr</sub> formula. **Conclusion:** CysC significantly higher in patients with cirrhosis than in the control group. More patients with decreased glomerular filtration rate (GFR) were identified based on CysC GFR than on creatinine GFR. Serum cystatin C represent sensitive indicators of renal dysfunction in liver cirrhosis.

**A-152**

**Strong Negative Interference by Calcium Dobesilate in 8 Sarcosine Oxidase Assays of Serum Creatinine Involving the Trinder Reaction**

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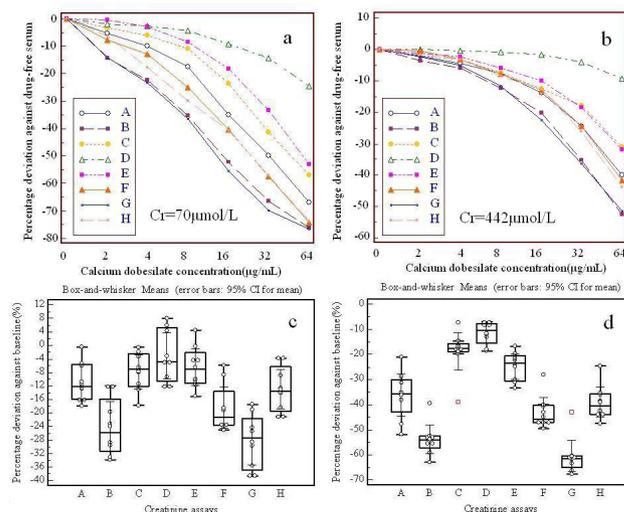
**Background:** Calcium dobesilate has been observed to interfere with creatinine (Cr) measurement using sarcosine oxidase assays. The aim of this study was to evaluate

the interference in 8 sarcosine oxidase Cr assays (Roche, Beckman, Siemens, Ortho Clinical, Maker, Merit Choice, Leadman, Biosino) and to determine its clinical significance.

**Methods:** In the in vitro experiments, we measured Cr in pooled serum with final concentrations of calcium dobesilate additions (0, 2, 4, 8, 16, 32, and 64 µg/mL) using 8 enzymatic assays. Bias (%) was calculated relative to the drug-free specimen. In the in vivo experiments, 8 participants were recruited and baseline serum were collected, then calcium dobesilate was given 3 times per day (each dose, 500 mg orally) for 3 days. Blood samples were collected at 0 hour and 2 hours after another 500-mg dose administration on the fourth morning. The Cr concentration quantified using different assays at 0 hour and 2 hours were compared with the level at baseline. Cr levels of 10 specimens from those who have taken calcium dobesilate were measured by Roche, Beckman, Maker, Merit Choice assays and the LC-IDMS/MS method.

**Results:** The exogenous addition of calcium dobesilate negatively interfered with the Cr concentration in all 8 enzymatic Cr assays, which was highly dependent upon the calcium dobesilate concentration (Figure 1a,b). The observed Cr concentrations for 8 participants measured by enzymatic assays were inhibited by -28.5% to -3.1% at 0 hour (Figure 1c) and by -60.5% to -11.6% at 2 hours (Figure 1d) relative to the level at baseline. The Cr values of 10 patients measured by Roche, Beckman, Maker, Merit Choice assays showed an average deviation of -20.0%, -22.4%, -14.2%, and -29.6%, respectively, compared with the LC-IDMS/MS method.

**Conclusion:** Calcium dobesilate produced significantly negative interference with Cr in all Cr assays based on the sarcosine oxidase method. Significant differences in the degree of interference were observed among assays.



Note: Panels a and b showed the effects of the exogenous addition of calcium dobesilate on Cr quantification in 8 enzymatic assays when basal Cr concentrations were 70 and 442 µmol/L, respectively. Panels c and d showed the percentage deviation plots for the creatinine concentrations at 0 hour, 2 hours after drug administration from 8 participants against baseline by Box-and-whisker plot, respectively. Creatinine concentrations were measured by 8 enzymatic assays: A, Roche, B, Beckman, C, Siemens, D, Ortho Clinical, E, Maker, F, Merit Choice, G, Leadman, H, Biosino

**A-154**

**Status of Harmonization of Serum Albumin Measurement Procedures: Comparison of 21 BCG and BCP Methods to an Immunochemical Method**

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**Background:**

Measurements of serum and plasma albumin are widely used in medicine, including their use as quality indicators for the care of patients in renal dialysis centers. We examined the current status of harmonization of measurement procedures for albumin.

**Methods:**

Pools (n=152) were prepared from non-frozen residual serum or plasma samples submitted for routine albumin testing at two academic medical centers during 3 weeks in the spring of 2014. Among these, 53 pools were predialysis serum samples from patients undergoing renal dialysis. Samples were distributed non-frozen on cold packs to manufacturers to perform quadruplicate albumin measurements by 21 measurement procedures: 9 used bromocresol green (BCG), 12 used bromocresol purple (BCP). The

Roche Tina Quant immunochemical procedure recovered 101.4% of the IRMM ERM DA470k/IFCC reference material value and was used for comparison.

**Results:**

Table 1 shows that, for each type of sample (renal serum, nonrenal serum, and plasma), the mean of all results of the 9 BCG methods was higher than the corresponding mean of results from the 12 BCP methods. In general, BCG methods had a positive bias and BCP methods had a negative bias when compared to the Tina Quant immunochemical procedure. Biases varied among BCP methods and among BCG methods. For all but one method, biases were statistically significantly different (P<0.02) for serum vs. plasma samples and for renal dialysis vs. non-renal patient sera.

**Conclusion:**

BCG methods had higher values than did BCP methods, and both BCG and BCP methods were biased when compared with an immunochemical method. Moreover, bias of both BCG and BCP methods varied among manufacturers' methods and depending on sample types (serum vs. plasma) and patient types (dialysis patient vs. non dialysis patient). Results from albumin measurement procedures are not harmonized and fixed decision values for clinical decisions or for quality indicators are not appropriate.

Recovery of reference material and mean values for pooled samples measured by the indicated methods			
Parameter	Immunochemical Method	12 BCP Methods	9 BCG Methods
Recovery of IRMM material (%), Grand mean (Min, Max Recovery within method group)	101.4	98.4 (95.5,102.3)	95.3 (80.5,100.7)
Mean Albumin Concentrations of Pools (g/dL)			
Renal Serum, Grand Mean (53 pools) (Min, Max Mean Concentrations)	3.42	3.29 (3.17, 3.40)	3.53 (3.34, 3.72)
Nonrenal serum, Grand Mean (50 pools) (Min, Max Mean Concentrations)	3.35	3.29 (3.19, 3.40)	3.53 (3.39, 3.76)
Nonrenal plasma, Grand mean (49 pools) (Min, Max Mean Concentrations)	3.05	3.11 (2.98, 3.21)	3.40 (3.21, 3.70)
Mean Bias vs Immunochemical Method (g/dL)			
Renal serum, Grand mean (Min, Max Mean Bias)	---	-0.13 (-0.26, -0.02)	0.10 (-0.08, 0.30)
Nonrenal serum, Grand mean (Min, Max Mean Bias)	---	-0.06 (-0.16, 0.05)	0.18 (0.04, 0.41)
Nonrenal plasma, Grand Mean (Min, Max Mean Bias)	---	0.06 (-0.08, 0.16)	0.35 (0.16, 0.64)

