Tuesday, August 1, 2017

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

Factors Affecting Test Results

A-226

Glucose-6-Phosphate Dehydrogenase Activity Deficiency - A Critical Indicator for Appropriate Interpretation of 'Normal' Haemoglobin A1c Result

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Backgrounds: Clinical decision limit of hemoglobin A1c (HbA1c) level below 6.5% (48 mmol/mol) as good glycemic control, adequate treatment and non-diabetes mellitus (DM) is valid only in patients with normal erythrocyte life span. Any condition reducing erythrocyte survival might associate with falsely low HbA1c level. Glucose-6-Phosphate Dehydrogenase (G6PD) protects red cells against oxidative damage and subjects with G6PD deficiency are prone to intravascular hemolysis when the red cells are exposed to oxidative stress thus shortened the life expectancy. Reports of G6PD deficiency that resulted in lowering of percentage glycosylated haemoglobin and HbA1c level were not only scanty, but also not investigated extensively.

Methods: Our laboratory determined HbA1c level by BioRad Variant II Turbo analyzer with a stringent in-house retention time system for correct HbA1c and HbAo peaks identification. Since April 2010 our laboratory adopted an HbA1c reporting algorithm with regard to in-house established reference range 4.9-6.5% (NGSP), 30-48 mmol/mol (IFCC), which was set-up by recruiting 8028 normal subjects of normal fasting blood glucose, 3607 female and 4421 male, age ranged from 4 to 104 years. Cases with unusually low HbA1c result, lower than 4.9% (NGSP), lower than 30 mmol/mol (IFCC), but incompatibly elevated fasting or random blood glucose were further investigated. After verifying transfusion history, eliminating inadequate fasting and haemoglobinopathy, glucose-6-phosphate dehydrogenase (G6PD) activity was determined by Trinity Biotech in Cobas c501, Roche Diagnostic.

Results: Among 61643 HbA1c requests from January 2012 to October 2016, we identified 195 G6PD deficiency patients, accounting for 505 (0.8%) requests, who presented with spuriously normal results of HbA1c of lower than 6.5% (lower than 48 mmol/mol) and estimated average glucose (eAG) of lower than 7.8 mmol/L, lower than 104.4 mg/dL but discordant elevation of fasting blood glucose level of higher than 5.6 mmol/L. Thirty nine patients had multiple visits, from twice to sixteen, and showed consistent observations. HbA1c and eAG ranged from 3.3 to 6.5% (NGSP), 13 to 48 mmol/mol (IFCC)), and 2.7 to 7.8 mmol/L, 48.6 to 140.4 mg/dL, respectively. Corresponding fasting blood glucose level ranged from 5.7 to 14.0 mmol/L, 102.6 to 252.0 mg/dL.

Conclusion: Patient with G6PD activity deficiency due to shortened erythrocytes life span and rapid red cell turnover might have 'normal' HbA1c level but not indicating good glycaemic condition due to inefficient and lowered degree of glycation. Laboratory should establish an effective algorithm to avoid reporting of spuriously low or normal HbA1c which was discordant with persistent elevation of fasting blood glucose level. To arouse awareness of requesting doctor to clinical relevance of the HbA1c result, upon verification of discrepancy, laboratory should supplement the HbA1c result with interpretative comments, such as in our laboratory *[The HbA1c level is likely spuriously lowered by the G6PD deficiency status of this patient. Please correlate the result clinically.]*

A-228

Implementation of Six Sigma metrics for the assessment and process improvement of the quality control program in 22 laboratories from the Secretary of Health in the State of Jalisco, Mexico

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Background: Six Sigma methodology is used to evaluate laboratory performance and improve assay quality by identifying inaccurate and/or imprecise assays in any process in a clinical laboratory. Sigma is a metric that measures the performance of a process as a rate of Defects-Per-Million opportunities. As a pioneer project in Mexico, particularly of the Secretary of Health in the State of Jalisco, our aim was to implement Six Sigma metric analysis as a first phase for the assessment and continuous improvement of the quality control program in laboratories belonging to the Laboratory Network of our state, in order to reduce the number of errors in laboratory results.

Methods: From May to December 2016, a total of 22 laboratories from regional and community hospitals as well as health centers were included in our study. In order to evaluate the total allowable errors in the internal quality control program of our laboratory system by sigma metric analysis, we included the performance of clinical chemistry analytes. Sigma metric was calculated for 23 analytes: glucose, total cholesterol, HDL-High Density Lipoprotein, ALP-Alkaline Phosphatase triglyceride, ALB-Albumin, DB-Direct Bilirubin, TB-Total Bilirubin, UA-Uric Acid, ALT-Alanine Aminotransferase, AMY-Amylase, AST-Aspartate Aminotransferase, Lipase, electrolytes: Ca-Calcium, Mg-Magnesium, P-Phosphorus, K-Potassium, Na-Sodium, Cl-Chlorine TP-Total Protein, urea and creatinine, at two control levels. Sigma metrics of each analyte was calculated using the formula [Sigma metric = (TEa-bias)/ CV-Coefficient of Variation]. We used the minimum specifications for total allowable error (TEa), imprecision and bias, based on the 2014 minimum specifications from the Biological Variation Database updated and compiled by Dr. Carmen Ricos and colleagues. The performance values for sigma (σ) were considered at three levels: σ >4: good to excellent, σ from 2-3.99: poor to marginal, and σ <2: unacceptable.

Results: A total of 3897 clinical chemistry tests were performed at both control levels (L1: Normal and L2: Abnormal). The σ value with the highest incidence was good to excellent (σ >4) for the analytes: Triglycerides, AST, DB, ALT, Urea, TB, and UA. The sigma value considered as unacceptable (σ<2) was for Na, Ca, Cl, ALB, TP, and Mg. For the rest of the analytes, poor to marginal σ values (2-3.99) were obtained.

Conclusion We conclude that analytes with a sigma value <2 require strict monitoring and adjustment of the quality control procedures. Sigma metric analysis provided a cumulative evaluation of the analytical process in clinical chemistry. We established acceptance/rejection criteria in laboratory results, as well as the implementation of standardized processes in clinical chemistry testing within the 22 laboratories of the Laboratory Network in the State of Jalisco. Furthermore, a collaborative and conscious environment was created among laboratory personnel and directors towards the desired goal of quality control.

A-229

National efforts to Improve Laboratory Quality and Safety in Clinical and Public Health Laboratories

C. Fitzgerald, N. L. Anderson, N. Cornish, J. Ridderhof, B. Chen, J. Chaitram, S. Howard, D. B. Lake, R. M. Salerno. *CDC, Atlanta, GA*

Background: Clinical laboratory testing is performed nationwide in more than 250,000 laboratories certified under the Clinical Laboratory Improvement Amendments (CLIA) regulations, ranging from simple tests performed at point-of-care sites to highly complex procedures in reference laboratories. The increasing diversity and importance of laboratory services in patient care and health management warrant national standards, guidance, and training to strengthen laboratory quality and safety. The mission of CDC's Division of Laboratory Systems (DLS) is to strengthen the nation's clinical and public health laboratory system by continually improving quality and safety, data and information science, and workforce competency. **Methods**: DLS accomplishes its mission through multidisciplinary collaborations and engagement with diverse partners and stakeholders, including professional societies, accrediting organizations, proficiency testing programs, healthcare systems, and government

agencies at federal and state levels. Examples include managing the Clinical Laboratory Improvement Advisory Committee (CLIAC) in partnership with CMS and FDA; collaborating with ASM, National Guideline Clearing House (NGC), and the American Society for Clinical Laboratory Science (ASCLS) as part of our Laboratory Medicine Best Practices (LMBP) Initiative to address quality improvement practices of interest to the clinical laboratory community; and partnering with the Association of Public Health Laboratories to improve quality, safety, and workforce of public health laboratories nationwide. The division's laboratory training website provides easily accessible learning resources. Through CDC TRAIN, laboratory professionals can register for live and on-demand courses, create learning plans, obtain continuing education credits, and sign up for notifications of new courses. **Results:** Since 2015, DLS has published guidelines on diverse topic areas, including developing an individualized quality control plan, quality practices in next generation sequencing, laboratory professional competencies, and an Informatics Self-Assessment Tool. Ten formal CLIAC recommendations have been submitted to HHS that address interoperability of laboratory information, laboratory safety, integration of laboratory medicine into health care, and non-invasive prenatal testing. Seven LMBP systematic reviews have been published including reviews on effectiveness of practices to reduce blood sample hemolysis in EDs, effectiveness of automated notification and customer service call centers for timely and accurate reporting of critical values and Decision Point Cardiac Troponin (cTn) Threshold Selection, assay selection, serial testing, and Point of Care Testing. In 2016, DLS distributed free tools to 3,798 laboratories that help assure the quality of waived testing and test results, and disseminated an educational booklet to 3,063 laboratories on recommended practices for provider-performed microscopy procedures. DLS' laboratory training website has >300 course offerings with >18,900 registrants in FY16; 92% indicated the training objectives aligned with their training needs. **Conclusion:** DLS and partners have developed practice guidelines and trainings for a national audience. This work has a substantial positive impact on the laboratory community. In 2017, among other activities, DLS will publicly release many new CDC biosafety courses, and expand its engagement on laboratory biosafety. Through continued collaboration and active engagement with the laboratory community, DLS can collectively strive for exemplary laboratory science and practice across clinical care and population health.

A-230

Adjusted calcium: Local application of observations from Big Data

E. Dewar, D. Robertson, I. Rothnie, H. Regan, K. Booth, M. Lum, F. Brandie, W. Simpson, B. Croal. *NHS Grampian, Aberdeen, United Kingdom*

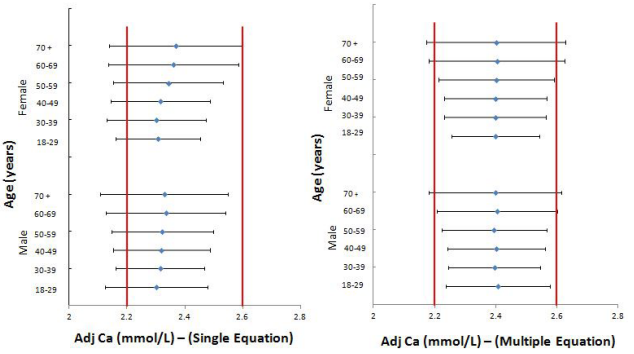
Background: Large variation currently exists across pathology services as to the method and choice of equation for adjusted calcium calculations. Recent observations from big data (NHS England) have demonstrated clear age and sex related differences in serum albumin levels which would have significance for choice of adjusted calcium equation in the population sub groups.

Objective: To use guidance on adjusted calcium equation generation along with knowledge from big data to construct a multi-equation approach to this problem.

Methods: Laboratory data was collected over a four month period (April –August 2015). Primary care results with a measured serum albumin and calcium were pulled using the following exclusion criteria (where available) of age (<18 years), calcium (<2.0 or >2.7 mmol/L), albumin (<20 or >50 g/L), creatinine (>200 µmol/L), urea (>15 mmol/L), ALT (>55 U/L), vitamin D (<25 nmol/L) and PTH (<1.7 or >9 pmol/L) – in line with national UK guidance. Results, divided into gender and 10-year age categories (from 18 to 70 years), were used to derive suitable adjusted calcium equations for each group. A comparison of the distribution of adjusted calcium using this multi-equation approach was made with the existing single adjusted calcium equation technique.

Results: The existing single adjusted calcium equation technique demonstrated significant misalignment with the target reference interval of 2.2 - 2.6 mmol/L (8.8-10.4 mg/dL) – with over diagnosis of hypocalcaemia being a particular concern (Figure). The multi-equation approach using 12 distinct sex/age related equations allowed much better alignment with all 95% C.I. distributions fitting within the target reference range.

Conclusions: The multi-equation approach for adjusted calcium calculation more closely aligns to targeted reference intervals and minimises inappropriate classification of calcium status. This strategy should replace the current single equation approach in order to better optimise direct and consequential costs to patient care and healthcare finances.



A-231

The Effects of Hydrocodone Rescheduling on Laboratory Urine Drug Testing

J. Akin. *University of California San Diego Health, San Diego, CA*

Background: Hydrocodone is one of the cornerstone medications of abuse in the current opiate epidemic in the United States. On October 26th, 2014 the DEA rescheduled hydrocodone containing products from schedule III to schedule II in hopes of deterring abuse and aberrant use. This rescheduling event has effectively decreased the number patients prescribed hydrocodone, but little is known about the impact on laboratory urine drug testing. We hypothesize that the overall volume and frequency of urine drug testing will have increased since the rescheduling of hydrocodone containing products. The positivity rate of hydrocodone should be significantly decreased, as well as the mean urine hydrocodone concentration since fewer people will be prescribed the drug. In addition, we speculate that the positivity rate of schedule II alternatives will have increased since the rescheduling event.

Methods: Laboratory urine drug results from 18 months prior (pre) and 18 months after (post) the rescheduling event were compared for statistical change. A total of 253,773 laboratory results were extracted from UC San Diego Health. Positive and negative results for thirty-five separate urine drug screen and confirmation tests were compared.

Results: There was an increase of 6.8% in the total number of tests, and the mean number of tests per patient increased 7.1% from 1.26 to 1.35. The mean number of tests order per provider increased by 38% from 14.3 to 19.8. The positivity rate for opiates, as a class, was decreased by -0.09% (X2=4.12, p=0.042), as were benzodiazepines at -1.1% (X2=7.62, p=0.006). Codeine increased significantly by 1.3% (X2= 3.99, p=0.046), as well as alpha-hydroxyalprazolam (5.4%, X2=12.1, p=0.00) and temazepam (4%, X2=6.12, p=0.013). Cannabinoids increased slightly by 1.7% (X2=14.1, p=0.000). Hydrocodone (-2.9%, X2=7.85, p=0.005) and hydromorphone (-4.0%, X2=11.8, p=0.001) were the only opiates that saw a decrease. The mean concentration of hydrocodone increased by 249 ng/mL (21.9%, p=0.000, CI 100.2, 407.3).

Conclusion: The results suggest a significant difference in the number and frequency of laboratory urine drug testing after the rescheduling event. As hypothesized, the positivity rate of hydrocodone significantly decreased after the hydrocodone rescheduling event. In contrast, the positivity rate of select schedule II alternatives, such as marijuana, select benzodiazepines and codeine increased after rescheduling. A decrease in mean hydrocodone urine concentrations was not observed as hypothesized, as the mean concentration increased after the rescheduling. Although we cannot fully account for all bias in this study, the data presented suggests that the rescheduling of hydrocodone from schedule III to schedule II significantly impacted laboratory urine drug testing results.

A-233

Effect of Bilirubin Interference on Hemoglobin Quantitation using the NanoDrop ND-1000 and the Roche Cobas Indices

S. Miller, A. Algeciras-Schimnich. *Mayo Clinic, Rochester, MN*

Background: Evaluation of hemoglobin and bilirubin concentrations in serum specimens is an important pre-analytical step to identify potential sources of error in clinical laboratory measurements. For ELISA based immunoassays, our laboratory performs determination of hemoglobin and bilirubin concentrations using the

NanoDrop ND-1000 spectrophotometer. Neuron Specific Enolase (NSE) and 14-3-3 are two immunoassays where hemoglobin concentrations as low as 20 mg/dL could lead to inaccurate results. For these assays, we have observed that in some samples bilirubin interferes with the NanoDrop measurement of hemoglobin, resulting in falsely decreased hemoglobin concentrations. The objective of this study was to evaluate the effect of bilirubin on hemoglobin quantitation using the NanoDrop spectrophotometer. The Roche Cobas serum indices were evaluated as an alternate method for hemoglobin quantitation in samples with an elevated bilirubin**.**

Methods: Correlation between the NanoDrop spectrophotometer and the Roche Cobas indices measured on a c501 instrument was determined using serum samples (n=95) with hemoglobin concentrations between 1.3-273 mg/dL. For bilirubin interference studies, serum pools with various concentrations of hemoglobin (3.5-200 mg/dL) were spiked with increasing concentration of bilirubin (0-15 mg/dL). Samples were measured on the NanoDrop spectrophotometer and the Roche Cobas c501serum indices. A change of 20% between the nonspiked and bilirubin spiked sample was considered a significant change.