**A-155**

**Evaluation of Sweat Collection Material for Sweat Chloride Testing**

C. A. Gallo, D. A. Payto, S. Wang. *Cleveland Clinic, Cleveland, OH*

**Background:** The Cystic Fibrosis Foundation guidelines on diagnostic sweat testing for cystic fibrosis specify that gauze or filter paper used for sample collection should have an area of 4 square inches. The aim of this study was to investigate various materials that could be used for sweat collection. **Methods:** Grades 1, 2, and 3MM filter paper squares (2 inch X 2 inch), as well as 4-ply, 2-ply, and 1-ply gauze squares (2 inch X 2 inch), were evaluated. Five chloride solutions with concentrations covering the analytical measurement range (10-160 mM) were added to vials containing the collection materials and were measured by the Schales and Schales chloride titration method. **Results:** Gauze squares were easier to manipulate with forceps and place into glass collection vials than filter paper squares. While 4-ply and 2-ply gauzes absorbed all of the titration volume making it hard to titrate, 1-ply gauze did not. All gauze tested contained chloride contamination, which was resolved by performing a blank on each lot of gauze. Chloride was linear from 9.7 to 161.0 mM, with recoveries of 97-103%. Simple Precision, performed at low, mid, and high levels, showed a %CV of <2.5%. **Conclusion:** 1-ply Gauze was determined to be the best material for our titration method, but was found to be contaminated with chloride that needed to be subtracted from the measured chloride concentrations. 1-ply gauze has been validated for patient testing.

Table 1. Recovery of chloride spiked on gauze.

Spiked Cl Conc. (mM)	Mean Yield without a Gauze Blank (mM)	Mean Yield with a Gauze Blank (mM)	Analytical Recovery without a Gauze Blank (%)	Analytical Recovery with a Gauze Blank (%)
160	148.0	161.0	93	101
80	80.7	82.0	101	103
40	40.7	40.0	102	100
20	25.0	20.0	125	100
10	15.0	9.7	150	97

**A-156**

**Establishment of Analyte- and Concentration-Specific Hemolysis Index Thresholds for Aspartate Aminotransferase (AST) and Direct Bilirubin (DBIL) Reduces Specimen Rejection and Recollection Rates**

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**Background:** Interference due to hemolysis is a common reason for specimen rejection in clinical laboratories. Assay manufacturers provide instrument- and test-specific hemolysis index (HI) limits for interference in their instructions for use, however laboratories may also perform interference studies to verify or establish hemolysis tolerance limits. Automated hemolysis detection using serum indices and rule-based algorithms based on analyte concentration and HI may increase laboratory efficiency and reduce specimen rejection rates. **Objectives:** The aims of the study were to (i) determine concentration-specific HI thresholds for aspartate aminotransferase (AST) and direct bilirubin (DBIL) on the Cobas® 6000/8000 chemistry platforms (Roche Diagnostics) and (ii) compare rule-based concentration-dependent algorithms to manufacturer recommended HI limits and measure the impact on specimen rejection and recollection rates. **Methods:** Residual specimens from physician-ordered AST or DBIL (serum) and hematology (EDTA whole blood) testing were used to prepare serum and hemolysate pools, respectively. Twelve serum pools with AST concentrations between 27-770 U/L and 19 serum pools with DBIL concentrations between 0.1-9.7 mg/dL were created. Hemolysate was prepared from washed red cells and lysed by freeze-thaw (-70°C). Varying hemoglobin concentrations were obtained by diluting hemolysate with water. Hemolysate (10% by volume) was then added to serum pools with known analyte concentrations to obtain HI ranging from 6 to 526. AST, DBIL and HI were measured using the Cobas 6000/8000 c501/c701 chemistry analyzers. The absolute and percent bias due to increasing HI was calculated. Recovery within ±5 U/L or ±10% of initial AST value and recovery within ±0.1 mg/dL (initial DBIL ≤0.3mg/dL) and ±0.2 mg/dL or 20% (initial DBIL >0.3 mg/dL) for DBIL were considered acceptable. Specimen rejection and recollection rates were calculated by applying manufacturer HI

limits and the established concentration-specific HI limits to results from physician-ordered AST and DBIL tests performed in the Central Clinical Laboratory at Mayo Clinic, Rochester, MN (January-February 2015). **Results:** Concentration-specific HI thresholds were established for AST <100 U/L, 100-200 U/L, 200-300 U/L and >300 U/L at HIs of 50, 100, 200 and 500, respectively. There were 10,605 orders for AST and 862 (8.1%) specimens would have been rejected and recollected based on manufacturer's recommendations (HI >20 for all AST concentrations). By applying concentration-specific hemolysis limits for AST, 154 (1.5%) specimens were rejected due to hemolysis, thus eliminating recollection of 708 specimens (82% reduction). For DBIL, HI tolerance limits of 70 and 50 were established for 0.0-0.3 mg/dL and >0.3 mg/dL, respectively. Out of 7062 orders for DBIL, 494 (7.0%) specimens would have been rejected based on manufacturer's recommendations (HI >30 for all DBIL concentrations). By applying the concentration-specific hemolysis thresholds for DBIL, 188 (2.7%) specimens were rejected due to hemolysis, thus eliminating recollection of 306 specimens (62% reduction). **Conclusions:** Analyte- and concentration-specific hemolysis index thresholds were established for AST and DBIL. Automated hemolysis detection using HI and rule-based algorithms based on analyte concentration significantly reduces specimen rejection and recollection rates due to hemolysis. Additional downstream benefits include improved turn around time and cost savings associated with not having to recollect patient specimens.

**A-157**

**Protein Interference with Common Laboratory Tests**

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Objective: Protein as an interferent is under appreciated. Therefore we evaluated interference by proteins on clinical laboratory tests. Methods: Base Pool was prepared using a diluted serum pool with low total protein. A High Pool was prepared by spiking proteins into the base pool (Concentrated human albumin and gamma-globulins 1:1; ASSURANCE™ Interference Test Kit, Sun Diagnostics, New Gloucester, ME). The base pool and high pool were intermixed to create 5 levels of total protein (3.5, 6.9, 9.4, 12.4, and 15.3 g/dL). Multiple analytes were measured on the Beckman AU5800. Results: Minimal effects were seen with ALP, AMY, DBIL, TBIL, CK, GGT, GLU, LDH, LIP, PHOS, BUN, UA, Lp(a), sLDL-C, hsCRP, ferritin, LDL-C, HDL-C, K, CL, TRIG, and CHOL. Positive bias with increasing protein concentration was seen with ALT, AST, CA, CRE, FE, MG, homocysteine (HCY), fructosamine, and non-esterified fatty acids (NEFA). Because proteins bind analytes such as calcium, magnesium, iron, homocysteine, and NEFAs, we believe these increases were artifactual. Negative bias was seen with CO2, apo AI, and apo B. Conclusion: Manufacturers and laboratorians need to pay more attention to proteins as a potential interferent. The mechanism may be photometric or a volume depletion effect, whereby a very high protein concentration will reduce the available water so that the analyte concentration is artifactually low.