Results: For hemoglobin concentrations >20mg/dL, the Nanodrop and Roche Cobas methods showed an excellent agreement with a Spearman correlation coefficient of 0.971, slope of 1.02 and intercept of -5.41 by Passing-Bablok regression fit. When the hemoglobin concentration was <20 mg/dL, the Spearman correlation coefficient was 0.699, slope of 0.95 and intercept of -1.49. Hemoglobin quantitation using the NanoDrop spectrophotometer was susceptible to bilirubin interference. At 20 mg/dL hemoglobin, a decrease of 30%, 42% and 55% from the unspiked sample was observed at bilirubin concentrations of 2.5, 3.75 and 5 mg/dL, respectively. A similar bilirubin dose dependent interference effect was observed for hemoglobin concentrations of 3.5 and 10 mg/dL. At 60 and 200 mg/dL hemoglobin, bilirubin concentrations up to 5 and 15 mg/dL, respectively, did not affect hemoglobin quantitation in the NanoDrop. The Roche Cobas hemolysis index was unaffected by up to 15 mg/dL of bilirubin at hemoglobin concentration of 20, 60 and 200 mg/dL.

Conclusions: Quantitation of low concentrations of hemoglobin (<20mg/dL) using the NanoDrop spectrometer is significantly affected by bilirubin concentrations as low as 0.5 mg/dL resulting in falsely low hemoglobin concentrations. The use of the Roche Cobas H-index is a good alternative method in these situations since this methodology is not susceptible to bilirubin interference.

A-234

Analyzer Maintenance Affects the Observable Sigma Metrics of Clinical Chemistry Analytes

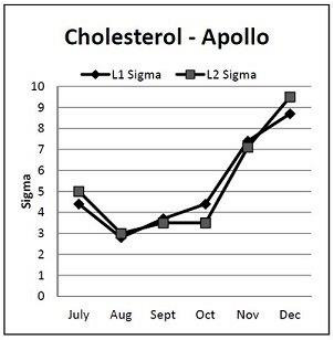
J. A. Montgomery, W. C. Miller, J. F. Licata. *Asante Rogue Regional Medical Center, Medford, OR*

Background: To calculate sigma metrics on paired Beckman Coulter AU5812 analyzers and evaluate the efficacy of measures to improve 3 sigma and 4 sigma analytes.

Methods: 22 CLIA-regulated analytes were examined across both platforms with sigma values calculated at two levels of concentration. CLIA total error allowable (TEa) limits and Unassayed Chemistry Control QAP (Bio-Rad--over four months) were used to calculate the initial sigma as: Sigma = (TEa%-Bias%)/CV%. Performance metrics followed a "Plan-Do-Study-Act" model.

Results: One AU5812 (nicknamed Zeus, maintained by day shift) had 35 of 44 analyte/levels at 5 sigma or better. The alternate AU5812 (nicknamed Apollo, maintained by night shift) had 36 of 44 analyte/levels at 5 sigma or better. Studying the performance and differences between the two instruments for 3 sigma and 4 sigma analytes, it was noted that for cholesterol (both levels), Zeus had a lower CV% (i.e., 2.0, 1.8) resulting in higher sigma values (i.e., 4.3, 5.1) than were observed on Apollo (i.e., CV% 2.7, 2.8; sigma 3.5, 3.5). Additionally, Zeus Level 2 QC for calcium had less variation and a higher sigma (i.e., CV% 1.4; sigma 5.5) than Apollo (i.e., CV% 1.7; sigma 4.5). Following the "Plan-Do-Study-Act "model, these observations inspired a "Plan" to increase the frequency and monitoring of Apollo's sample probes, syringe replacement and instrument maintenance. The plan was implemented ("Do") and a "Study" of the next two months' QAP data showed significant improvement in Apollo's CV% and sigma values for both cholesterol and calcium, raising the sigma to above 6 for two consecutive months. Following the study, the "Act" is to continue monitoring the system.

Conclusion: An assay's sigma is greatly influenced by CV% and can be improved with increased maintenance and observation. Changes in instrument performance over time indicate sigma values are not static and should be monitored periodically.



A-235

Reporting real gene coverage resolution for clinical diagnostic tests

R. L. M. Guedes[1], M. A. Pereira[1], F. S. V. Malta[1], M. C. M. Freire[2], A. C. S. Ferreira[1], E. Mateo[1]. *[1]Hermes Pardini Institute (Research & Development Division), Vespasiano, Brazil, [2]Hermes Pardini Institute (Progenética Laboratory), Rio de Janeiro, Brazil*

Background: Next-generation sequencing (NGS) technology allowed users to analyze thousand to million of DNA sequences in a short time, revolutionizing the field of genomics and medical diagnosis. Build a custom panel targeting specific genes or genomic regions of interest is more cost-effective and useful for studies of disease- or phenotype-related genes when compared to broader approaches (e.g. whole-genome and whole-exome sequencing). It is expected that all genes/regions are completely covered and it is known that the panel coverage is highly dependent on a wide range of factors such as the sequencing platform, sequence complexity, nucleotide composition (GC content), location of variants, uniqueness of regions and primers specificity, efficiency and interference. **Objective:** Report a simple tool to analyze the actual coverage achieved across the targeted regions in each patient panel report. **Methods:** The tool was developed in PERL scripting language. Samtools was used to count sequencing coverage at base-level resolution for a BAM alignment output file. A BED format file used to design the panel was parsed to extract every gene coordinates and sizes. Human genome annotation GTF files was used to extract all gene isoforms coordinates. **Results:** Two output files are generated: a text file reporting the percentage of bases above the established coverage threshold for each gene and a total percentage for the whole panel; and a BED file reporting the coordinates of all bases below the coverage threshold. The report can be performed for the complete gene or any specified isoform. The tool tooks only 3'40'' minutes to process ~1 million reads from a panel with 40 gene isoforms with ~130k bases using a single processor in a common desktop computer. **Conclusions**: The coverage threshold is an essential parameter to detect variants in any NGS sequencing experiment. Furthermore, if full coverage is not achieved, it is recommended to provide the actual values obtained across the targeted regions for each patient report. We developed a tool to calculate the percentage of bases above a minimum established coverage threshold for each gene/region and for the whole panel. This tool is compatible with all sequencing platforms and can be used to guide primer design for further Sanger sequencing or simply used to incorporate as essential information in the final individual report.

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Comparability of two different urine total protein methods in patients with monoclonal proteinuria

C. D. Giesen, J. C. Lieske. *Mayo Clinic, Rochester, MN*

Background: Quantitation of urine total protein has long been problematic. No gold standard method exists as many protein species are present in varying amounts in the urine under normal physiological conditions and disease states. All available assays reliably detect albumin but have well-documented limitations for detecting monoclonal and other non-albumin proteins. The present study compared two commercial methods for quantifying urinary total protein in a cohort of patients with and without kidney disease and with and without known monoclonal M-spikes.

Methods: Waste urine samples were selected for analysis from among those submitted to the Mayo Clinic Renal Testing Laboratory for random urinalysis (RUA, n=99) and to the Mayo Clinic Protein Immunology Laboratory for monoclonal protein testing (n=61). Urine total protein was measured using pyrogallol red (QuanTtest Red,

Redondo Beach CA) and benzethonium chloride (Roche Diagnostics Total Protein Gen. 2, Indianapolis IN) on a Roche Cobas 6000 c501. The pyrogallol red assay was modified by the addition of 30 mg/L sodium dodecyl sulfate (Sigma-Aldrich, St. Louis MO) previously observed to increase detection of monoclonal proteins. The modified pyrogallol red assay, currently used in our laboratory, served as the reference assay for this analysis

Results: Among the RUA samples without known monoclonal species there was excellent correlation between the two methodologies by Passing & Bablok analysis (n=99; y=0.99x+0.79; range 0.9-229.9 mg/dL). Overall bias by Bland-Altman analysis was also acceptable (10.8% [3 mg/dL]) but was somewhat higher in samples with a low protein concentration of <10 mg/dL (22.2%) than those in the more clinically relevant ranges (10-30 mg/dL [4.5%]; >30 mg/dL [3.9%]). Among the samples with known M-spikes, the Roche assay yielded higher results than the modified Quantimetrix assay (Passing & Bablok regression y=1.30x+0.32; range 3.5-623.9 mg/dL; Bland-Altman % Bias = 35.1%).

Conclusion: Both urine total protein assays gave comparable results in random urine samples obtained from clinic patients with and without kidney disease, a population in which albumin is usually the major protein present in urine. However, quantitation of monoclonal proteins was quite variable between these 2 assays, with the Roche benzethonium chloride assay yielding values approximately 30% greater than the modified pyrogallol red method. These results have implication regarding the sensitivity of total protein assays for detecting and quantifying urine monoclonal proteins. Individual laboratories should take this into account together with their local patient population when choosing a urine total protein assay.

A-237

Equivalence of urine albumin to creatinine ratio measurements in 12h overnight urine and first morning urine

L. J. Tolentino[1], C. B. Maluf[1], R. C. Fiqueiredo[2], R. C. P. dos Reis[1], P. G. Vidigal[1]. *[1]School of medicine. Universidade Federal de Minas Gerais, Belo Horizonte, Brazil, [2]School of medicine. Universidade Federal de São João Del'Rei, São João Del'Rei, Brazil*

Background: Sustained albuminuria is a criteria for diagnosis of chronic kidney disease. Its early identification with adequate management reduces cardiovascular and renal risk, especially in hypertensives and diabetics. Albuminuria measured with timed urine is the gold standard, but its collection is laborious and susceptible to errors. Urine albumin to creatinine ratio (UACR) in random sample is an alternative to the gold standard. The aim of this study was to evaluate the equivalence between albuminuria in 12h urine (overnight) compared to first void urine considering the urinary stasis interval and the collection time. **Methods:** 123 participants collected urine for 12h, beginning in the evening. The urine overnight was stored in bottle 1, and kept in the refrigerator. First morning urine, identified as the urine performed after waking up, was collected in bottle 2. The hour of all urine collections were registered. Samples were analyzed using the Vitros 5600 Ortho Clinical Diagnostics Integrated System (Raritan, New Jersey), at the same analytical run. Equivalence between two samples was evaluated using Lin's, Pearson's and Bland Altman's methods. **Results:** Strong correlation and accordance between tests in samples with a 2 to 4 h stasis interval was found, being stronger if collected between 06:30 and 08:15 a.m (TAB.1). **Conclusion:** Although UACR is recommended for detection of albuminuria, lack of standardization of sample characteristics is a barrier to its reliability. Our results indicate that the best sample is obtained between 06:30 and 08:15 a.m. after a retention interval of 2 to 4 hours which may contribute for better standardization of albuminuria dosages.TABLE 1Equivalence of UACR measurements in 12h overnight and first morning urine

	N	CORRELATION COEFFICIENT		BLAND ALTMAN		
		Lin	r's Pearson	Mean difference	Standard deviation	Limits of agreement
UACR 12 h vs UACRm	123	0,558	0,646	-4,927	29,57	-62,88
UACR 12 h vs UACR 2-4h	41	0.958	0.974	2.67	11.76	-20.38
UACR 12h vs UACR 6:30	26	0.994	0.997	0.48	5.16	-9.631

UACR: Urine albumin to creatinine ratio; UACR 12 h: in 12h overnight urine samples; UACRm: in morning urine samples; UACR 2-4h: in samples with stasis time of 2 to 4 h; UACR 6:30: collected between 06:30 and 08:15 am.

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Establishing and adjusting the calibration interval based on reagent stability

E. Cho, K. Park, J. Kim, E. Shin, H. Hong, W. Lee, S. Chun, W. Min. *Asan Medical Center, Seoul, Korea, Republic of*

Background: The calibration criteria in the guidelines follow the manufacturer's recommended interval, but there are many differences in the volume of test between laboratories, applying the manufacturer's calibration interval uniformly is unreasonable. We tried to verify the calibration interval recommended by the manufacturer by investigating the in-use stability of the reagents in this study. We compared the point of time when the percent change of calibration factors exceeds the acceptance criteria with the manufacturer's recommended calibration interval. **Methods:** Using the equipment of Roche cobas 8000 (Roche Diagnostics, Switzerland), 22 general chemistry items (albumin, ALP, ALT, amylase, AST, BUN, Ca, cholesterol, CK, creatinine, direct bilirubin [DB], glucose, iron, LD, lipase, Mg, γ-GT, phosphorus [P], total bilirubin [TB], triglyceride [TG], total protein [TP], uric acid [UA]) were examined. The allowable range of the calibration factor variation was set as the optimal, desirable, minimum imprecision goal based on the biological variability and the coefficients of variation (CV) of the cumulative internal quality control results. For instruments using calibration only reagents, two lot numbers of reagents were used to evaluate each for 7 days. Only one reagent cassette was used in each test items and reagent was used only for calibration. The average of the calibration factor and the 95% confidence interval (CI) were calculated 12 times a day for each lot number of reagents. In addition, the calibration factors from the day when the reagent cassette was installed on the instrument to the maximum 7 days were analyzed using the instrument performing high-volume and small-volume tests in the actual laboratory. The time at which the 95% CI of the mean percent change deviates from the acceptance criteria was obtained. **Results:** Among the instrument that use calibration-only reagents, instrument performing high-volume and small-volume tests, there were no differences in the 15 items such as ALT, amylase, BUN, Ca, cholesterol, CK, creatinine, glucose, iron, LD, lipase, Mg, P, TB and UA at the time of exceeding the allowable limit. However, in albumin, ALP, AST, DB, γ-GT, TP and TG, it is observed that the earlier time point exceeding the allowable limit. When applying the optimal goal or the CV, the time point when the limit is exceeded obtained from the instrument using a calibration-only reagent, compared with the manufacturer's recommendation. The albumin, ALP, DB, LD, Mg and TP were required to shorten the recommended interval of the manufacturer, but Ca needs to increase the recommended interval. The cholesterol, creatinine, iron, and TG were found to be consistent with the manufacturer 's recommendation. The 11 items such as ALT, amylase, AST, BUN, CK, glucose, lipase, P, γ-GT, TB and UA did not exceed the limit for 7 days. **Conclusion:** When the desirable or minimum imprecision goal are applied as the acceptance criteria, the time point exceeding the allowable limit can be changed. Therefore, it is necessary to determine the calibration interval according to the appropriate acceptance criteria and the volume of test rather than the uniform calibration interval suggested by the manufacturer in the laboratory.