Analytes Affected by Protein						
Protein, g/dL	ALT, U/L	AST, U/L	CO2, mmol/L	CA, mg/dL	CRE, mg/dL	FE, µg/dL
3.5	8.3	8.4	8.5	4.7	0.44	35.8
6.9	9.2 (11.6%)	9.7 (15.1%)	8.0 (-5.8%)	6.0 (25.5%)	0.60 (35.4%)	55.3 (54.2%)
9.4	9.1 (10.1%)	10.1 (19.2%)	6.6 (-22.9%)	6.5 (36.3%)	0.67 (51.1%)	69.0 (92.5%)
12.4	9.8 (18.8%)	11.0 (30.5%)	5.7 (-32.7%)	7.4 (56.5%)	0.80 (79.7%)	87.5 (144%)
15.3	10.8 (30.4%)	11.7 (39.2%)	5.9 (-30.4%)	8.3 (75.5%)	0.93 (110%)	105.2 (194%)
Protein, g/dL	Apo AI, mg/dL	Apo B, mg/dL	MG, mg/dL	HCY, µmol/L	NEFA, mg/dL	NA, mmol/L
3.5	77.8	38.1	1.0	5.9	0.44	151
6.9	80.9 (3.9%)	38.4 (0.8%)	1.3 (23.3%)	10.9 (84.4%)	1.73 (293%)	148 (-1.8%)
9.4	68.6 (-11.9%)	27.6 (-27.5%)	1.4 (35.0%)	15.1 (156%)	2.89 (557%)	147 (-2.3%)
12.4	63.2 (-18.9%)	18.2 (-52.3%)	1.6 (53.4%)	19.8 (236%)	4.12 (836%)	146 (-3.2%)
15.3	55.4 (-28.8%)	11.1 (-70.8%)	1.8 (73.8%)	24.5 (316%)	5.29 (1102%)	146 (-3.4%)

**A-158**

**Effects of Urine Specimen Containers with Metal Caps on Urine Test Results**

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**Background:** Specimen collection containers must be carefully evaluated to ensure that the analytes being tested are not affected by the materials present in the containers. Due to the better sturdiness our institution intended to switch from plastic urine collection containers with plastic screw-caps to plastic containers with metal screw-caps. The aim of this study was to determine if this switch would cause any adverse impact on our current tests. **Methods:** Urine from each of 5 leftover plastic collection jugs was aliquotted to one plastic container with metal cap and another with plastic cap, for a total of ten aliquots. The urine samples were spiked at levels spanning the analytical measurement ranges if the original concentrations were low. The cups with metal caps were placed refrigerated upside-down for a minimum of 18 hours to allow for urine contact with the caps, while the corresponding cups with plastic caps were stored at the upright position side by side with those with metal caps. These samples were analyzed for citrate, oxalate, metanephrine, normetanephrine, 5-hydroxyindole-3-acetic acid, vanillylmandelic acid, copper, magnesium, zinc, porphyrins, porphobilinogen, morphine, codeine, 6-acetyl-morphine, hydromorphone, hydrocodone, dihydrocodeine, oxycodone, oxymorphone, O-desmethyl tramadol,

tramadol, fentanyl, norfentanyl, buprenorphine, norbuprenorphine, methadone, EDDP, amphetamine, methamphetamine, benzoylcegonine, 11-nor-9-carboxy-delta-9-THC. **Results:** No significant difference (<20%) was observed between urine refrigerated in all-plastic containers and those in plastic containers with metal caps for all analytes tested except for 11-nor-9-carboxy-delta-9-THC (THC) which yielded significantly lower results for the samples in the containers with metal screw-caps (Table 1). **Conclusions:** The plastic containers with metal screw-caps offered similar results as the ones with plastic screw-caps for all analytes tested except for THC, which warrants further investigation.

Table 1. Difference in THC results.

Samples Refrigerated in Plastic Tubes (ng/mL)	Samples Refrigerated in Plastic Containers with Metal Screw Caps (ng/mL)	Difference (%)
1479	1207	-18
728	511	-30
312	151	-52
65	51	-22
24	12	-50

**A-160**

**Long-Term Stability of Novel Urinary Acute Kidney Injury Biomarkers**

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**Background**

Acute kidney injury (AKI) is an abrupt loss of kidney function induced by multi-organ failure, sepsis, and nephrotoxicity caused by drugs in development. Several novel urinary biomarkers outperform creatinine as early and more sensitive indicators of AKI; however, their long-term stability (LTS) is unknown and is extremely crucial for consistent reliable results when evaluating drug treatment effects in clinical trials.

**Objective**

Standardizing LTS criteria across assays is complicated by factors including assay precision, robustness, matrix variation between subjects and protein post-modifications. This study compared two LTS methods and established stability for the following biomarkers: Cystatin C (Cys-C), KIM-1, NGAL, clusterin, and n-acetyl-beta-D-glucosaminidase (NAG).

**Methods**

Three pools, each containing urine from three individuals, was collected and aliquots were left at ambient temperature for ≤2h (baseline), then stored at -70°C until assayed at 1, 3, 15, 18 and 28 months. The between-run precision (BRP) of each biomarker assay was determined from QCs used over the 28-month LTS study. Stability was estimated using two methods; 1) Standard Method rendered a sample unstable if it exceeded the acceptance criteria (2\*CV% of BRP from baseline) for two consecutive time points, and 2) Regression Method generated a linear regression through each time point with the y-intercept set as baseline; instability occurred when the rate of degradation (slope) intersected the acceptance criteria (1.5\*CV% of BRP from baseline).

**Results**

The BRP for the urinary assays measuring Cys-C, KIM-1, NGAL, clusterin and NAG ranged from 6-25%CV. The assigned acceptance criteria ranged from 15-50% for the Standard Method and 10-30% for the Regression Method. The stability for Cys-C and KIM-1 in three separate pooled samples was variable when estimated by the Standard Method, ranging from 3-28 months and 1-28 months, respectively. The Regression Method narrowed the estimated range of Cys-C stability down to 12-28 months and 12-21 months for KIM-1. Clusterin's stability ranged from 1-3 months (Standard Method) and 8-11 months (Regression Method). Clusterin stability rapidly decreased within the first month (up to -28%), resulting in an overestimation of stability by the Regression Method due to a shift in baseline (y-intercept). NAG and NGAL were stable for all three samples during the entire 28 months regardless of the estimation method (up to 15% and 7% difference from baseline).

**Conclusion**

The Regression Method more precisely estimated stability when large gaps between time points were observed, such as with Cys-C and KIM-1. In addition, this method applied the assay imprecision to the initial baseline time point (y-intercept). The Standard Method may be more appropriate when early time points are lacking or if a linear rate of degradation cannot be established, such as with clusterin. Applying both methods provides a more precise estimation of stability. In this study, NGAL and NAG were the most stable AKI biomarkers, Cys-C and KIM-1 displayed mid-range stability and clusterin was the least stable biomarker.

**A-161****Achieving Reduction of Lot-to-Lot Variation of the  $\kappa$ - and  $\lambda$ -Freelite Assay (Binding Site) through Standardization**

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**Background:** Maintaining consistent results over multiple batches of immunoassay reagents can be a challenge for the clinical laboratory but is important for providing the best patient care. The complexity of generating antibodies and manufacturing kits for the Freelite assays can introduce more variation than observed with traditional chemistry assays due to the highly variable nature of monoclonal Free Light Chains (FLC). In a multi-site reference laboratory, it is critical to evaluate each new lot of a reagent to determine if the reagent stays within the total allowable error (TE<sub>a</sub>) and to maintain standardization across testing locations. Lot-to-lot validation entails measuring QC material and random patient serum samples before and after each Freelite lot change. The use of patient samples, however, can introduce bias if they contain monoclonal proteins that do not dilute linearly or if they contain free kappa or lambda concentrations that fall outside of the standard measurement range of the assay. The goal of this project was to develop a standardized lot evaluation scheme that minimized the impact of instrument imprecision and sample selection bias for a multi-site reference laboratory.

**Methods & Results:** Quest Diagnostics partnered with The Binding Site (TBS) to achieve standardizations, including instrumentation and patient sample panels to limit introduction of imprecision and bias. In addition, 2 Quest Diagnostics laboratories (Chantilly, VA and Lenexa, KS) were used as pre-validation sites before the implementation of Freelite kits across all subsidiaries. Qualifications of approval for the new FLC reagent (percent bias determined by fraction of TE<sub>a</sub>) were also agreed upon by Quest Diagnostics and TBS. Patient panels of serum samples containing FLC concentrations spanning the analytical measuring range ( $\kappa$  1/10: 2.7-127 mg/L,  $\lambda$  1/8: 5.2-139 mg/L, Integra 800) were exchanged between TBS and Quest Diagnostics to standardize testing at all locations. Lot-to-lot analysis by TBS was conducted using Passing and Bablock regression analysis and Spearman's Rank Correlation Coefficient; Quest Diagnostics then utilized the bias with acceptability criteria based on a 1/4TE<sub>a</sub> or 7.5% or 4 mg/L.

Prior to this work, differences between TBS and Quest Diagnostics evaluations could be as high as a 30% bias. Standardization of instrumentation to minimize instrument differences when evaluating new reagent lots and the exchange of patient panels reduced the differences between TBS and Quest Diagnostics kit evaluations to a maximum observed 11% bias; on average, the bias has only been ~6%. In addition, use of this protocol has allowed problems to be identified prior to widespread implementation across laboratories. Finally, standard patient panels are in development to be used for monitoring long-term bias across multiple lots. The use of these panels will prevent sampling bias from introducing error into the evaluation process.

**Conclusions:** The partnership between Quest Diagnostics and TBS helped reduce error by standardizing equipment and exchanging patient samples for lot evaluations. Reducing error can help streamline the evaluation of a new reagent product, improve efficiencies of testing of new reagent lots, and allow for clear observation of true analytical and clinical variation introduced by use of new Freelite lots.

**A-162****The effects of interfering substances on Free Plasma Hemoglobin values measured using Hemocue Plasma/ Low Hemoglobin spectrophotometer**

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**Background:**

Red blood cell hemolysis can be the result of a mechanical destruction with a ventricular device or extracorporeal membrane oxygenation (ECMO) system. The resulting free plasma hemoglobin is monitored using Hemocue™ Plasma/Low hemoglobin spectrophotometer. According to the manufacturer listed limitations for analysis of free plasma hemoglobin, the presence of lipids and bilirubin up to 30mg/dl may interfere with measurement of the free plasma hemoglobin and give erroneous results. Objective: The purpose of this study is to evaluate to what extent the assay is affected by the presence of bilirubin and lipids, and how each specimen received should be handled by the technologist prior to testing.

**Methods:**

Bilirubin interference was determined using 6 patient samples with known plasma hemoglobin results spiked with conjugated bilirubin. Samples were created to contain 0mg/dL, 10mg/dL, 20mg/dL and 30mg/dL additional conjugated bilirubin. Similarly another 6 samples were spiked for the unconjugated bilirubin study. Total and direct bilirubins were confirmed using a Roche Cobas analyzer. Each sample was measured again for free plasma hemoglobin concentration. Lipemia interference was determined using 12 patient samples. 6 samples with known values of lipemia, hemolysis and plasma hemoglobin were recorded at baseline. These samples were then centrifuged using the Vivaspin™ method at 1000k Daltons cut-off and results recorded post centrifugation. The next 6 samples were tested for plasma hemoglobin at baseline noting the levels of lipemia, hemolysis and triglyceride prior to spiking a small amount of harvested lipids (10,000mg/dL triglycerides and 1000mg/dL cholesterol) to yield 100, 250, 500, and 750mg/dL triglycerides respectively. Each sample was tested again for free plasma hemoglobin.

**Results:**

There was no significant interference associated with direct bilirubin up to 30mg/dl. However, there is a positive interference when indirect bilirubin is greater than 20 mg/dl. The interference led to falsely elevated results. Samples containing harvested lipids showed a constant upward trend of falsely elevated results at concentrations of triglycerides as low as 100mg/dL. Results after processing with the Vivaspin demonstrated significant decrease in measured plasma hemoglobin. However, we are not able to validate that all free plasma hemoglobin is recovered in the filtrate from lipemic sample post centrifugation.

**Conclusion:**

Samples tested for free plasma hemoglobin using the Hemocue Plasma low device should be checked for lipemia and icterus prior to analysis. Given the interference seen with triglycerides occurs at levels that may not be visually determined; the use of a spectrophotometric lipemia indice may be required. Result comments should include the information regarding icteric and lipemic interferences.

**A-163****Sigma-metrics to Assess Analytical Quality of Certified HbA1c Methods**

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**Background:**

The National Glycohemoglobin Standardization Program (NGSP) was launched in 1996 with the aim of harmonizing HbA1c testing to get more accurate and precise results for the care of diabetic patients. Most of the methods used by the clinical laboratories are NGSP certified and many of the laboratories that perform the test participate in the accuracy based GH2 PT program administrated by the CAP.

**Methods:**

This study was undertaken to calculate the Sigma-metrics for 26 manufacturers' HbA1c methods using imprecision and bias estimates derived from CAP surveys GH-2b 2013, GH-2a 2014, and GH-2b 2014. This approach was recently described by S. Westgard (<http://www.westgard.com/six-hba1c-methods.htm>). We used a total allowable error (TE<sub>a</sub>) of 6%, the quality requirement specified for HbA1c methods by the NGSP/CAP, and calculated Sigma-metrics as: Sigma = (TE<sub>a</sub>-bias)/CV (all expressed as %'s).

**Results:**

Table 1 shows the Sigma-metrics for a sample of the method groups that reported HbA1c results for the 3 samples from survey set GH-2b. The metrics for sample GH2-04, which had an expected value of 6.58 %, and is close to the clinical decision threshold for the diagnosis of diabetes, ranged from 0.74-3.27. The within-method average Sigma-metrics across the 3 survey specimens ranged from 0.10-9.91. Only 5.2 % of the 228 Sigma-metrics calculated for the 9 PT challenges over the 3 survey sets were > 3.

**Conclusion:**

Based on the metrics calculated from CAP survey data, the average defect rates for most of the HbA1c methods that are currently being used for the diagnosis and monitoring of diabetes were > 5 %, and exceeded the range of defect rates (1 to 5%) (Blumenthal D. Clin Chem 1997;43:1305) that are generally considered to be acceptable in healthcare.