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Evaluation of hemolysis interference for high sensitivity cardiac troponin I immunoassay on ABBOTT Architect i2000 system

Y. Huang, L. Jiang. *Sichuan Academy of Medical Science & Sichuan Provincial People's Hospital, Chengdu, China*

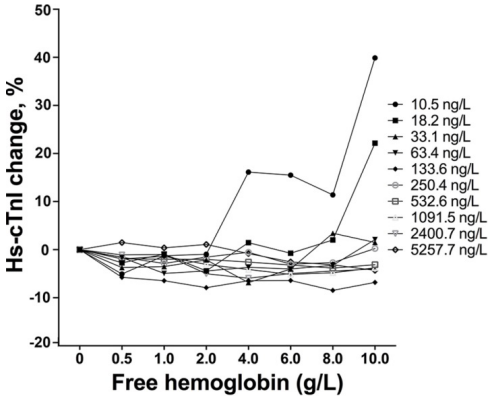
Background: Hemolysis is the most common endogenous interference that effects clinical laboratory test results. Our study aims to investigate hemolysis interference on ABBOTT immunoassay of high sensitivity cardiac troponin I (hs-cTnI), one of the most important markers for acute myocardial infarction diagnosis.

Methods: None-hemolyzed serum with high concentrations of hs-cTnI was pooled and spiked with hemolysate. Basal and seven hemolysis groups were constituted with free hemoglobin levels at 0.0, 0.5, 1.0, 2.0, 4.0, 6.0, 8.0 and 10.0 g/L, respectively. Each group contains 10 samples with serial hs-cTnI concentration at 10.5 ng/L, 18.2 ng/L, 33.1 ng/L, 63.4 ng/L, 133.6 ng/L, 250.4 ng/L, 532.6 ng/L, 1091.5 ng/L, 2400.7 ng/L and 5257.7 ng/L. Hs-cTnI concentrations for all 80 samples were analyzed in triplicate on i2000 analyzer (ABBOTT, USA) to evaluate the hemolysis interferences.

Results: ABBOTT hs-cTnI immunoassay had minimal interference with hemolysis. When applied a previously proposed serial cTnI change criteria to our spiked samples, no hs-cTnI results were effected by more than 20%, except for 10.5 ng/L (40.0%) and 18.2 ng/L (22.0%) at the free hemoglobin concentration of 10.0 g/L, which is rarely observed clinically. According to another suggested cTnI change criterion, results

changed by 28 ng/L (All give lower levels) at the hemoglobin concentration above 2.0 g/L. The cTnI concentrations in these samples were at 1091.5 ng/L, 2400.7 ng/L and 5257.7 ng/L, respectively, which were far above cutoff value for clinical diagnosis.

Conclusion: Our results demonstrated that hemolysis has minimal effect on the immunoassay of hs-cTnI concentration on ABBOTT i2000 system, except for at very high cTnI levels or at extremely high hemoglobin levels. Thus, hemolysis is not a confounding factor of the hs-cTnI assay on ABBOTT i2000 system for clinical interpretation at most of clinical situations.



A-240

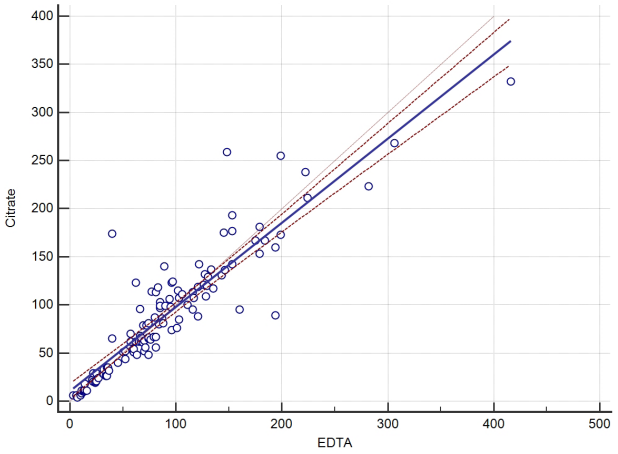
Effect of Sodium Citrate on Automated Platelet Count

M. O. Nakashima. *Cleveland Clinic, Cleveland, OH*

Background: Platelet clumping due to EDTA-induced antibodies can falsely decrease automated platelet counts. Mechanical disaggregation, such as vortexing, may be strictly prohibited by hematology analyzer instructions for use. Using sodium citrate as an anticoagulant has been suggested as an alternative to EDTA to reduce likelihood of clumping. We did a retrospective analysis to see how results from platelet counts ordered on citrated samples compared to those obtained from EDTA-anticoagulated samples.

Methods: Orders for platelet counts in EDTA and citrate which were simultaneously placed over a period of four months were retrieved from the laboratory information system. Platelet counts were obtained on Sysmex XE-5000 analyzers (Kobe, Japan). A 1.1x correction was applied to the citrated count to correct for dilution by the anticoagulant. Regression analysis was performed (MedCalc v 14.8.1, Ostend, Belgium).

Results: There were 164 concurrent orders for EDTA and citrate platelet counts. Platelet clumps were reported in the EDTA samples only for 11 orders (6.7%), the citrate sample only in 27 orders (16.4%), and in both citrate and EDTA samples in 7 (4.3%) . Linear regression yielded an equation of $y = 0.874x + 10.6399$ (95% CI for slope 0.8012-0.9467) with $R^2 = 0.8289$ (figure; thick solid line indicates regression, dashed lines are 95% CI, thin line is $y = x$). When analyzing only thrombocytopenic samples (defined as EDTA platelet count <150 x 10^9/L), $y = 10.259x + 0.9201$ (95% CI for slope 0.9092-1.1425) with $R^2 = 0.7528$.



Conclusion: Use of citrate anticoagulant does not prevent platelet clumps, and citrate samples were more likely to contain platelet clumps than EDTA samples. Although there was a relatively linear correlation between the results, there was bias in the citrate result. In thrombocytopenic samples, bias was smaller, however there was higher variability. These counts should be interpreted with caution especially in thrombocytopenic patients.

A-241

Automated urinary NGAL assay for early acute kidney injury detection

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Background: Urinary Neutrophil Gelatinase-Associated Lipocalin (NGAL) is one potential biomarker for early detection of acute kidney injury (AKI), since it increases before serum creatinine or even cystatin C. However, previous NGAL assays were not available on platforms that would allow rapid and cost efficient turnaround for clinical use. Here we validated an NGAL assay developed for a chemistry autoanalyzer

Methods: An enzymatic NGAL assay (BioPorto Diagnostics A/S, Denmark) was studied using a Cobas c501 chemistry analyzer (Roche Diagnostics, Indianapolis, IN). Validation was performed using residual urine samples from patients with and without documented kidney damage. All samples were centrifuged to remove white blood cells which are known to artificially increase NGAL values. A reference value study was completed in healthy volunteers without known kidney disease (54 females, age 24-85 years old; 42 males 23-85 years old). Accuracy was assessed via spike recovery. Mixing recovery was performed using high and low concentration samples in 1:1 ratios. Mean (range) % recovery was calculated as (measured/expected x100%) for each experiment with recoveries of 100+/-10% considered acceptable. Serial dilution was performed in water.

Results: This automated urinary NGAL assay was analytically robust between 40 ng/mL and 3000 ng/mL. Intrassay precision was acceptable (5%) at 112 ng/mL, and improved to 2% at 2084 ng/mL. Average recovery with serial dilutions using water, calculated as (measured/expected x100%), was 94%. Recovery upon mixing high and low samples 1:1 (n=3 pairs) was 101%. The upper 95% reference value in the healthy male and female donors was 109ng/mL. Samples were stable after centrifugation for up to 7 days ambient, 4ºC, -20ºC, or -70ºC, with toluene or sodium bicarbonate preservatives, and with up to 3 freeze-thaw cycles. The analytic turnaround time is 12 minutes.

Conclusion: An automated NGAL assay can be used on a chemistry analyzer to rapidly and accurately quantitate NGAL in urine. This platform should facilitate widespread clinical validation studies and implementation into practice if clinical utility is demonstrated.

A-242

Evaluation of BD Vacutainer® Barricor™ Plasma Blood Collection Tubes Under Various Transport Conditions

K. McDougall, B. Gormley. *BD, Franklin Lakes, NJ*

Objective: Two studies were performed to assess the integrity of the barrier in BD Vacutainer® Barricor™ Plasma Blood Collection Tubes (BD Barricor) under various transport conditions. One study evaluated filled tubes transported via a pneumatic transport system (PTS) pre- and post-centrifugation to determine if there was any effect on the mechanical separator in BD Barricor Tubes; the second study evaluated filled tubes during simulated ground transportation conditions post centrifugation for maintenance of the separator barrier.

Background: Although pneumatic tube systems (PTS) provide a rapid means of tube transport to the laboratory, the samples withstand forces of pressure, such as changes in air pressure, movement or shaking of blood in the test tube, vibrations, and sudden accelerations and decelerations. Studies tested the effect of PTS on the mechanical separator in BD Barricor Tubes. Barrier integrity was also assessed under simulated ground transportation conditions that were designed to emulate conditions that centrifuged blood specimens collected in BD Barricor Tubes would be subjected to during transport from remote clinics and physicians' offices to a testing laboratory.

Methods: Three hundred 3.0 mL, 13x75 mm and 300 4.5 mL, 13x100 mm BD Barricor Tubes were filled with sheep's blood prior to centrifugation, inspected for correct positioning of the separator and transported in the PTS. Six hundred varied draw BD Barricor Tubes were filled with sheep's blood and centrifuged at 4000g for 3 minutes. Tubes with no or trace hemolysis were subjected to the PTS and re-inspected for plasma color change, an indicator of barrier leakage and loss of barrier integrity. To simulate ground transportation conditions, bagged human blood in 300 BD Barricor Tubes each of 3.0 mL, 13x75 mm and 4.5 mL, 5.5 mL, 13x 100 mm, was centrifuged at 4000g for 3 minutes and inspected at four time intervals.

Results: Pre-centrifugation, post PTS transport showed no separator movement from the original position. There was no loss of barrier integrity in 300 3.0 mL and 299 4.5 mL BD Barricor Tubes and post PTS transport for varied draw tubes. The 95% confidence limit for the failure rate was less than 1.0% for all tube configurations. This demonstrated compatibility for PTS transport, as all tubes maintained barrier integrity through simulated transportation. No deterioration was observed in barrier performance, which met the acceptance criterion of 95% confidence of 95% reliability to maintain barrier integrity at each time interval.

Conclusions: The mechanical separator in BD Barricor Tubes maintained its position at the top of the uncentrifuged tubes through pneumatic tube transport. The separator formed a robust barrier between cells and plasma in centrifuged tubes, which remained integral throughout the transport conditions.

A-243

Differential interferences of a kinetic Jaffe creatinine method by three ketones

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Objective:

It is well-known that ketones interfere with creatinine measurement by Jaffe methods. However, which ketone(s) are responsible for this interference have not been previously elucidated. Here we report the effects of beta-hydroxybutyrate, acetoacetate, and acetone on creatinine analysis by a Jaffe method and an enzymatic method. We also propose the use of a creatinine gap in the differential diagnosis of an excess osmolal gap.

Methods:

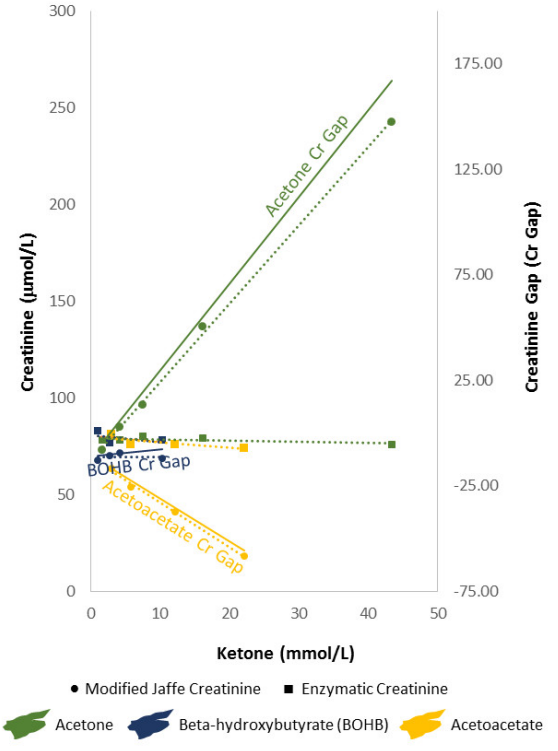
DL-beta-hydroxybutyric acid sodium salt and lithium acetoacetate were purchased from Sigma-Aldrich, acetone from Thermo Fisher Scientific. Stock solutions of beta-hydroxybutyrate, acetoacetate, and acetone were prepared in distilled water. Plasma samples with creatinine concentration of ~80 umol/L were pooled. The pooled plasma was spiked with distilled water (control) or with the prepared stock ketone solutions to create specimens with varying ketone concentrations. Creatinine levels in these spiked samples were measured by both a kinetic Jaffe method (Siemens Vista) and an enzymatic method (ABL800). The creatinine gap was calculated as the Vista creatinine concentration - the ABL800 creatinine concentration. Beta-hydroxybutyrate was measured on the Vista, acetone by gas chromatography; acetoacetate level was estimated by the sample's lithium concentration, as measured on the Vista platform.

Results:

Ketones do not interfere with the enzymatic creatinine method. In contrast, ketone-specific interferences are observed with the kinetic Jaffe creatinine method: acetone causes concentration-dependent falsely high creatinine levels, acetoacetate causes concentration-dependent falsely low creatinine levels, and beta-hydroxybutyrate does not interfere with the creatinine measurement (see figure).

Conclusion:

Ketones interfere with creatinine analysis by the kinetic Jaffe method. This interference is ketone-specific. Osmolal gap, anion gap, and lactate gap are often used to expedite diagnosis and treatment of toxic alcohol ingestions. As acetone is the metabolite of isopropanol, calculation of the creatinine gap, as determined by creatinine measurements between Vista and ABL800 analyzers, can be used as a surrogate marker for isopropanol ingestion.



A-244

Negative interference by Calcium Dobesilate in Five Trinder Reaction Assays

X. Guo, L. Qiu. *Peking Union Medical College Hospital, Chinese Academic Medical Science and Peking Union Medical Col, Beijing, China*

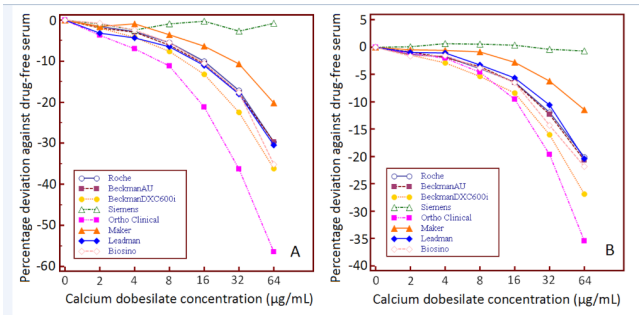
Background: Previously, we reported the strong negative interference of calcium dobesilate, a vasoprotective agent, in creatinine assays involving the Trinder reaction. It is hypothesized that a similar effect occurs in the detection of uric acid (UA), total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C). The interference of calcium dobesilate in the five serum analytes was investigated.

Methods: Calcium dobesilate standard was added into two sets of the blank serum of each analyte at final concentrations of 0, 2, 4, 8, 16, 32, and 64 µg/mL. Each analyte was measured using eight different assay systems. The percentage deviation of each analyte value was calculated between each drug concentration and the drug-free samples, and the effects were compared among eight different assay systems for each analyte.