Sigma-metrics for selected methods: CAP survey GH-2b 2014					
Method Group	N	GH2-04 [6.58 (6.51-6.66)]	GH2-05 [8.39 (8.32-8.47)]	GH2-06 [5.65 (5.58-5.72)]	Mean
Roche Cobas c500 series	350	1.87	2.14	1.59	1.87
Tosoh G8 Auto HPLC	330	2.10	1.66	2.08	1.95
Siemens DCA Vantage	307	1.37	0.98	0.73	1.02
Siemens Dimension Vista	269	0.85	2.35	1.59	1.60
Bio-Rad D-10	217	2.03	2.40	1.96	2.13
(Ortho Clin Diag) Vitros 5,1 FS, 4600, 5600	209	1.52	2.24	1.44	1.74
Beckman UniCel DxC Synchron	172	2.17	1.72	2.03	1.97
Bio-Rad Variant II Turbo	127	1.91	1.91	1.97	1.93
Bio-Rad Variant II Turbo 2.0	124	1.96	1.85	1.94	1.91
Beckman AU systems	67	0.74	0.78	0.88	0.80
Siemens Dimension RxL	67	1.06	2.16	1.10	1.44
Siemens Dimension Xpand	59	1.11	1.66	0.95	1.24
Abbott Architect c System	47	0.88	0.60	1.25	0.91
Siemens Advia Chemistry Systems	32	1.18	1.26	0.55	0.99

**A-164****Evaluation of Technopath Controls on the ARCHITECT Family of Instruments**

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**Introduction:** Quality controls are an important part of laboratory testing to ensure that released results meet the required quality in regards to accuracy and precision of patient results. Consolidation of controls is a current trend in laboratories to simplify QC testing. Multi-constituent control panels (MCCs) offered by Technopath Manufacturing Ltd. cover a wide range of clinical chemistry and immunoassay analytes for both serum and urine.

**Objective:** The goal of this study was to evaluate the performance of the Multichem S Plus, Multichem IA Plus and Multichem U control panels on the ARCHITECT family of instruments. Precision and accuracy compared to the target value were evaluated.

**Methods:** The three control panels were evaluated for a minimum of thirty days. Testing was performed on two ARCHITECT c8000 and three ARCHITECT i2000<sub>SR</sub> instruments. Data presented here are from the following serum clinical chemistry analytes: ALT, AST, total bilirubin, chloride, total cholesterol, creatinine (picrate), glucose, potassium, total protein, sodium, triglycerides and urea; the following immunoassay analytes: CEA, total PSA, free T3, free T4, TSH, troponin-I, total beta HCG, estradiol, ferritin, FSH, vitamin B12 and vitamin D; and the following clinical chemistry urine analytes: chloride, creatinine (picrate), glucose, potassium, sodium and urea. The Multichem S Plus and IA Plus panels are serum based with three control levels; the Multichem U panel is prepared from human urine with two control levels. All data were collected via

AbbottLink, allowing for automated data retrieval. Means, standard deviations and ranges were calculated for all controls. Sigma Metrics were also calculated for each analyte.

**Results:** The %CV for the 12 clinical chemistry analytes with the Multichem S Plus control ranged from 0.46 to 5.33%. The %CV for the 6 clinical chemistry urine analytes with the Multichem U control ranged from 0.51

to 3.2%. For both control panels, the majority of the CVs were less than 2%. The %CV for the 12 immunoassay analytes with the Multichem IA Plus control ranged from 1.34 to 18.87% (TnI, Level 1); however the majority of the CVs were less than 5%. Overall, little variation was seen from instrument to instrument.

**Conclusions:** The Technopath S Plus, IA Plus and U controls

performed well and demonstrated similar performance to the routine internal laboratory quality controls. The use of these MCCs reduced the number of controls required for the analytical quality control testing of both clinical chemistry and immunoassay analytes with no compromise to quality.

**A-166****Evaluating the Effects of Preanalytical Factors on Common Biochemistry Parameters: Duration Before/After Centrifugation, Recentrifugation, and Storage Conditions**

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Preanalytical factors should be considered for the samples conveyed from peripheral to central laboratories. Our aim was to evaluate the effects of duration before/after centrifugation, re-centrifugation, storage conditions on the stability of 19 serum parameters.

Blood samples (n=10) were collected from healthy volunteers into vacutainer tubes with gel separator (Becton Dickinson, USA) which were allowed to clot for 30 min and allocated to 6 groups. First and second groups were centrifuged at 1300xg for 10 min immediately and stored at room temperature (RT) or 4°C until analyzed at 0, 2, 4, and 6 hrs. Third and fourth groups were stored at RT or 4°C for 2, 4, 6 hrs, then centrifuged and analyzed. Fifth and sixth groups were centrifuged after clotting, stored at RT or 4°C and re-centrifuged at 2, 4, and 6 hrs before analysis. We measured below parameters (AU 680, Beckman Coulter, USA), calculated percentage relative bias from the baseline [(concentration of any hour-concentration of baseline)/concentration of baseline]x100 and compared with desirable bias (Westgard QC database) to determine clinically significant variations.

ALP, phosphorus, total bilirubin, and uric acid levels were not affected. The desirable bias for direct bilirubin was 12.2% and only 4th hour of fourth group exceeded this limit. Waiting at RT before and after centrifugation increased LDH concentrations when waiting time extended up to 6 hrs. Results for other parameters are given in Table 1.

Duration between centrifugation and analysis, storage before centrifugation, storage conditions, and re-centrifugation may cause serious biases in analytes which should be taken into consideration for central laboratories receiving samples from peripheral units.

Table 1. Relative bias values for groups that exceeded desirable bias (DB)

Test DB (%)	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
AST 6.54			2h -8.70	4h 10.00	4h 13.33	4h 10.00
ALT 11.48				4h 13.33	4h 13.33	4h 12.50
GGT 11.06		6h 11.11	2h 16.67 6h 14.86	2,4,6h >11.1	2,4,6h >13.3	2,4,6h >11.6
Glucose 2.34	6h -3.11	6h -2.97	2,4,6h <-9.52	2,4,6h <-5.71	4,6h <-5.38	4,6h <-3.23
BUN 5.6		6h -6.67		4h -6.67	4h -6.67	
Creatinine 3.96		4h -6.67	4h -6.67	4h -6.67	4h -6.67	4h -6.67
T. prot. 1.36	2h -2.21 4h -3.64	2h -1.59 6h 1.99	4h 2.75	2h -2.31 6h 3.15	2h -2.70 6h 2.01	2h -3.40 4,6h >1.69
Albumin 1.43	4h -1.53 6h -3.31	4h -1.43 6h -1.46	4h 2.22	4h -2.45 6h -1.80	6h -3.23	6h -4.62
Na+ 0.23	2,4,6h <-0.72	4h -0.72 6h -0.73	2h -0.72 4h -0.72	2,4,6h <-0.35	2h -0.72 4h -0.72	4h -0.73 6h -0.35
K+ 1.81	4h -2.35 6h -2.38	2h 2.27	2h -2.44 4,6h >2.33	2,4,6h >7.14	6h 2.38	2,4,6h >2.38
Cl- 0.50	2,4,6h <-1.01	4h -1.01 6h -1.99	4h -1.01 6h -1.98	2,4,6h <-0.98	4h -1.01 6h -1.01	4h -0.73 6h -0.35
Ca2+ 0.82	2h 1.08	2h 0.98 6h 1.98	4h -0.93 6h -1.02	4h -1.05	2h 1.01	2h 1.01 4h -1.05
Mg2+ 1.80	6h -3.04	2h 1.87 6h -1.84	2,4h >3.68 6h -1.99	6h -2.94	6h -3.67	6h -5.19