Results: Considering the clinically acceptable deviation of ±4.5% for UA, the exogenous addition of calcium dobesilate clearly exhibited dose-dependent negative interference with the determination of UA in all seven Trinder reaction-based assays (Figure 1). In the presence of 16mg/mL calcium dobesilate, all seven Trinder reaction-based UA assays exhibited deviations ranging from -6.3% to -21.2% in the low UA serum group (Figure 1A). As a control assay, the Siemens system using the Uricase-

UV method did not show any interference (Figure 1). The clinically acceptable error levels for TC, TG, HDL-C, and LDL-C were defined as±4.0%, ±5.0%, ±5.2%, and ±6.8%, respectively. At 16 µg/mL calcium dobesilate, six TG assay systems, three HDL-C assay systems and one TC assay system exhibited negative drug interference. The deviations for all eight LDL-C assay systems were less than 6.8% at calcium dobesilate concentrations ≤64 µg/mL.

Conclusion: Calcium dobesilate negatively interferes with the detection of UA, TG, TC, and HDL-C in assay systems based on the Trinder reaction. The effect was most significant in UA and TG detection but imperceptible for LDL-C detection.



A-245

Calcium Dobesilate can Negatively Interfere with the Detection of Glycated Albumin

X. Guo, L. Qiu. *Peking Union Medical College Hospital, Chinese Academic Medical Science and Peking Union Medical Col, Beijing, China*

Background: Glycated albumin (GA) is considered as a potential intermediate-term glycation index to fill the gap between self-monitoring of blood glucose and haemoglobin A1c testing in diabetes management ADDIN NE.Ref.{6B0F0033-2506-4A2A-AF78-F095247FA411}. The enzymatic method has been the primary method for assessing GA in medical laboratories owing to its high sensitivity and specificity, and ability for automated analysis. However, recently, we noted that calcium dobesilate, which is a vasoprotective agent widely used for the treatment of diabetic retinopathy, can negatively interfere with GA detection using the enzymatic method.

Methods: We quantified this analytical interference using both in vitro and in vivo methods. A calcium dobesilate standard was added to 3 serum samples (GA levels: 39.4%, 23.3%, and 17.6%, respectively) to prepare dose-response series according to the CLSI EP7-A2 guidelines. For in vivo interference experiments, baseline serum samples were collected from 8 healthy participants. Calcium dobesilate (500 mg) was then administered orally 3 times daily for 3 days to achieve a steady state according to pharmacokinetic information. Fasting blood samples were collected at trough drug levels on the morning of the 4th day (0 hours), and at 2 hours after administering another 500-mg dose of calcium dobesilate. GA levels were measured with the Asahi Kasei GA assay (Asahi Kasei, Inc., Japan) using a Beckman AU5800 analyser (Beckman Coulter, Inc., Brea, CA), according to the manufacturer's recommended procedure. Calcium dobesilate (mg/mL) concentrations were measured using a high-performance liquid chromatography method.

Results: The exogenous addition of calcium dobesilate clearly exhibited dose-dependent negative interference with GA determination. In the presence of 16 mg/mL calcium dobesilate, the percentage deviations from drug-free serum were -9.9%, -11.6%, and -10.2% for the high-, medium-, and low-GA interference samples, respectively. The degree of interference reached about 30% at a calcium dobesilate concentration of 64 µg/mL. In the in vivo interference experiments, after 3 days of calcium dobesilate administration, the mean calcium dobesilate concentrations at 0 and 2 hours were 7.33 (range, 4.63-9.55; interquartile range [IQR], 6.25-8.18) and 18.52 (range, 7.04-20.2; IQR, 15.90-19.67) mg/mL, respectively, resulting in a decrease in GA values of -4.8% and -12.8%, respectively, relative to that in the baseline control samples.

Calcium dobesilate is believed to remain mainly in its original form after excretion through the kidney and intestinal tract. Currently, the recommended clinical dose of calcium dobesilate is 500 mg TID, and its steady-state plasma concentration is estimated to be above 15 µg/mL. We previously reported that calcium dobesilate interfered in creatinine assays using the Trinder reaction method. We also noted that the calcium dobesilate concentration in patients administered this drug reached 63.35 µg/mL, and interference is expected to be more substantial at such high concentrations.

Conclusion: In conclusion, calcium dobesilate was confirmed to negatively interfere with GA determination in both in vitro and in vivo experiments, which may result in false glycaemic status evaluations in the management of diabetic patients. Extra care should be taken in the evaluation of GA levels in patients receiving calcium dobesilate.

A-246

Evaluation of Sekisui LDL-C Reagent against Wako LDL-C Reagent on Beckman Coulter AU5800 and its Impact on Patient and External Quality Assurance Results

S. Phun, Z. Yang, S. Saw, S. Sethi. *National University Hospital, Singapore, Singapore*

Background: National University Hospital(NUH) has participated and achieved satisfactory results in College of American Pathologists(CAP) surveys for LDL-Cholesterol(LDL-C) for many years. In April 2014, we changed to Beckman Coulter AU5800 and observed higher recovery than our peers for all our CAP General Chemistry and Accuracy-Based surveys. Our investigations revealed that Sekisui Medical Co. Ltd LDL-C reagent was supplied to US customers and Wako Pure Chemical Industries Ltd LDL-C reagent to customers outside US. Both LDL-C reagents passed the Centre for Disease Control and Prevention(CDC) Lipid Standardisation Program and both calibrators were traceable to CDC Reference method. However, the differences in method performance between the two reagents were not evaluated in detail.

Methods: The study was performed over a period of 5 days with daily calibrations and valid QC runs. Three methods were evaluated- Sekisui(Reference method), Wako(Test Method 1) and Wako with adjusted LDL calibration factor(Test Method 2). A total of 200 fresh patient samples with Triglyceride values less than 2.25mmol/L and LDL-C values across analytical measurement range were tested. The correlation results were evaluated for percent bias from Reference Method across clinically significant decision levels.

Results: Both Test Methods 1 and 2 had an over-recovery ranging from 10.9-20.3% and 2.4-10.9% respectively. Test Method 2 achieved closer recovery compared to Reference Method using calibrator adjustment alone. The table below shows the percent bias of the Test Methods.

Percent Bias from Reference Method Based on Best Fit Line		
LDL-C Clinical Decision Levels	Test Method 1	Test Method 2
2.6 mmol/L	20.3%	10.9%
3.4mmol/L	15.6%	6.6%
4.1 mmol/L	13.0%	4.3%
4.9 mmol/L	10.9%	2.4%

Conclusion: This study highlights the problems faced by international customers when diagnostic companies provide different reagents to different parts of the world, leading to the laboratories' inability to obtain appropriate performance (and sometimes failure) when assessed against peers in CAP surveys.

A-247

Multivariate Models for Combinations of Hemolysis, Icterus, and Lipemia Interference

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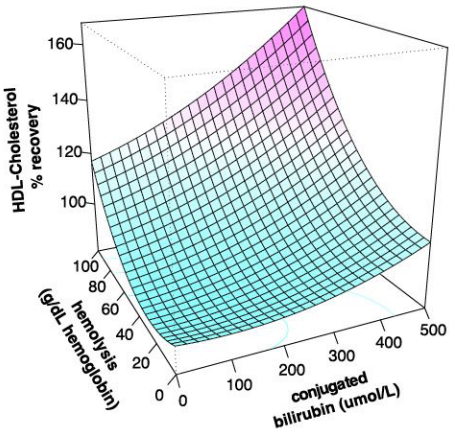
Background: Hemolysis, icterus, and lipemia interferences can affect the accuracy of patient results. Both manufacturers and laboratories spend considerable financial and personnel resources performing interference studies, but there remains limited data on the effect of combinations of interferences. The objective of this study was to use multivariate experimental design to efficiently determine the effect of combinations of hemolysis, icterus, and lipemia interferences on common biochemistry test results.

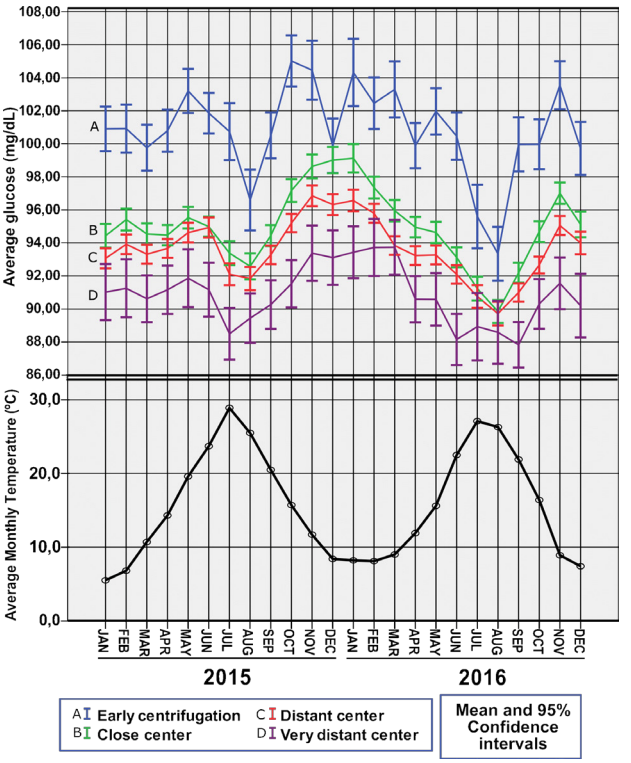
Methods: To model the combined effects of hemolysis, icterus, and lipemia on creatinine, HDL-cholesterol, AST, and ALT, we used a face-centered central composite design (CCD). CCD is an efficient experimental design method using limited experimental data to model individual factors on a response; in this study, factors included hemolysis, icterus (conjugated and unconjugated), lipemia, and analyte concentration and the response was %recovery. Interferences were added in combination to represent low, medium, and high values for each. The design yielded a mathematical model for each analyte and interference combination with only 30 samples. Analytes were tested on the Siemens Vista 1500, where %recovery was

calculated as: (expected - measured)/expected. Results were analyzed using response surface modeling to identify linear and exponential effects and well as interactions (e.g. hemolysis:lipemia in combination).

Results: At low concentrations, AST showed markedly increased recovery due to hemolysis. Also at low analyte concentrations, ALT was subject to positive bias due to lipemia interference. There was significant positive bias on HDL-cholesterol recovery in the combined presence of hemolysis and conjugated bilirubin. Enzymatic creatinine showed positive bias with icterus and hemolysis in combination.

Conclusion: Creatinine and HDL-cholesterol showed an until now unrecognized synergistic positive bias from hemolysis and icterus. ALT and AST analysis confirmed existing package insert information supporting that there is no multivariate effect from interference combinations. Multivariate experimental design is an efficient way to obtain complete data for multiple interferences in combination.





A-249

Glucose preanalytical variation: Influence of time-to-centrifugation and environment temperature

L. Bautista Balbás, R. Gómez Rioja, M. Duque Alcorta, C. Eisman Valdés, L. Alba Valdivia, A. Buño Soto. *Hospital La Paz, Madrid, Spain*

Background: Glucose determination in samples collected in external centers is very susceptible to preanalytical errors; particularly due to glucose consumption. In this observational study we want to describe these variations in our particular conditions (Northern area of Madrid, Spain).

Materials and methods: We retrospectively gathered the serum glucose results of outpatients (primary care and specialized care) from 2015-2016; all these were drawn outside our hospital and delivered by road in portable non-electric coolers.

We calculated the time-to-centrifugation (TTC) according to our daily preanalytical unit logbook and we estimated the approximate TTC for each centre. We obtained the Average monthly temperatures in our area from Spanish Meteorological Agency (AEMET). We classified the samples in early centrifugation (before road transport, in less than 30 minutes), close centers (30-90 minutes), distant centers (90-120 minutes) and very distant centers (more than 120 minutes). Glucose was measured in Advia 2400 (Siemens Healthineers) (hexokinase).

Results: We gathered 361,268 results: 39228 in Early centrifugation, 134311 in Close centers, 164245 in Distant centers and 23485 in Very distant centers. Slight differences were observed in the mean age between the groups (52-56), and in sex (37-40% males).

In a general linear model age, sex, the average TTC of the centre and Average monthly temperature (ºC) in our area were significantly associated with the glucose value (although, as we expected, these factors only explained a small percentage of the patient results variability: R^2=0.109).

Conclusions: Observed glucose variations of up to 10 mg/dL between groups of samples are coherent with described glycolysis rates of 2-7% per hour. This could be prevented by using tubes with glycolysis inhibitors or centrifuging samples in collection centers.

We can conclude from these observational results that in part due to preanalytical factors serum glucose results could vary up to 10% in samples from external centers.

A-250

Assessment of Lipemia Interference using Human Triglyceride-Rich Lipoproteins

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Background: IntraLipid® continues to be used as a lipemia interferent despite data that suggests it fails to replicate the lipemia seen with human lipoproteins. Therefore, we evaluated interference by triglycerides on common chemistry tests and compared results to manufacturer's claims based on IntraLipid. **Methods:** Base Pool was prepared using serum pools with low triglycerides. High Pool was prepared by spiking concentrated triglyceride-rich lipoprotein (TRL) into the Base Pool (Assurance™ Interference Test Kit, Sun Diagnostics, New Gloucester, ME). Base Pool and High Pool were intermixed to create five levels of triglycerides (107, 397, 677, 964, and 1227 mg/dL). Multiple analytes were measured on the Beckman AU5800. **Results:** Minimal interference was seen with alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), amylase (AMY), bicarbonate (CO2), Calcium (CA), creatine kinase (CK), gamma-glutamyltransferase (GGT), lactate dehydrogenase (LDH), lipase (LIP), magnesium (MG), phosphorus (PHOS), total protein (TP), apolipoprotein AI (ApoAI), uric acid (UA), adiponectin (ADN), cystatin C (CYSC), high sensitivity C-reactive protein (hsCRP), ferritin (FER), 1,5-anhydroglucitol (1,5-AG), potassium (K), or chloride (CL), consistent with manufacturer claims using IntraLipid. We saw less lipemia interference with TRL compared to manufacturer's claim using IntraLipid for total bilirubin (TBIL), direct bilirubin (DBIL), iron (FE), urea nitrogen (BUN), and creatinine (CRE). We saw no TRL interference with K or CL, although higher triglyceride concentration likely support manufacturer's claim of potential decrease due to volume displacement. Sodium (NA), however, did display decreasing values with increased triglyceride concentrations. Albumin (ALB), low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C) concentrations increased with increasing triglyceride interferent concentration, in contrast with manufacturers' claims of minimal lipemia interference using IntraLipid. **Conclusion:** The use of TRL versus IntraLipid as an interferent offers a more accurate depiction of the potential for triglyceride interference during laboratory testing.

A-251

Recognition of Rare Hemoglobin Variants during Hemoglobin A1c Analysis by Capillary Electrophoresis

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Background: Hemoglobin A1c (HbA1c) is the result of a non-enzymatic glycation of hemoglobin A and is measured by multiple analytical techniques, including immunoassay, boronate affinity chromatography, HPLC, and capillary electrophoresis. Few studies have investigated the effects of rare variants on HbA1c analysis by high resolution capillary electrophoresis (CE).

Methods: During routine HbA1c analysis by CE in our laboratory, potential hemoglobin variants were identified by examination of CE electropherograms. Variants were confirmed by sequencing and A1c was subsequently measured in selected samples by 3 additional HbA1c measurement procedures (2 ion-exchange HPLC and 1 boronate HPLC).

Results: During a 13-month period, 35 patients were identified as having an "atypical profile" with the CE method. Of those, 11 samples were confirmed to have hemoglobin variants and 6 samples appeared to harbor a hemoglobin variant, but additional blood samples for sequencing were unavailable. Four variants (Hb Roanne, La Desirade, Nouakchott, and A2 prime) had not been reported previously to influence the quantification of HbA1c by the CE method. Three of these variants (Hb Roanne, La Desirade, and Fannin-Lubbock) were further tested by 3 additional methods for measurement of HbA1c, and the results were compared to the CE results. HbA1c as measured by the CE method differed by 0.8%, 0.7%, and 2.2% for Hb Roanne, La Desirade, and Fannin-Lubbock, respectively, from the mean of the other 3 methods. Hb Roanne and Fannin-Lubbock were resolved from HbA only by the high resolution CE method and not by either HPLC method (ion-exchange or boronate affinity), while Hb La Desirade displayed aberrant peaks only in the CE and the G8 Tosoh HPLC methods.

Conclusions: Failure to identify these silent variants could lead to misleading HbA1c results and negatively impact patient outcomes. The high resolution of the CE method allows for better identification of hemoglobin variants in patients requiring routine HbA1c analysis. For patients with a hemoglobin variant that prevents measurement by HPLC or CE, another method is needed or alternative assays, such as measurement of fructosamine or glycated albumin, may also be required for appropriately monitoring glycemic control.

A-252

Effect of Seasonal Temperature on Specimens Stored Outside in Courier Lock Boxes

J. R. Wiencek, J. H. Nichols. *Vanderbilt University Medical Center, Nashville, TN*

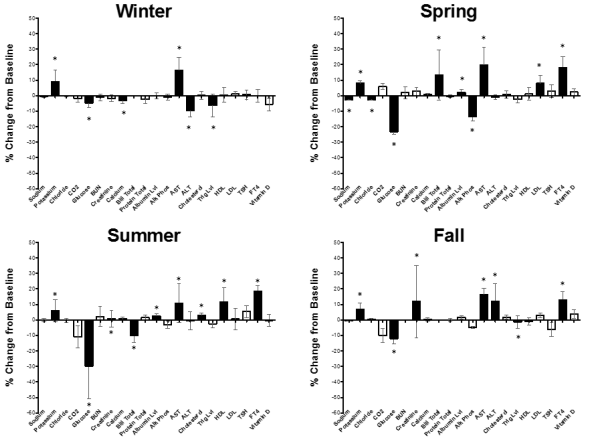
Background: Lock boxes for patient specimens are commonly used by outpatient clinics to store processed specimens awaiting pick-up for courier transport to a laboratory. Temperature conditions and delays in courier pick-up are critical factors in the preanalytic quality of these specimens and have not received rigorous investigation.

Objective: To determine the effects of sample exposure to ambient temperature changes and delayed shipping conditions (over a weekend) on several commonly measured analytes in the clinical laboratory at different times of the year.

Methods: Paired lithium heparin BD PST Vacutainer® tubes were drawn from healthy volunteers (n=4) during four seasons (winter, spring, summer, fall) in the Southern US. Specimens were processed immediately after collection. Paired specimens were analyzed either immediately by Abbott Architect analyzers or after 72 hours of holding at ambient temperature in an outside courier lock-box. Temperature was continuously monitored every 15min with an INKBIRD Temperature Data Logger. Each sample was analyzed for a comprehensive metabolic panel, lipid profile, thyroid panel and Vitamin D. Acceptable tolerance limits for analytes were determined by significant change limit (SCL) analysis. For each analyte, SCLs were calculated from 12-months of cumulative performance data (quality control coefficient of variation) multiplied by ±2.8.

Results: The average temperatures were: winter -3.1°C (Range: -9.9-22.3°C), spring 16.1°C (Range: 7.0-25.2°C), summer 25.0°C (Range: 21.4-29.8°C) and fall 10.6°C (Range: 2.2-22.8°C). Large deviations were observed for glucose, K^+, and AST when compared to initial measurements across all seasons. Colder temperatures seen in the fall and winter experiments showed 7/21 and 6/21 of the analytes were outside of the SCL tolerance limits (black shaded bars in seasonal graphs $p<0.05$ from t_0). More analytes were affected by warmer seasons with 10/21 in spring and 9/21 in summer outside SCL tolerance limits.

Conclusion: Delayed sample delivery and fluctuating seasonal temperatures in the Southern US are important sources of preanalytical error.



A-253

Surface Characterization and Free Thyroid Hormone Measurements of Chemically Modified Polyethylene Terephthalate Films and Blood Collection Tubes (BCT) to Eliminate Potential BCT Surfactant Interference on Some Immunoassay Tests

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Background: Blood collection tubes (BCTs) are not inert specimen carriers, as shown by some studies reporting significant differences in serum hormone concentrations collected in polyethylene terephthalate (PET) BCTs due to surfactants. We recently developed chemically modified BCTs (chemoPETs) that have an interior surface similar to glass BCT and lacks surfactant(s). However, the chemoPET surfaces were not fully characterized and free triiodothyronine (FT_3) and free thyroxine (FT_4) concentrations were not studied. Free thyroid hormones in blood is important because they are metabolically active and useful for the diagnosis of thyroid diseases. This study's first objective is to characterize and compare unmodified PET and chemoPET film surfaces to determine if the latter is significantly hydrophilic. The second objective is to determine if the biases in FT_3 and FT_4 concentrations from chemoPET and other serum BCTs compared to glass tubes are statistically and/or clinically significant. **Methods:** PET and chemoPET film surfaces were characterized by X-ray photoelectron spectroscopy (XPS), time-of-flight secondary ion mass spectrometry (ToF-SIMS), and scanning electron microscopy (SEM). The PET film contact angles and surface energy were determined with liquid probes. For serum specimens, one glass (control BCT) and five plastic (Vacuette™, SST™, RST™, plain red-top, and chemoPET) BCT types were used. The chemoPET film and tubes were made from a base-catalyzed transesterification reaction. Blood samples were drawn in a randomized order from 25 volunteers (10 males, 15 females; age range: 25-70 years), mixed, and allowed to clot for 1 hour. Following centrifugation, serum specimens were transferred to plastic tubes and stored at -70 ºC until analysis. The concentrations of FT_3 and FT_4 in the BCTs were analyzed in singleton on an Immulite 1000 instrument. Repeated measures ANOVA with Bonferroni correction were used to determine significant differences in FT_3 and FT_4 concentrations. To assess whether the BCT-related biases (deviation from glass tubes) were clinically significant for FT_3 and FT_4 concentrations, each BCT bias was compared to the desirable allowable bias, derived from biological variation. **Results:** XPS analysis results indicate that the carbon/oxygen ratio on the PET film surfaces decreased from 2.9 for unmodified PET to 2.1 for chemoPET surfaces. Tof-SIMS analysis shows a higher peak intensity of oxygen and hydroxyl groups on the chemoPET compared to unmodified PET film surface. The surface topography by SEM demonstrated that the chemoPET film surface was smooth without any holes. Water contact angle measurements show a

decrease from 70° for unmodified PET to 44° for chemoPET film surfaces and the surface energy and polarity increased from 37.9 to 51.9 (mJ/m^2) and 25% to 71%, respectively. These results clearly indicate that the chemoPET surface is more hydrophilic than the unmodified PET. No statistically significant differences in FT_3 (p=0.01) and FT_4 (p=0.77) concentrations were observed when chemoPET and other BCTs were compared to glass BCTs. However, compared to glass tubes, clinically significant differences were found in FT_3 (6.71%; desirable bias: 4.80%) and FT_4 (3.36%; desirable bias: 3.30%) concentrations in SST and RST tubes, respectively. **Conclusion:** ChemoPET tubes may be used to eliminate BCTs that contain surfactants known to interfere with some immunoassays.

A-256

Analysis of serum indices measurement results as partial evaluation of preanalytical phase

M. S. Gungoren, D. I. Topcu, C. Zungun. *Duzen Laboratories Group, Ankara, Turkey*

Background: Serum indices are calculations of spectrophotometric measurements that represent levels of icterus (I), hemolysis (H), or lipemia (L) in serum samples. Main purpose of serum indices is to evaluate sample integrity and avoid analytical errors due to inappropriate sample. Additionally, measuring the presence and the extent of hemolysis, icterus and lipemia of a serum sample can give us some idea about patient's health and nutrition conditions and quality of phlebotomy. As these are several preanalytical factors, we decided to evaluate preanalytical errors related with blood sample collection and inadequate collection of patient information by analysing results of serum indices together with demographical features of patients.**Methods:** Patient records with serum indices results of last 12 months were imported from Laboratory Information System. Lower limits for H, I and L indices were determined as 15, 1 and 15, respectively. Total of 38 tests were selected for evaluation (23 for H, 6 for I and 9 for L). Serum indices were measured with Roche SI2 Reagent using Roche Cobas c501 (Roche Diagnostics, Mannheim, Germany). Statistical analyses were performed with R 3.3.2 (R Working Group, Vienna, Austria).**Results:** Total number of samples with selected 38 tests was 34394. Firstly, ratio of out-of-range results were found. 20.3% of all H index results, 12.0 % of all I index results and 11.0 % of all L index results were above lower limits. Positive results were evaluated by categorizing patients as 8 age groups (<1, 1-3, 4-11, 12-17, 18-64, 65-74, 75-84, >85). Rates of icterus (19.3 %) and lipemia (57.8 %) were higher among babies under 12 months of age. Additionally, rates of hemolysis were remarkably high among samples from patients aged 0-12 months (43.8 %) and >85 years (20.8 %).**Conclusion:** Improving quality of sampling by training phlebotomists especially for working with babies and elderly may reduce rate of hemolysis, whereas adequate questioning of feeding status of babies may improve rate of lipemia.

A-257

Reduce the impact of hook effect on hCG immunoassay by proactive backtracking confirmation

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Aim: Immunoassay is a general methodology for hCG measurement in clinical laboratories. Hook effect occurs when hCG concentration is extremely high, resulting in an falsely low result. In our study, we develop a proactive backtracking confirmation strategy to reduce the error rate caused by hook effect.

Methods: SIEMENS Immulite2000 XPi was used to quantitate hCG in our laboratory. The hCG calibration range is 0.4~5,000mIU/mL. For samples with hCG concentration greater than 5,000mIU/mL, the instrument was set up to switch to auto-dilution program in order to quantitate hCG correctly. The vendor claimed that there is no high-dose hook effect at concentrations up to 600,000 mIU/mL. There is no appropriate way to identify the extremely high-concentration samples if the instrument did not switch to auto-dilution mode. The laboratory could report the falsely low results. Therefore, to decrease the error rate, we developed a backtracking confirmation rule: When an over 5,000mIU/mL hCG result was shown, laboratory technologists should proactively search 7-day history results of this patient. If hCG 7-day history results were below 5,000mIU/mL, technologists retested the previous sample in a dilution mode. An over 5,000mIU/mL retested result implied that due to hook effect, the previous result was underestimated. We then corrected the result in laboratory information system (LIS) and notified clinicians. To verify the practicality of the backtracking confirmation rule, we applied it to history hCG results retrieving from LIS during the period from January through November 2016. This retrospective study was confirmed by resident doctors.

Results: Among 5,045 prescribed hCG tests from January to November in 2016, 19 cases met the inclusion criteria of the backtracking confirmation rule. In these cases, one patient was diagnosed as having hydatidiform mole, and hCG results of this patient before and after surgery were 2,247 mIU/mL and 101,105 mIU/mL respectively. Hook effect may explain this phenomenon. We further modified the inclusion criteria, extended the history data search setting from 7-day to 14-day. No cases revealing possible evidences of hook effect were observed. According to medical records, all cases were pregnant women or in vitro fertilization. By applying the backtracking confirmation rule, we screened out one case in December 2016. The patient was diagnosed as having hydatidiform mole. These hCG results before and after the surgery were 2,279 mIU/mL and 81,779mIU/mL respectively. The 400-fold diluted of the first sample was retested, the final result was 1,610,533 mIU/mL. The hCG results were revised and the clinician was informed timely.

Conclusion: A proactive backtracking confirmation rule was applied to hCG measurement. Our laboratory can timely recall and revise incorrect results caused by hook effect. Through proactive notification from laboratory technologists to clinicians, patient safety will be improved.

A-258

Increasing the reliability of variant reports

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Background: Variant Callers (VC) are programs designed to detect sequence variants in genomic datasets. They have several parameters that define conditions that must be fulfilled for confirming a candidate as variant. The parameters set used in VC can be adjusted according to each strategy, for example, less stringent parameters may decrease false negatives, but increase false positives. The challenge is to define a parameter set to obtain the lowest error rate. In our laboratory routine we noticed that some parameters have more influence to the number of false negatives. These parameters (e.g., total minimum depth or minimum depth per strand) assume that a minimum number of reads must be available in the dataset, but the minimum depth parameter is not achieved, the VC will not consider these regions for the variant calling, regardless of the presence of an eventual variant. This event cannot be considered an algorithm interpretation error. In fact, those positions were not even assessed because they did not satisfy the established threshold. Here we describe a simple workflow that can be applicable by any health service laboratory that wants to increase the accuracy of variant reports. **Methods:** The dataset used was obtained from the sequencing of nine genes of NA12878 human reference sample using the Ion PGM platform. The variant calling process was performed using Torrent Variant Caller v5.0 (TVC). Our workflow basically comprised in the use of modified "low stringency" default parameters of TVC with more stringent adjustments (mapping quality, minimum total coverage and minimum coverage per strand) combined with the use of a bioinformatics tool (developed in PERL). Our tool was designed to recover regions that did not fill the minimum depth adjusted parameter in the VC. And then these regions were evaluated by manual curation and/or Sanger sequencing. The result obtained by this workflow was compared with the results obtained using the "low stringency" default parameters in the same variant caller. **Results:** Using the default parameter on TVC, we obtained 11 concordance, 2 false negatives and 4 false positives while in the analysis using our workflow, we obtained 12 concordance, 1 false negative and 1 false positive. **Discussion:** Considering those results, our workflow had better performance than the "low stringency" default. The workflow was able to reduce the number of false positives and negatives, allowing the user to recover the information about the discarded regions. This workflow will allow users to use more rigorous parameters to ensure better quality, without the risk of ignoring target regions and variants that could be better evaluated by another methodology. **Conclusion:** We consider that our analysis workflow is an alternative to ensure more reliable results. Furthermore, other parameters can have the same behavior like the minimum depth and therefore can also be treated in the same way. Therefore we believe that improvements in the tool still can be made and enhance this workflow.