**A-167**

**Stability of Extracted Samples for LC-MS/MS Analysis of Free Plasma Metanephrines**

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**Background:** Plasma free metanephrines is the recommended first line laboratory testing for the diagnosis and follow-up of pheochromocytoma. The low physiological concentrations and potential interference make the quantification of these analytes challenging. Due to the labor intensive SPE extraction stability of the processed samples was investigated to determine how long the processed samples can be stored in case of instrument breakdown. **Method:** Stability was studied by running 3 levels (low, mid, and high), in triplicate, of spiked EDTA plasma using a previously validated LC-MS/MS method. All samples were extracted at time 0 in the final matrix of 1 mM ammonium formate + 0.1% formic acid. Set 1 was analyzed immediately after extraction and the remaining sets were left at 4 °C protected from light to mimic the conditions in a typical HPLC autosampler. Sets 2 and 3 were removed and analyzed immediately at 72 hours and 168 hours, respectively. **Results:** Processed plasma samples in 1 mM ammonium formate + 0.1% formic acid stored at 4 °C for 72 and 168 hours showed recoveries ranging from 85% to 113% compared to the baseline results for both free normetanephrine and metanephrine (Table 1). **Conclusion:** Processed plasma samples in 1 mM ammonium formate + 0.1% formic acid for plasma free metanephrine and normetanephrine are stable for up to 168 hours when stored at 4 °C protected from light.

Table 1: Stability Data

Normetanephrine	Temperature	Timepoint (Hours)	n	Mean	%CV	%Difference
Low		0	3	310	3%	NA
	4°C	72	3	318	2%	3%
		168	3	345	4%	8%
Mid		0	3	1520	6%	NA
	4°C	72	3	1417	2%	-7%
		168	3	1507	4%	6%
High		0	3	3933	6%	NA
	4°C	72	3	3343	1%	-15%
		168	3	3793	2%	13%
Metanephrine	Temperature	Timepoint (Hours)	n	Mean	%CV	%Difference
Low		0	3	67	7%	NA
	4°C	72	3	64	3%	-4%
		168	3	66	2%	3%
Mid		0	3	576	5%	NA
	4°C	72	3	502	3%	-13%
		168	3	507	1%	1%
High		0	3	1038	10%	NA
	4°C	72	3	930	3%	-10%
		168	3	904	2%	-3%

**A-168**

**Improved testing options through evaluation of specimen temperature stabilities.**

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**Objective:** The specimen stability study was performed to determine how storage temperatures and length of storage affected result integrity.

**Background:** Specimen stability over time and varied storage temperatures is essential for a laboratory to understand in order to make process improvements for patient care, client service and laboratory operations. It allows the laboratory to improve patient care by offering the clinician an extended period to order add-on testing following the preliminary diagnosis without having to recollect from the patient. This will improve patient care delivery by accelerating time to result and improve the experience by limiting recollections. In addition, understanding specimen stability limits allows modification of shipping conditions, storage conditions and sample processing workflows. In order to fully understand potential areas of variability in qualitative changes of specimens over time, rigorous analysis is important to understand the potential areas of variability both within patient and between patients to determine acceptability of the new stability times and temperatures.

**Materials and Methods:** 10-15 specimens were selected based on assay methodologies, order volume and the frequency of Add-on testing. 39 analytes (albumin, alkaline phosphatase, ALT, AST, Bilirubin(conjugated and unconjugated), cholesterol, LDL, Ferritin, HDL, CRP, hsCRP, LD, phosphorous, PSA, TSH, sodium, potassium, chloride, calcium, parathyroid hormone, Lipoprotein (a), N-telopeptides type 1, hemoglobin A1C , total IgA, Gliadin antibodies (IgG, IgA), Endomysial IgA , Tissue transglutaminase antibodies (IgG, IgA), cardiolipin immunoglobulins (IgM, IgG, IgA) beta-2 glycoprotein immunoglobulins (IgM, IgG, IgA)) requiring frozen transport and specimens with less than one week refrigerated stability were selected for analysis. Residual patient samples were identified over low, normal and high result ranges for each analyte tested. Measurements were taken on sequential days for up to 14 days and results compared to the day 0 measurements. Stability acceptance criteria was based on the reading being within the accepted level of variance as published by the manufacturer and the CAP proficiency testing.

**Results:** In an analyte specific manner, room temperature stability was extended between 3 to 7 days allowing all 23 analytes to be shipped refrigerated. This has

several benefits to both the client and the laboratory. Dry ice shipments require special handling and the elimination of the “ship frozen” requirement improves logistics by simplifying shipments and reducing costs. In addition, extension of the stability in most cases will allow for add-on testing to be accommodated from an existing specimen thereby minimizing the need to redraw the patient which improves the time to result.

**Conclusion:** As methodologies and instrumentation change over time, laboratories should periodically evaluate testing sample requirements for stability as a means to improve the patient and client experience, logistics, work flows and to save on costs.

**A-169**

**Enrichment of Nucleic Acid Purification via Process Optimization of Glass Fiber Manufacturing**

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Boom chemistry is a solid phase extraction technology that has long been exploited as a common platform for nucleic acid purification. The mechanism of action has been established as being due to the chaotropic effect between the silica substrate and the nucleic acid. Glass fiber matrices are frequently utilized as a binding structure for the capture and purification of nucleic acids in a rapid and reliable manner. Disparity between glass fiber matrices of current manufacturers results in varying levels of recovery of nucleic acids from samples. A study has been undertaken between glass fiber manufacturers to compare performance of capture, purification and elution of nucleic acids from a spiked model sample. Results indicate significant improvement of Porex glass fiber over commonly utilized glass fiber matrices with 91.2% recovery of 20ug of gDNA versus 74.7% and 77% by the competition respectively using the Qiagen DNeasy Blood and Tissue Kit as a standard protocol. Similarly, standard deviation in recovery rates decreased from 8.3% and 11.8% by the competition to 3.6% using a Porex glass fiber matrix. Recovery also improved using a plasmid DNA sample from 91.6% and 94% to 97.8% with Porex glass fiber. Standard deviation varied from 6.3% and 3.9% in the competitive samples to 2.9% in Porex glass fiber utilizing the same protocol. The results obtained by this study indicate significant differences in the performance of glass fiber membranes with similar properties. Factors effecting performance will be discussed as well as future areas of improvement of the glass fiber binding matrix.

**A-170**

**N-linked Glycosylation in Human Corin, a Potential Factor in Diagnosis**

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**Background:**

Corin is a type II transmembrane serine protease mainly expressed in heart. As the only known physiological activator of atrial natriuretic peptide, corin plays an important role in maintaining blood pressure. People with deficiency of corin activity may suffer high risks of hypertensive diseases.

Corin is synthesized in cardiac myocytes and anchored on the cell surface. The anchored corin may be cleaved from cardiac myocytes to generate three different kinds of soluble fragments. These soluble fragments will enter circulation system and be detected in blood. Previous studies showed plasma corin levels reduced significantly in patients with heart failure (HF) and may act as a biomarker for the diagnosis of HF.