A-259

National Survey of Adult and Pediatric Reference Intervals in Clinical Laboratories across Canada: A Report of the CSCC Working Group on Reference Interval Harmonization

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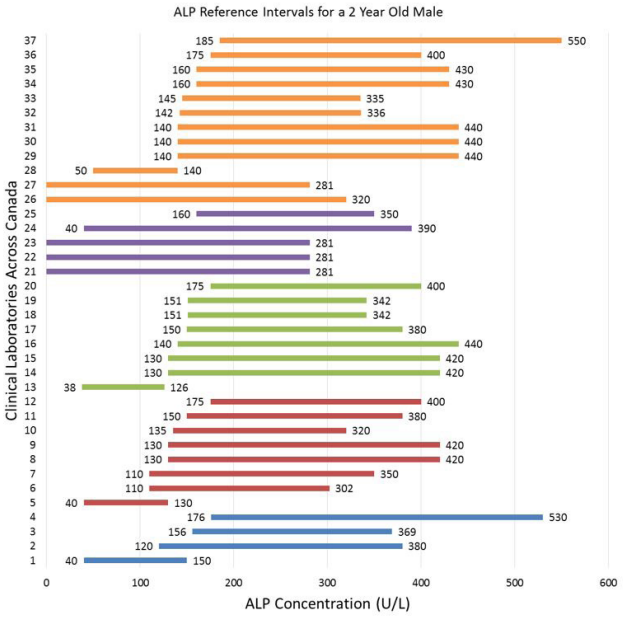
Background: Reference intervals are health-associated benchmarks used to interpret laboratory test results in clinical practice. There is evidence suggesting that there is wide variation in reference intervals, even between laboratories using the same analytical equipment and the same reagents. This poses a major problem for test result interpretation and patient safety, contributing to the lack of consistency in test result interpretation and decreased transferability of test results between labs. Here, we present data from a recent national survey conducted by the Canadian Society of Clinical Chemistry (CSCC) Reference Interval Harmonization (hRI) Working Group that examines the variation in pediatric and adult reference intervals, as well as a reference sample measurement in Canadian laboratories.

Methods: Reference intervals currently used by 37 Canadian laboratories were collected through a national survey for seven biochemical markers. Additionally, 40 clinical laboratories measured six analytes in a reference sample as a baseline assessment. The CVs and percent biases for reported reference intervals and test sample measurements were calculated and compared.

Results: Reference intervals for alanine aminotransferase, alkaline phosphatase (**Fig. 1**), and creatinine were most variable. As expected, reference interval variation was more substantial in the pediatric population and varied between laboratories using instruments/reagents from the same manufacturer. Test sample results differed between laboratories, particularly for alanine aminotransferase and free thyroxine. Reference interval variation was greater than test result variation for most analytes and was often not related to the observed bias of the assay.

Conclusion: The survey data highlights the critical lack of standardization in laboratory reference intervals across Canada, particularly for the pediatric population. The CSCC hRI Working Group aims to address this critical issue by establishing and recommending harmonized reference intervals across Canada, based on evidence-based reference data.



A-260

Assessment of Clinical Significance of Differences Between K2 and K3 EDTA Tubes for Routine Hematological Analyses and Glycated Hemoglobin Measurements

C. Züngün, D. I. Topcu, M. S. Gungoren. *Duzen Laboratories Group, ANKARA, Turkey*

Background: K_2 and K_3EDTA are both most frequently preferred additives for hematological analysis and glycated hemoglobin (A1c) measurements. The aim of this study is to compare the routine hematology test results obtained by the K_2 and K_3EDTA tubes and to investigate the analytical significance of differences between two additives for four hours stability. A1c results were also compared between K_2- and K_3EDTA tubes. **Methods:** Blood samples of 50 patients for hematological analyses and of 47 patients for A1c analyses were collected into both BD Vacutainer K_2 and K_3EDTA containing tubes considering the sufficient blood volume. For routine hematological analyses K_2 and K_3EDTA tubes were analyzed at 0th minute and four hours after the initial analysis by using Sysmex XN-1000. White blood cell, red blood cell, platelet counts and hematocrit, mean corpuscular volume and mean corpuscular hemoglobin indices obtained from both tubes which are related to cell shrinkage or dilution effect were compared to ascertain the differences between K_2 and K_3EDTA. The samples were evaluated for 6 complete blood count parameters. For short term stability testing, results of four hours preserved samples were compared against initial results. A1c analyses were performed within one-hour after sampling by Roche Cobas c501. The calculated differences were compared with total allowable error values based on biological variation to assess clinical significance. Statistical analyses were performed with R 3.3.2 (R Working Group, Vienna, Austria). **Results:** At 0th minute, the percentage of differences between K_2 and K_3EDTA tubes were WBC:0.02%, RBC:1.85%, PLT:4.39%, Hct:4.10%, MCV:2.24% and MCH:0.17%. The percentage of differences between K2 and K3-EDTA tubes at 4th hour were WBC :0.75%, RBC:0.71%, PLT:0.66%, Hct:2.96%, MCV:2.30% and MCH:0.19%. The results of four hour stability studies for K2-EDTA WBC:0.24%, RBC:1.17%, PLT:4.63%, Hct:0.04%, MCV:1.26% and MCH:0.13% and for K3-EDTA were WBC (0.97%), RBC (0.02%), PLT (0.93%), Hct (1.18%), MCV (1.19%) and MCH (0.22%). For all of 6 parameters the percentage of differences between K_2 and K_3EDTA tubes at both 0th minute and 4 hours were found below the total allowable error limits. Mean of HbA1c results of K2-EDTA samples was 38.32±10.16 mmol/mol, whereas mean of HbA1c results of K3-EDTA samples was 38.47±10.55 mmol/mol. HbA1c results of K_2 and K_3EDTA samples were positively correlated (r=0.99). The mean difference between K2 and K3 samples was calculated as 0.4%. Analytical coefficients of variation (CV's) for A1c measurements were calculated with measurement results of manufacturer's internal quality control samples. **Conclusions:** Our results demonstrated that BD Vacutainer K_2 and K_3EDTA tubes performed equivalently for routine hemataological parameters. Both tubes are found convenient to be used interchangeably for routine hematological analysis with Sysmex XN-1000. For the stability test, all of the results were found comparable in the aspect of clinical signficance of differences when compared against initial analysis. As mean difference between K_2 and K_3EDTA samples was much lower than analytical CV's, it can be concluded that there is no significant difference between BD Vacutainer K_2 and K_3EDTA in A1c results for analysis with Roche Cobas c501.

A-261

Correction of Lactate Dehydrogenase and Potassium Values of Hemolyzed Specimens

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Background: Test cancellation on hemolyzed samples is routinely performed to avoid reporting falsely elevated lactate dehydrogenase (LDH) and potassium (K) concentrations. However, this common practice is associated with an increase in turnaround time and a delay of patient treatment. This study aims to use mathematic models to predict the true values of these two analytes in hemolyzed specimens in order to reduce associated test cancellations.

Methods: Non-hemolyzed patient plasma containing LDH (126 U/L -1792 U/L) or K (2.6 mmol/L-5.9 mmol/L) was spiked with free Hb (0-666 mg/dL) prepared from fresh blood, with a corresponding hemolysis index ranging from 0 to 4 as determined by the Abbott Architect analyzer. Multivariate regression analysis based on concurrent measurement of LDH or K and Hb was used to establish their correction equations. Prediction validation was performed using 100 paired hemolyzed and non-hemolyzed patient samples to assess their predictive performance. Either the predicted

or measured value was used to determine the sample rejection by comparing the difference between the paired samples to the total allowable error as defined by CLIA.

Results: Average increase in LDH and potassium were 1.332(±0.052) U/L and 0.0031(±0.0002) mmol/L per mg/dL spiked Hb, respectively. Correction equations for the true LDH or K value (Fig. 1A, B) were established via multivariate regression, with R^2 of 0.993 and 0.996, respectively. The predicted LDH and K values of hemolyzed sample corresponded well with their non-hemolyzed counterparts (Fig. 1C, D). Moreover, the correction significantly reduced the difference in LDH or K values between the paired samples (P<.0.01), and consequently led to 83% and 78% reduction of sample rejection for LDH and K, respectively (Fig. 1E, F).

Conclusion: Our data demonstrate that mathematic corrections of LDH and K values might be a beneficial alternative to report reliable values of hemolyzed specimens and reduce unnecessary test cancellations.

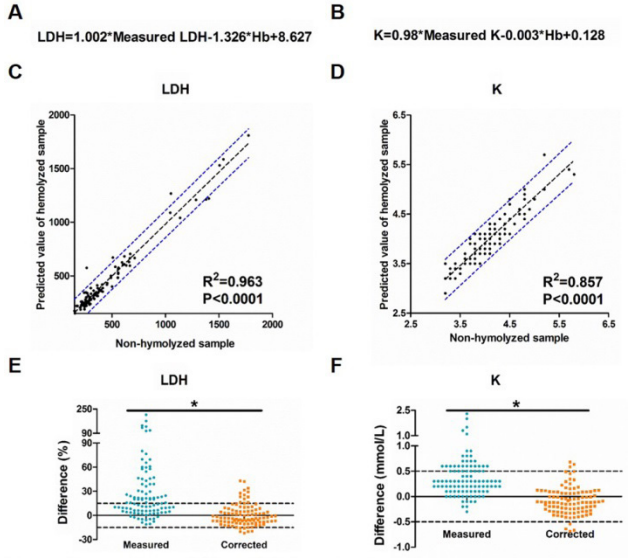

Figure 1. Correction of LDH and K in hemolyzed specimen. Correction equation for LDH (A) and K (B). Linear correlation between predicted values for for LDH (C) and K (D) of hemolyzed samples and its non-Hemolyzed counterparts. Dash lines indicate 95% prediction interval. Difference in LDH (E) and K (F) between the paired samples. Dash lines indicate total allowable errors.

A-262

Effect of non-fasting and non-morning samples on results of serum TSH and fT4 tests

C. Zungun, M. S. Gungoren, D. I. Topcu. *Duzen Laboratories Group, Ankara, Turkey*

Background: Sample collection requirements known to date for thyroid function tests are sampling in the morning and after an overnight (8-12 hours) fasting. However, laboratories encounter inappropriate demands from some patients who cannot meet these requirements from time to time. Although morning sample after an overnight fasting has been accepted as an acknowledged information, the number of studies found in scientific literature is limited with controversial findings. With this study, it has been aimed to compare results of fasting morning samples with non-fasting and non-morning samples for serum TSH and fT4 tests.**Methods:** This study was conducted with left-over samples of 50 individuals who was admitted to Duzen Laboratories Group for serum fasting and postprandial (120th min.) glucose tests. Samples for fasting glucose test were drawn in the morning (08.00-10.00 a.m.) whereas samples for postprandial glucose tests were drawn after 12.00 a.m. Individuals being at the age of 18 to 70, who were not on medications and had no chronic systemic disease were included. Samples were scanned from Laboratory Information System every day at around 16.00 and any eligible sample was analysed at the same day with Roche Cobas e601. Descriptive statistics and paired sample t-test were performed with R 3.3.2 (R Working Group, Vienna, Austria). **Results:** 100 samples from 50 patients were analysed. Average interval between fasting and postprandial samples were found to be 192±73 minutes. Means of fasting and non-fasting TSH results were 2.41±1.75 and 1.87±1.14 µIU/mL, respectively. There was significant difference between fasting and non-fasting samples (p<0,001). Means of fasting and non-fasting fT4 results were 16.56±2.84 and 16.51±3.28, respectively. There was no statistically significant difference between fasting and non-fasting samples (p=0.73).**Conclusion:** These results prove that non-fasting and non-morning samples cause variations in TSH levels. On the other hand, changes in conditions do not effect fT4 levels.

A-263

Between-generation Hemoglobin A_{1c} discrepancies for the Roche Tina-quant assay

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Background: The N-terminal glycated hemoglobin A (HbA_{1c}) is an important biomarker for diabetes mellitus. During an in-house verification process, two generations of the Roche HbA_{1c} assays exhibited significant discrepancies at lower values.

Objective: The study was aimed to compare the performance characteristics of the two generations of HbA_{1c} assay and investigate the significant differences of HbA_{1c}% measurement by the two assays observed in patient samples containing low concentrations of HbA_{1c}.

Method: The HbA_{1c}% in the assay is calculated based on separate measurements of total Hb and HbA_{1C}, by absorption spectrophotometry and turbidimetric immunoassay respectively. The 3rd generations of assays were both evaluated based on analytical measuring range, precision, sensitivity, and method comparison. In addition, samples displaying significant discrepancies between the two generations of assays were further evaluated by comparison to an ion-exchange chromatography method traceable to the DCCT reference method and certified NGSP samples.

Results: Overall, the analytical characteristics of the new generation (3rd) HbA_{1c} test were comparable with the existing older generation (2nd) assay. In a patient comparison study (n=55) performed between the two generations, the Passing-Bablok regression analysis for HbA1c% showed a slope of 1.14 and an intercept of -1.12%. The average bias between the two methods were -0.21%. More detailed analysis was performed on samples with HbA_{1c}% values closer to the prediabetes and diabetes diagnostic cutoff values of 5.7% and 6.5%, based on the American Diabetes Association (ADA) guidelines. In those samples (n=33), we observed a more significant difference between the two methods. Specifically, the Passing-Bablok regression analysis showed a slope of 1.29, an intercept of -2.00%, and an overall mean bias of -0.34%. Of which, 6 patient samples showed differences greater than -0.5%. Samples with biggest discrepancies were measured using a method traceable to the DCCT reference method and the reported values were between the results obtained from the 2nd and 3rd generation assay. This indicated a negative bias for the 2nd generation assay, and a positive bias for the 3rd generation. Because HbA_{1c}% is reported based on the measured HbA_{1c} and total Hb concentration, we also compared the measured HbA_{1c} and total Hb results obtained from both methods. It was identified that the significant discrepancies between the two methods were observed in samples with low amounts of HbA_{1c} (<0.45 g/dL, near the LOQ of the assays). For the certified NGSP materials, the two methods reported comparative results across the measuring range (4.7% - 22.4%). Nevertheless, NGSP samples with lower HbA_{1c} (<0.45 g/dL) were reported with more significant discrepancies (0.8%) between the two methods.

Conclusion: In patient samples with low HbA_{1c} (<0.45 g/dL), significant systematic bias (1.0%) in HbA_{1c}% were observed between two generations of assays. As 5.7% and 6.5% are utilized as the diagnostic cutoff values for prediabetes and diabetes according ADA guidelines, such biases between the two methods can lead to different clinical interpretations for diagnosis and management of diabetes when a laboratory switches from one generation to another generation of assay.

A-264

Midodrine, an Antihypotensive Medication, May Produce Falsely Elevated Free Plasma Metanephrine Levels due to Analytical Interference

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Background: Free plasma metanephrines (Met) and normetanephrines (Normet) are biomarkers that aid in the diagnosis of catecholamine-secreting tumors. If left untreated, these tumors may cause severe to life-threatening repercussions. Our laboratory provides Met and Normet testing on a liquid chromatography tandem mass spectrometry (LC-MS/MS) platform. The sample preparation involves the addition of deuterated internal standards to the plasma specimen followed by weak cation exchange solid phase extraction. Recently, upon routine chromatography review a small shoulder was noticed on a Met peak in a patient specimen. Furthermore, there

was a slight retention difference from the internal standard (0.03 minutes), which is atypical for this assay. The patient's medication list was reviewed and one possible interferent was found, midodrine, an antihypotensive agent. This medication is a prodrug that biotransforms via amide hydrolysis to desglymidodrine. This active metabolite is an isobar of Met with a similar structure. Herein, is the investigation of desglymidodrine interference with the Met assay. **Methods**: An EDTA plasma pool was spiked with 25 ng/mL desglymidodrine and serially diluted to 0.78 ng/mL with blank EDTA plasma. Along with the blank each serial dilution was extracted and analyzed in triplicates. Next, desglymidodrine and a Met standard were separately infused into the mass spectrometer to obtain fragment ion spectra. A unique fragment for desglymidodrine was identified and the transition added to the panel. Samples from additional patients, midodrine-presribed and otherwise, were then analyzed. **Results**: The desglymidodrine spiking study demonstrated a linear rise (R^2=0.972) in Met results with increasing desglymidodrine levels. The blank plasma pool (0 ng/mL desglymidodrine) was found to have an average endogenous Met value of 14 pg/mL, but the highest spiked desglymidodrine level (25 ng/mL) averaged a Met level of 358 pg/mL. During the fragmentation studies, a 180/117 transition for desglymidodrine was found to be unique with respect to Met. No unique transitions were identified for Met with respect to desglymidodrine. The desglymidodrine transition was added to the Met assay, and monitored in midodrine-prescribed patients and midodrine-free patients. The midrodrine-prescibed patients demonstrated peaks, near the Met internal standard, in the desglymidodrine (180/117) and Met (180/148) chromatograms. For the midodrine-free patients, the desglymidodrine chromatograms did not have peaks near the internal standard. Our routine chromatogram review, for the met assay, was amended to include monitoring the unique desglymidodrine transition. If similar peaks are present in both the desglymidodrine and Met chromatograms, and they co-elute with the Met internal standard, prompt communication with the ordering physician is warranted. **Conclusions**: Patients prescribed midodrine may have falsely-elevated free plasma Met results in LC-MS/MS assays. This is due to an active metabolite, desglymidodrine, sharing the same transition as Met with a similar retention time. The same interference was not found in our laboratory's urinary Met assay. Laboratories should be aware of this potential analytical interference to avoid reporting erroneously-high Met levels.

A-265

Comparison of automated immunoassays across different platforms to evaluate combinability for clinical trials

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Background

In global clinical trials, pharmaceutical sponsors rely on central laboratory systems to deliver harmonized results regardless of testing location. This is typically achieved by deploying the same instrument platforms across central laboratory sites; however, scenarios arise where different platforms are utilized across a laboratory network. Here, we performed correlation studies to investigate the combinability of immunoassays performed on different analyzers.

Methods

A minimum of 30 human serum or plasma samples spanning the analytical measurement range were tested in parallel over a three-day period for beta-hCG, testosterone, follicle stimulating hormone (FSH), thyroid stimulating hormone (TSH), total and free thyroxine (TT4 and FT4), total triiodothyronine (TT3), prostate-specific antigen (PSA), B-type natriuretic peptide (BNP), folate, ferritin, C-peptide, insulin, immunoglobulin E (IgE), troponin I (TnI), and estradiol at the Indianapolis, IN and Cranford, NJ central laboratory locations on Siemens (Malvern, PA), Beckman Coulter (Brea, CA), and Roche (Indianapolis, IN) analyzers. Estradiol, TnI, and IgE served as positive controls for like platforms (Siemens Centaur). All assays were calibrated twice over the three day period. Quality control samples were assayed on each day prior to testing. Data reduction was performed using the Alternate Method Comparison Module on Data Innovations EP Evaluator® Version 9.4.0 software (South Burlington, VT). Methods were considered combinable if they met standard operating procedure acceptance thresholds for slope, y-intercept, and correlation coefficient calculated from a Deming linear regression.

Results

Correlation outcomes are listed in the table below. Positive control assays (IgE, TnI, and estradiol) tested on the Centaur CP or XP platforms demonstrated acceptable statistical correlation.

Assay	Cranford Method	Indianapolis Method	Correlation Outcome	Average Bias (%)
beta-hCG	Centaur CP	cobas e601	Fail	-21
Testosterone	Centaur CP	Access DXI	Fail	-14
FSH	Centaur CP	Access DXI	Pass	+1
TSH	Centaur CP	Access DXI	Pass	-1
TT4	Centaur CP	Access DXI	Pass	+6
FT4	Centaur CP	Access DXI	Fail	-6
TT3	Centaur CP	Access DXI	Fail	-22
PSA	Centaur CP	Access DXI	Fail	+11
BNP	Centaur CP	Access DXI	Fail	+16
Folate	Centaur CP	Access DXI	Fail	+10
Ferritin	Centaur CP	Access DXI	Fail	-29
Insulin	Immulite 2000	Access DXI	Fail	+55
C-peptide	Immulite 2000	Centaur XP	Pass	-7

Conclusions

This study highlights the gap in standardization across automated immunoassays offered by different vendors. The lack of correlation between some vendors could not be attributed to experimental design, as assays evaluated on two generations of the Siemens Centaur platform demonstrated acceptable statistical correlation.

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Hematuria Without Microalbuminuria

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Background: Microalbuminuria is a condition in which a small amount of albumin is excreted in the urine in the absence of overt nephropathy. It is an early marker of kidney injury in diabetes, hypertension, and glomerular disease. Microalbuminuria is defined as persistent albumin excretion between 30-300 mg/day, 20–200 mcg/min, or 30-300 mcg/mg creatinine. Laboratory analysis of microalbuminuria is clinically utilized to detect early kidney injury, when treatment is more effective in slowing down the progression of the disease. Since urine dipstick and protein precipitation methods lack sensitivity, immunoassays are used for the measurement of trace amount of albumin in urine. It is commonly believed that in the presence of hematuria, testing for microalbuminuria is not valid. Based on this belief and manufacturers' recommendations, many laboratories cancel microalbumin testing if the urine dipstick is positive for blood. During routine clinical testing, we have encountered several urine samples that were dipstick positive for blood but tested negative for microalbumin when the clinician requested the testing be completed. **Methods:** Although this does not mimic true patient samples, to study this aspect more objectively, several urine samples with a typical creatinine concentration were spiked with 5, 10, 20 and 50 uL of whole blood (total volume 10 mL). Using a dipstick, these aliquots were tested for blood and protein. One of these samples is described here. **Results:** All of the aliquots except the 5 uL aliquot were visually positive for blood. All aliquots were dipstick positive for blood (3+), and aliquots with 10, 20 and 50 uL were positive for proteins (trace, trace and 1+, respectively.) After centrifugation, these aliquots were tested for microalbumin. All of the aliquots were negative for microalbuminuria (< 30 mcg/mg creatinine) except the 50 uL aliquot. **Conclusion:** These findings suggest that samples with hematuria may be negative for microalbumin, and therefore, laboratories should not automatically cancel microalbumin testing if a sample is dipstick positive for blood. A better strategy may be to test the sample for protein using a dipstick and cancel the test for microalbumin only if the sample is positive for greater than trace protein. Further studies using non-spiked patient samples are underway.

A-267

Assessment of Body Fluid Testing Requests and Validation of Methods on the VITROS® 5600

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Background: Chemistry testing of body fluids can be useful in clinical and post mortem investigations. However, these sample types are often not validated by assay manufacturers. The objective of this study is to catalogue fluid testing requests

received by the laboratory at BC Children's and Women's Hospital (BCCW) to guide in-house validation studies.

Methods: *Part A*: Fluid testing practices at BCCW were evaluated by retrospective review of test orders and results in the laboratory information system (LIS) spanning a 21-month period (January 2015 through September 2016). The data query was limited to fluid-specific test codes as well as miscellaneous test requests (requests without dedicated LIS codes) and included sample (fluid) type and test result.

Part B: The most frequently encountered body fluids (excluding CSF) and analytes were identified as targets for initial matrix validation studies. Residual clinical samples were saved from discard and stored at -30ºC for up to 8 months for use in linearity and accuracy experiments on an Ortho-Clinical Diagnostic VITROS 5600. Due to limited sample volume, linearity studies used an admixture design. Linearity in one clinically relevant fluid type was assessed per analyte (as determined in Part A). To expand the linear range tested, for some analytes a high concentration fluid was created by spiking with urine, plasma or dextrose solution, or by mixing two different fluid types. Accuracy was assessed by spike-recovery using calibrator materials. The volume of spiking material was limited to 10% of total sample volume and percentage recovery was calculated relative to the expected concentration. Multiple samples of 2-4 fluid types were included in recovery studies for each analyte. Recovery from fluids was also compared to recovery from plasma specimens. Data analysis was performed using Excel (2010).

Results: A total of 4525 body fluid tests were reported over the query period. CSF and vitreous fluids accounted for 59.4% and 17.1% tests, respectively. Drain, abdominal, pleural, stomal and dialysate fluids accounted for the majority of remaining orders. Most frequently requested analytes (defined as >10 orders in 21 months) included: glucose, sodium, creatinine, urea, potassium, protein, chloride, triglyceride, lactate dehydrogenase (LDH), phosphate, albumin, amylase, bilirubin, cholesterol and lactate.

Linearity studies were performed for amylase, lipase, chloride, albumin, cholesterol, triglyceride, sodium, potassium, LDH, protein, urea, creatinine and glucose. Good linearity over the range spanned by available samples was seen for most analytes (R^2>0.995), minor non-linearity was seen for sodium, potassium and LDH (R^2>0.98), although the latter experiments consisted of admixtures of different fluid types. Maximum deviation from a linear regression line was < 7% for all analytes.

The majority of spiking experiments showed recovery within 10% of the expected concentration. Larger deviations were seen in some samples for triglyceride, LDH, lipase and creatinine (up to 30%).

Conclusions: A significant number of body fluid test requests are received by the laboratory at BCCW. Linearity and accuracy studies suggest acceptable performance of many assays in multiple fluid types. Further evaluation of factors affecting electrolytes, LDH, triglyceride and creatinine measurement in select fluids is warranted.

A-268

Stability of Glucose and Lactate in Samples with and without Glycolytic Inhibitors: Fluoride and Citrate

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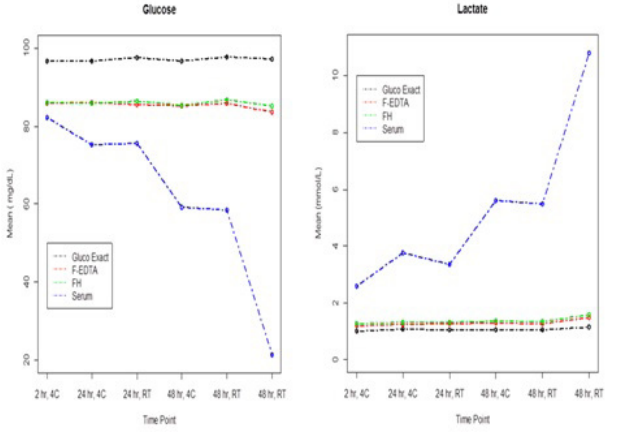
Background: Glycolysis decreases Glucose and increases Lactate concentrations in blood collected at room temperature (RT) in the absence of stabilizers. Although cold temperatures (4°C) inhibit glycolysis, processing blood samples at this temperature is difficult to achieve in routine clinical practice. This study evaluated the effect of different tube types and storage temperatures on Glucose and Lactate measurements. **Methods:** Fasting blood samples were collected from 34 healthy volunteers into 3 sets each comprising of Serum, Fluoride/Heparin (FH), Fluoride/EDTA (FE), Fluoride/EDTA/Citrate (GlucoEXACT) Sarstedt S-Monovette tubes. Set 1: Samples stored at 2-8°C throughout the study period and measured at 2, 24 and 48 hours post collection. Set 2: Samples stored at 2-8°C before centrifugation but after centrifugation stored at RT for the rest of the study period and measured at 24 and 48 hours. Set 3: Samples stored at RT throughout the study period, centrifuged and measured 48 hours post collection. All Glucose and Lactate measurements were performed on Abbott ARCHITECT c8000 systems using Glucose (LN 3L82) and Lactic Acid (LN 9P18) reagents. **Results:**Mean measured Glucose concentration is ~12% higher and Lactate is ~20% lower in GlucoEXACT tubes when compared to FH tubes at 2 hours (Figure 1). Samples in tubes with glycolytic inhibitors demonstrated comparable recovery for up to 48 hours compared to the corresponding tube type at 2 hour. In contrast, samples stored without glycolytic inhibitors showed ~74% lower and ~315% higher recovery at 48 hours after collection for Glucose and Lactate, respectively. **Conclusion:** The study demonstrates that in routine clinical practice, compared to Fluoride additive tubes, use of Citrate additive tubes may result in overestimation of Glucose and underestimation of Lactate. Use of Citrate additive tubes may cause higher number of Glucose determinations above the decision limit for diabetes. This may necessitate decision limits and reference intervals to be redefined for effective patient management.



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Lipemic Interference of Ceruloplasmin Assays - An Evaluation of Lipid Removal Methods

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Background: Ceruloplasmin (CERU) is a ferroxidase and a major copper-containing protein in the circulation. CERU is typically measured on automated chemistry and/or immunoanalyzers using nephelometric or turbidimetric methods. Interference due to lipemia with some CERU assays has previously been described. It is unknown, however, whether CERU assays from different manufactures have different susceptibility to lipemic interference, or if methods to remove interferent (e.g. dilution, ultracentrifugation, or lipemia-clearing reagent) are effective when applied to specimens across different platforms. The present studies were conducted to characterize lipemic interference of CERU assays and to evaluate procedures designed to address lipemia while minimizing analytical interference with lipemic and non-lipemic patient specimens.

Methods: Residual human serum specimens were obtained from frozen storage (-20°C) and de-identified according to an IRB-approved protocol. Three FDA-cleared CERU assays were evaluated on instruments from their corresponding manufacturers: ARCHITECT *ci*8200 (Abbott; Abbott Park, IL); AU5800 (Beckman Coulter; Brea, CA); and Cobas Integra 400 Plus (Roche Diagnostics; Indianapolis, IN). Lipemic index was measured on each instrument concurrently with CERU. Precision studies (low and high; 2x/day in duplicate over 5 days) were conducted to assess assay imprecision. As diluents referenced in package inserts differed by assay, a diluent evaluation study comparing distilled water (dH_2O), 0.85% saline, and 9% saline was conducted to evaluate linearity and % recovery of CERU with serial dilutions of human AB sera (Mediatech, Inc., Manassa, VA) enriched with human CERU (SigmaAldrich, St. Louis, MO). Lipemic interference was evaluated by performing serial dilutions of human sera enriched with human CERU after spiking with triglyceride-rich lipoproteins (Sun Diagnostics, New Gloucester, ME). Methods for minimizing lipemic interference - 1:5 dilution, ultracentrifugation (AirFuge; Beckman Coulter), and LipoClear (StatSpin; Norwood, MA) - were then evaluated using human serum specimens with varying degrees of lipemia.

Results: CERU assays across platforms demonstrated acceptable imprecision (%CV; low, high): *ci*8200, 1.2%, 1.1%; AU5800, 3.3%, 1.5%; Integra 400 Plus, 6.1%, 2.0%. Diluent evaluation showed linearity and acceptable % recovery (85-115%) for most diluent/analyzer combinations; although a trend toward slight over-recovery (+17%) was observed on the *ci*8200 at greater than 1:4 dilution for each diluent. The CERU assay on the *ci*8200 showed greater susceptibility to interference by spiked triglyceride-rich lipoproteins than the AU5800 or Integra 400 Plus. Ultracentrifugation was more effective than LipoClear or dilution at removing lipemic interference without impacting baseline CERU results observed in non-lipemic specimens.

Conclusions: The present study provides comparative data for three methods of addressing lipemic interference in three different CERU assays. These findings demonstrate that the ultracentrifugation method of lipemia elimination was the least likely to interfere with underlying CERU concentration in specimens regardless of lipemia.