Glycosidase digestion reveals that corin is abundantly N-glycosylated and one of the predicted N-linked glycosylation site (site 80) is located at the cleavage region for one soluble fragment. It is not clear whether the specific N-glycosylation affects the application of corin in the diagnosis. Here we evaluated the potential influence of the specific N-glycosylation to plasma corin levels.

**Methods:**

Plasmids expressing human corin mutants N80Q, F77N/K78G/N80Q, F77N/K78G/S79A/N80Q, N80Q/E83N/P84G/L85S and N80Q/E83N/P84G were made by PCR-based mutagenesis. Plasmids for WT corin and mutants were transfected into HEK293 cells, respectively. After two-day culture, soluble corin in conditioned medium was immunoprecipitated and analyzed by SDS-PAGE and Western blotting. HEK293 cells were collected and lysed. Corin in the lysate was also analyzed as an evaluation of plasmid expression.

**Results:**

Corin zymogen levels in the cell lysates were similar for WT corin and mutants. Compared with WT, F77N/K78G/N80Q and N80Q/E83N/P84G/L85S, levels of ~180-kDa soluble fragment, rather than the other two soluble fragments, in N80Q, F77N/K78G/S79A/N80Q and N80Q/E83N/P84G increased significantly. The total amounts of all the three soluble fragments increased in N80Q (270 ± 26% vs. WT), F77N/K78G/S79A/N80Q (281 ± 34% vs. WT) and N80Q/E83N/P84G (243 ± 10 % vs. WT) while those in WT, F77N/K78G/N80Q (93 ± 26% vs. WT) and N80Q/E83N/P84G/L85S (103 ± 22% vs. WT) were normal. These results indicate that N-glycosylation at or close to site 80 protects corin from ectodomain shedding.

**Conclusions:**

Lack of N-glycosylation at site 80 causes increase in the total amount of all the three soluble fragments, which may lead to an abnormally high plasma corin level. In HF patients, this abnormally high plasma corin level may give a false negative diagnosis result if corin is used as the biomarker. Till now, no human corin variant corresponding to N-glycosylation at site 80 has been found. However, disorder of N-linked glycosylation is a possible reason for abnormally high plasma corin level and, since N-linked glycosylation occurs to plenty of glycoprotein in most of human cells, N-linked glycosylation may affect other biomarkers containing N-glycans in diagnoses. Therefore, further studies about disorder of N-linked glycosylation and N-glycosylation related genetic polymorphism are urgent in diagnoses using N-glycosylated biomarkers.

**A-171**

**Case Report: Delta bilirubin as an “interference” in direct bilirubin assays**

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**Background:** Delta bilirubin (B<sub>δ</sub>) forms slowly and non-enzymatically from conjugated bilirubin (Bc) and albumin in circulation, is normally undetectable in adults as Bc is excreted into the biliary tract and eliminated through the gut. Traditional direct bilirubin (DBIL) assays, often assumed to be equivalent to Bc, includes Bc & B<sub>δ</sub>. BuBc assays measures conjugated and unconjugated bilirubin (Bu) quantitatively.

**Methods:** BuBc was measured using the Vitros 5600 Chemistry system. TBIL and DBIL on are measured using 3,5-dichlorophenyldiazonium tetrafluoroborate (DPD) method on Beckman Coulter AU5800 Chemistry system. Repeat analyses were performed using a bilirubinometer and vanadate oxidase method on Siemens Advia Chemistry system.

**Case Report:** A 56 year old female patient undergoing cancer treatment with presented with a Total Bilirubin (TBIL) of 29 μmol/L, Bu of 13 μmol/L and Bc of 16 μmol/L. On the following day, her bilirubin results were 124 μmol/L, 61 μmol/L and 63 μmol/L for TBIL, Bu and Bc respectively, with “mild icterus” tagged to her results. 12 hours later, her bilirubin results were 22 μmol/L, 12 μmol/L and 10 μmol/L for TBIL, Bu and Bc respectively. An investigation started to determine if there had been any erroneous results. Visual inspection of the samples showed mild icterus. Audit trail records revealed that the first and third bilirubin measurements were performed on the Vitros while the second set of results were from the AU5800. A repeat sample from the patient was then analysed using various methodologies and compared:

Analyser	TBIL (μmol/L)	Bu (μmol/L)	Bc (μmol/L)	Icterus Index
Vitros 5600 Chemistry	78 (measured)	12	9	Normal
Bilirubinometer	76	-	-	-
Beckman Coulter AU5800 Chemistry	96	50	46	Mild icterus
Siemens Advia Chemistry	82	20	62	Mild icterus

**Conclusion:** This is a case of B<sub>δ</sub> due to drug-induced hepatobiliary obstruction. B<sub>δ</sub> acts as an “interference” in traditional direct bilirubin assays. TBIL (measured) should be reported for adults when using the Vitros method. B<sub>δ</sub> has a maximum absorption at 440 nm which is not measured in the serum indices on Vitros. Visual inspection of sample condition is as valuable as automated serum indices measurements.

**A-172**

**Validation of lithium heparin tube for Cardiac markers in a Clinical Laboratory and its benefits.**

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**Background:**

Accurate laboratory testing requires an understanding of the complex interactions between collection devices and blood specimens. Clinical laboratories must consider the pre-analytical challenges in laboratory testing. Proper blood collection and timely processing are critical pre-analytical steps required for the integrity of laboratory results. Although the influence of blood collection devices on laboratory tests is often overlooked, correct pre-analytical handling is essential. In this study, we discuss the use of plasma to chemistry analysis with an emphasis on heparin tube.

**Objective:**

Currently the clot activator with separator gel tube is the first option for biochemical analysis, the objective of the study is to evaluate the possibility and benefits of the introduction of lithium heparin tube for holding some biochemical studies.

**Methods:**

Twenty paired samples using the two tubes were carried out simultaneously. Both tubes were centrifuged before analysis with the difference that the clot activator tube with gel separator needs a time for the formation of clot before the centrifugation procedure. Samples were analyzed for CK mass and Troponin by two different methods: electrochemiluminescence (ECL), in the E411 Roche platform and fluorescence enzyme immunoassay (ELFA), in the Vidas 30 bioMerieux instrument.

**Results:**

For the ECL CK mass analysis, the Test F, T and the Pearson correlation showed up as expected. 5.14% of systematic constant was observed for level I and 0.82% for Level II that proved to be insignificant. Proportional systematic error was -3.37% with no impact on levels of clinical decision. The comparative test conducted with clot activator tube with gel separator and plasma from lithium heparin tube showed satisfactory results with total error obtained 7.39% and 8.17% for levels 1 and 2, respectively, less than the total allowable error of 30.6%. The troponin, F test and Pearson correlation showed results as expected. Constant systematic error was 4.06% for level 1 and 0.37% for level 2 which were considered not relevant. Proportional systematic error was -6.57% with no impact on levels of clinical decision. The comparative test conducted from gel x lytic plasma serum samples showed satisfactory results with total error obtained 7.63% and 11.32% for levels 1 and 2 respectively less than the total allowable error 48.9%

For ELFA, CK Mass showed a correlated and Kappa index within the references, F test and Pearson correlation as expected. We observed constant and proportional systematic error of zero for the two levels. Comparative tests conducted between serum samples x lytic gel showed satisfactory results with plasma total error of 8.39% obtained for the two lower levels of total allowable error 27.91%.

**Conclusion:**

We observed that the use of both tubes had good performance in the evaluation of cardiac markers, CK Mass and troponin. The final result for the doctor had a shorter TAT, with high quality and efficiency, as the tube with plasma showed the benefit to be processed before the tube with the clot activator and separator gel.

**A-173**

**Storage stability of selected nutritional biomarkers at -20°C for up to 12 months**

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**Background:** Storage stability is an important preanalytical variable that affects the quality of biospecimens and integrity of results. Little is known about how frozen storage at suboptimal temperature affects nutritional biomarkers. We used quality control (QC) pools to investigate the stability of selected nutritional biomarkers at -20°C for up to 12 months. **Methods:** Multiple sets of QC vials (low, medium, and high concentration) were stored at -20°C. Three sets of vials were removed from the freezer after 3, 6, 9, and 12 months and placed into a -70°C freezer until analysis. Three separate sets of vials were stored at -70°C for the entire study period and used as the optimally stored reference condition. The content of each vial was analyzed in duplicate for a total of six replicates per condition. All replicates from one condition were analyzed in the same analytical run. We measured four iron-status indicators, one indicator of inflammation, seven biomarkers of water-soluble

B vitamins, vitamin C, and five biomarkers of fat-soluble vitamins. We calculated least squares geometric mean concentrations (to account for the right-skewed distributions and for the increasing variance with increasing concentration) using 2-way (time and pool) ANOVA. Since time by pool interactions were generally not significant, differences relative to the reference condition were expressed as mean percent change of geometric means across the three pools. Results: The majority of nutritional biomarkers showed no difference in concentrations after 12 months of storage at -20°C (Table 1). Ferritin (1.2%) and transferrin receptor (-1.7%) showed a small but significant change in concentration. Folate (-12%), pyridoxal-5'-phosphate (-33%), and vitamin C (-49%) showed large and significant decreases. **Conclusions:** Most nutritional biomarkers showed remarkable stability when stored at -20°C for up to 12 months. This information is important for the low-resource setting, where access to -70°C freezers may be limited.

Biomarkers (Serum)	Change, %	P value
Iron-status indicators		
Ferritin, ng/mL	1.22	0.0468
Transferrin receptor, mg/L	-1.70	0.0233
C-reactive protein, mg/L	-0.27	0.80
Water-soluble vitamins		
Folate, nmol/L	-11.7	0.0156
Vit B6 (PLP), nmol/L	-33.3	<0.0001
Vit B12, pg/mL	1.97	0.09
Vit C, mg/dL	-49.3	<0.0001
Fat-soluble vitamins		
Vit A, ug/dL	-1.25	0.27
Vit E, ug/dL	-0.97	0.33
Vit D (25OHD), ng/mL	-0.75	0.65

**A-174**

**The Performance of Chemically Modified Plastic Blood Collection Tubes To Achieve Interior Hydrophilic Surface on Clinical Chemistry Analytes**

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**Background:** Blood collection tubes (BCTs) cannot be regarded as inert specimen carriers as evident by previous studies reporting significant differences in particular serum hormone concentrations collected in different types of BCTs. Surfactant(s) are added to plastic [poly(ethylene terephthalate); (PET)] BCTs to prevent adsorption of cells, platelets, and proteins onto its hydrophobic interior surface. Such interferences from BCT surfactant(s) may not be detected by current laboratory quality control and external proficiency testing programs. The assay interference issue remains unresolved despite reducing the surfactant(s) amounts inside the tubes. Recently, we developed chemically-modified BCTs via a base-catalyzed transesterification reaction that has an interior surface similar to glass which do not contain surfactant (ChemoPET). We demonstrated that the ChemoPET tubes when compared to glass tubes, produced significantly lower biases for cortisol, total triiodothyronine (TT3), and total thyroxine (TT4), compared to other BCT types. However, this study was performed with BCTs containing the same volume of blood but with different tube blood draw volumes. The objective of this study is to determine whether there would be any significant differences in analyte concentrations when BCTs are completely filled to their pre-determined volume by vacuum.

**Materials and Methods:** Seven types of evacuated BCTs were used in this study: (1) plastic Vacuette™ tube; (2) glass tube; (3) plastic SST™ tube; (4) plastic rapid serum tube (RST™); (5) plastic red-top tube; (6) unmodified PET tube; and (7) ChemoPET tube. Blood samples were drawn in a randomized order after written informed consent from 50 healthy volunteers (18 males, 32 females; age range: 25-70 years). BCTs were inverted eight times after blood draw and allowed to clot for 60 minutes. Following centrifugation, serum specimens were transferred to plastic tubes and stored at -70°C until analysis. Serum cortisol, TT3, and TT4, were measured in random order on a Siemens Immulite™ 1000 analyzer and routine chemistry analytes were measured on a Siemens Dimension RxL™ analyzer. The means of triplicate results for cortisol, TT3, and TT4 measurements were used for statistical analysis. All other analytes were analyzed in singleton. A Student t-test and ANOVA were used to analyze serum specimen test results among the different BCTs. All P values were adjusted for multiple comparisons using a Bonferroni correction.

Results: When BCTs are completely filled to their draw volume by vacuum, we found that some individual BCTs when compared to glass tubes showed statistically and clinically significant differences in analyte concentrations ( $P < 0.007$ ), which was not observed when analyte concentrations from different BCT types were averaged.

Conclusions: It is conceivable that the quality and/or quantity of surfactant(s) in each BCT may be different and that a threshold of surfactant concentration to blood volume must be exceeded before significant changes in test results may be observed. Because the clinical laboratory frequently receives BCTs that are partially-filled, the findings of the current and previous studies with these BCTs have important implications for alterations in hormone analyte concentrations. We anticipate that the use of ChemoPET BCTs that do not contain surfactant would produce more accurate and consistent test results regardless of blood draw volumes.

## A-176

### Reducing Adhesion of Proteins on Stainless Steel Components by the Application of a Carboxysilane Coating.

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#### **Background:**

Protein binding and carryover in analytical systems is a challenge that can reduce accuracy and throughput of analysis. This work presents data on the use of coatings over stainless steel surfaces to compare analytical accuracy, reduction of carryover and increase of throughput for a range of proteins. Comparisons are made against other substrates and surface coatings. The areas of use are broad from all sample-contacting components of medical diagnostic equipment to HPLC columns, transfer tubing, and more.

#### **Methods:**

The protein-resistant properties of a carboxysilane coating (deposited via chemical vapor deposition) was studied using quartz crystal microbalance with dissipation monitoring (QCM-D) and compared to that of bare stainless steel and a Teflon-like fluoropolymer coating (AF1600).

#### **Results:**

With the assistance of a nonionic surfactant-containing wash solution, the carboxysilane coating was found to facilitate 100% removal of adsorbed proteins (BSA, mouse IgG and NHP), whereas these proteins remain adsorbed on the bare stainless steel surface under the same conditions. Compared to the carboxysilane coating, AF1600 showed similar resistance to plasma protein adhesion at initial use, but the AF1600 performance degraded due to mechanical wear-induced surface delamination. The carboxysilane coating, on the other hand, maintained the same level of protein resistance through harsh chemical washes and multiple sonication cycles, demonstrating excellent chemical stability and physical durability of the CVD coating.

#### **Conclusion:**

A carboxysilane coating on stainless steel components will improve reliability, reduce carryover and cycle time, and increase the durability of analytical systems.