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Building consensus in the Wild West: The Alberta reference interval harmonization project

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Background: Reference intervals (RI) are fundamental laboratory decision-making tool necessary for healthcare providers to appropriately interpret patient test results. RI should ideally represent the range of values that are present in a healthy population of a corresponding laboratory. Results that fall outside of the RI may be interpreted as abnormal and indicate the need for additional medical follow-up. Unfortunately, many labs depend on outdated, inappropriately determined or incomplete RI when reporting test results. To address this issue, there is growing interest in harmonizing RI across large geographic areas. Clinical laboratories in the province of Alberta include high volume community laboratories, rapid response laboratories located in secondary and tertiary care urban hospitals, and rural hospital laboratories. Many patients visit more than one laboratory during their clinical follow-up for testing of the same analyte, providing opportunity for result interpretation confusion and error. As such, standardizing RI within and/or across analytical platforms will reduce medical errors and provide improved patient care. The objective of this project is to develop harmonized references intervals, including appropriate age and sex partitions, for twelve frequently ordered clinical chemistry tests in the province of Alberta, Canada.

Methods: A posteriori indirect sampling approach was used to develop harmonized RI for glucose, creatinine, sodium, potassium, chloride, CO2, total bilirubin, calcium, total protein, albumin, alkaline phosphatase, and phosphorous. For each analyte, a survey was initially used to determine the current RI, sex and age partitions, as well as the analytical testing platform employed in every clinical laboratory in Alberta. Subsequently, de-identified patient results were obtained from the lab information systems. Due to the high volumes of tests performed in the province (>50 million/year), adult RI were developed solely from test values measured in community patients. For pediatric partitions, additional results from hospitalized patients or out-patients were required. Bhattacharya analysis was used to determine the statistically appropriate RI. Bhattacharya analysis requires large number datasets (N>3000) and excludes outliers by sampling the central distribution. This data was then used to help build clinically relevant RI by assessing clinical significance and through group consensus.

Results: Platforms measuring the twelve analytes are from Roche Diagnostics, Beckman Coulter, Ortho Clinical Diagnostics, and Siemens. The survey of current RI reinforced the need for our study as several out of date/incomplete RI were identified. After appropriate partitioning of the data according to age, sex and patient location, Bhattacharya analysis was performed using data sets of N=3,400-25,000 results, depending on the analyte. Consensus is being reached for all analytes; implementation is ongoing following final clinical laboratory approval and laboratory information system resource allocation.

Conclusion: Large data sets, a posteriori indirect sampling, Bhattacharya statistical analysis, and clinical judgment have enabled frequently ordered chemistry tests to be harmonized across multiple laboratories and analytical platforms. This not only benefits the small rural hospitals that often have minimal clinical support, but will also provide significant improvement to the care of patients within Alberta.

A-271

Evaluation of monoclonal peaks migrating in beta zone for the analysis of transferrin isoforms

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Background: The congenital disorders of glycosylation were originally called carbohydrate-deficient glycoprotein syndromes, affecting primarily N-glycans. In CDG, serum glycoproteins have altered glycosylation. Transferrin contains 2 complex type N-glycans with terminal sialic acid residues. Therefore the current screening test for CDG based on analysis of serum transferrin isoforms. Transferrin isoforms can be identified by using isoelectric focusing electrophoresis (IEF), high performance liquid chromatography (HPLC) and capillary electrophoresis (CE). In this study we analysed the effect of beta migrating monoclonal components on the analysis of Tf isoforms. Our aim was to compare HPLC and two different CE methods' performances in presence of monoclonal band interferences

Methods: Total 27 serum samples with monoclonal gammopathy migrating in beta zone were evaluated according to their transferrin isoform pattern. HPLC system was performed on a gradient HPLC (Shimadzu Europe, Germany), using column and reagents provided in kit (EUREKA-CDT test in serum by UV/VIS-FAST). First CE system analyses were carried out with Sebia 2 Capillarys™ (Capillarys™, Sebia, France) and second CE system analyses were carried out with Helena Biosciences' V8® E-class analyser (Helena Biosciences Europe, UK). The study was approved by the Hacettepe University Clinical Research Ethics Committee

Results: Transferrin isoform patterns were compared with this systems for the monoclonal band interferences. We observed that CE systems were effected by interferences, but HPLC system were minimally effected (Table)

		1.CE System		2. CE System		HPLC System	
		Interference					
	Total	+	-	+	-	+	-
IgG-K	3	-	3	1	2	1	2
IgG-L	3	-	3	3	-	2	1
IgA-K	11	8	3	7	2*	-	11
IgA-L	7	6	1	5	1**	1	5
IgM-K	2	2	-	2	1	1	2
IgD-L	1	1	-	1	-	-	1
Total	27	17	10	19	3	5	22

* 2 and ** 1 samples cannot be analysed due to insufficient serum

Conclusion: In this study we evaluated serum samples with monoclonal gammopathy migrating in beta zone and showed significant interferences on the CE systems. We demonstrated that analysis with HPLC system were more reliable for CDG diagnosis in terms of monoclonal gammopathy migrating in beta zone.

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Testosterone Content in Hyaluronidase Powder: Evaluation of Commercially-Available Sources for the Pretreatment of Viscous Body Fluid Specimens

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Background: Hyaluronidase (HYAL) is frequently used for the treatment of viscous body fluid specimens prior to chemical and cellular analysis. By catalyzing the hydrolysis of hyaluronic acid, addition of HYAL helps to liquefy viscous specimens and facilitate accurate aspiration by automated analyzers. Most commercially-available HYAL used for this purpose is sourced from mammalian testes, although potential contamination by testes-derived hormones in commercial HYAL has not been investigated. The present study was therefore designed to characterize the presence and/or relative quantity of testosterone (T) in commercially available HYAL with the goal of identifying a source that is relatively free of T while still remaining effective in reducing body fluid viscosity.

Methods: Five HYAL powders were evaluated (3 bovine, 2 ovine): HYAL-1) bovine type I-S (Sigma Aldrich; St. Louis, MO); HYAL-2) bovine (VWR / MP Biomedicals; Santa Ana, CA); HYAL-3) ovine type V (Sigma Aldrich); HYAL-4) ovine (VWR / MP Biomedicals); and HYAL-5) ovine type II (Sigma Aldrich). Each HYAL source was reconstituted in Universal Diluent (Roche Diagnostics; Indianapolis, IN) to yield a 20 mg/mL solution. T was measured using liquid chromatography-tandem mass spectrometry (LC-MS/MS; in-house assay). HYAL with low T content was then used for serum and body fluid spiking studies to exclude potential assay interference. These experiments used human AB sera (Mediatech; Manassa, VA) and residual, clinical body fluids (biliary/hepatic, cerebrospinal, drain, pancreatic, peritoneal, pericardial, pleural, synovial, and vitreous) that were de-identified according to an IRB-approved protocol. Assays investigated included amylase, blood urea nitrogen, cancer antigen 19-9, carcinoembryonic antigen, chloride, creatinine, glucose, lactate dehydrogenase, lipase, potassium, rheumatoid factor, sodium, and uric acid on Roche cobas 8000 instrumentation. Interference studies for body fluids were limited to assays available on our test-menu for the particular fluid type. HYAL with low T content was further

investigated for its ability to liquefy gels (0.5-2% in distilled H_2O) prepared from hyaluronic acid powder (Alfa Aesar; Ward Hill, MA) and viscous human body fluid specimens.

Results: T amounts in the corresponding commercial powders were as follows (ng T per mg HYAL powder): HYAL-1) 0.4272 ± 0.0108; HYAL-2) 0.3565 ± 0.0076; HYAL-3) 0.0004 ± 0.0001; HYAL-4) 0.0590 ± 0.0016; HYAL-5) 0.0018 ± 0.0002. HYAL-3 was therefore used in spiking studies of human serum. All serum assays investigated showed % differences within acceptable limits after HYAL-3 treatment (acceptability threshold ±15%). Interference was also not observed using HYAL-3 for assay/body fluid combinations evaluated. HYAL-3 was able to effectively liquefy hyaluronic acid gels and body fluid specimens with similar performance to our previous reagent (HYAL-1).

Conclusions: The present study identified a commercial HYAL source that was free from T contamination and without significant interference for the laboratory assays investigated.

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Evaluation of a New HbA1c Analyzer Mindray H50 against Bio-Rad Variant II Turbo2.0 and Interference Assessment of HbE and HbF

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Background: Hemoglobin A1c (HbA1c) is integral for monitoring long-term glycemic control i, and to diagnostic diabetes. It is imperative that methods used to measure HbA1c meet established performance goals and readily differentiate

hemoglobin variants that may affect HbA1c quantitation. This study was aimed to evaluate the analytical performance of a new IE-HPLC analyser to measure HbA1c (Mindray H50) . and evaluate Mindray H50 in comparison to boronate affinity HPLC method (Primus Ultra2) in patients with normal and abnormal Hb(HbE and HbF)

Methods: All studies were evaluated using CLSI guidelines. The precision, accuracy, linearity and interference were evaluated according to CLSI protocols EP5-A2, EP9-A3, EP6-A and EP7-A2 respectively. Measurements of HbA1c by the three methods were made in blood from 141 patients with normal Hb(HbA)and 41 patients with abnormal Hb(Hb E, n=30, 11 HbF,n=11).Primus Ultra2 was used as comparative system. Comparative analysis and bias evaluation were conducted on the results from two detection systems, Appropriateness of data for linear regression analysis was checked regards CLSI EP9-A3 document, then performed both linear regression and difference plot analyses.

Results: The within-run imprecision values(CV%) were less than 0.6% and the total imprecision values(CV%) were less than 1.3%**.** Bias using reference samples from NGSP ranged from -1.68 to 2.11%. The linearity of was excellent in the range between 3.9% and 16.9%.Comparison of against Primus Ultra2 demonstrated significant correlation (r = 0.994; slope= 1.030; intercept =0.017). The differences of the 95% confidence interval (95%CI) between the test systems and the comparative system in normal HbA samples and HbE samples,were within ±0.70% HbA1c, bias% were less than 6%, (P >0.05).The results showed that the Mindray H50

was not affected by CM,Hb,F-Bil,C-Bil,Acetal,HbE and HbF(<7.1%).

Conclusion: The Mindray H50 shows excellent analytical performance,demonstrated high analytical performance similar to previous systems such as Primus Ultra2 and Bio-Rad Variant II turbo2.0widely used HPLC systems. were not affected by HbE and HbF(<7.1%). and is therefore suitable for its utilization in modern clinical laboratories.

A-274

Elimination of False Positive ELISA Signals in RF Positive Patient Specimens

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Background: Heterophilic antibody interference remains a prevalent and persistent source of potential error in immunoassays. Spuriously high signals may lead to further diagnostic tests or procedures that are unnecessary, costly, and potentially detrimental to the patient. Rheumatoid factor (RF) is a well known interfering substance present in the majority of rheumatoid arthritis (RA) patients. This study was designed to identify false positive signals in commercial ELISA test kits observed with RF-positive serum and plasma specimens. Two biomarkers, Human Cardiac Troponin I (cTnI) and Human Mucin 16 (CA125), were selected for their clinical importance. Human Cardiac Troponin I is a critical biomarker for assessing myocardial disease, and Human Mucin 16 is the most widely used biomarker for detection of ovarian cancer.

Methods: Ten RF-positive serum specimens (4 male, age 35-88 and 6 female, age 29-75, RF titer 20-160 IU/mL) and nine RF-positive plasma specimens (9 female, age 43-76, RF titer 40-900 IU/mL) were tested in commercial ELISA kits, Human Cardiac Troponin I and Human Mucin 16, per the manufacturer's protocol. Kit assay diluent was also prepared according to the manufacturer's instructions. HeteroBlock®, a commercially available blocking reagent, was added directly to the assay diluent without any additional steps such as filtering or heating. Patient specimens were diluted per the manufacturer's recommendations (2- to 2.5-fold) just prior to testing with and without HeteroBlock present in the assay diluent.

Results: For the Human Cardiac Troponin I ELISA test kit, elevated signals were observed for seven of the ten RF-positive serum specimens; the seven elevated signals were eliminated in the specimens prepared with assay diluent containing HeteroBlock. Nine of the nine RF-positive plasma specimens produced elevated signals; the nine elevated signals were eliminated in the specimens prepared with assay diluent containing HeteroBlock.

For the Human Mucin 16 ELISA test kit, elevated signals were observed for six of the ten RF-positive serum specimens; specifically, four of the six specimens produced a result greater than the clinically significant level of 35 U/mL. None of the ten serum specimens prepared with assay diluent containing HeteroBlock generated a signal above the limit of detection. All nine RF-positive plasma specimens gave elevated CA125 results; seven of those nine results were greater than the clinically significant level of 35 U/mL. The elevated signals were reduced below the clinically significant level of 35 U/mL when the plasma specimens were prepared with assay diluent containing HeteroBlock.

Conclusion: This study reinforces the need for vigilance regarding the potential for false positive results caused by heterophilic antibody interference. In this study, the addition of HeteroBlock to the assay diluents for Human Cardiac Troponin I and Human Mucin 16 commercial ELISA kits demonstrated a simple and effective means of blocking heterophilic antibody interference.

A-275

A Central Laboratory Interlaboratory Comparison Program to Assess the Comparability of Data of Five Chemistry Tests from Four Regional Laboratories Involved in Global Clinical Trials over a Six Month Period

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Objective: The objective of this study was to develop and support a process for assessing the comparability of data used in global clinical trials from four individual laboratories, wholly owned by the same central laboratory entity, and that the same five chemistry tests on the same samples are under statistical control and acceptable limits of variation.

Relevance: Data from central laboratories have been key for assessing safety, tolerability, and efficacy of new drugs in clinical trials. With the increasing complexity and global scale of many clinical trials, it is important to maintain harmonization among the regional laboratories as part of a central laboratory participating in the same study. This is usually accomplished by maintaining standardized operating procedures (SOPs) and identical assay platforms, calibrators, and quality control material across all laboratories. However, continuous monitoring of same samples run at all laboratory locations may not be common practice.

Methodology: The interlaboratory comparison program was established in 2006 and involves locations in the US, Europe, Singapore, and China. The program involves multiple platforms to assess over 40 analytes tested globally, encompassing several therapeutic areas including Oncology, Cardiometabolic, Infectious disease and others. Pooled serum/plasma/urine samples were aliquoted, frozen at -70ºC, and distributed quarterly to each laboratory for analysis. Here we present the dataset for five chemistry tests using the Beckman Coulter AU series chemistry analyzers. Samples were analyzed weekly on the same day at each laboratory for six months (N= 260). College of American Pathologist evaluation limits, Westgard database, and Royal College of Pathologist of Australasia were sources used to establish bias criteria. The percent bias was calculated for each result using the US laboratory as the reference laboratory. The percent bias for each week and month was also calculated. A Bland-Altman plot was created between each laboratory and the reference laboratory for the six month period, and a student T-test run using a relative bias limit for each test and a significance level of 5%.

Results: Overall, during the six month period all five chemistry tests had a mean bias within the acceptable bias limit for the individual test compared to the reference laboratory. When comparing the mean weekly bias for the five chemistry tests from all laboratories, any week where the bias was outside acceptable limits, investigation and

corrective action was warranted. One week during the six month period two chemistry tests from the same laboratory had a mean weekly bias outside the acceptable limit. After investigation it was determined that a pre-analytical issue with thawing/mixing of frozen samples was the cause of the bias.

Conclusion: An interlaboratory program where frequent monitoring of identical samples run at all laboratories involved in clinical trials can provide valuable information into the harmonization of data reported by the central laboratory, and help mitigate pre-analytic, analytic, and post-analytic issues that may arise when assessing data used in the development of new therapeutics.

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Stability of 5-Hydroxyindole-3-acetic Acid and Vanillylmandelic Acid in Urine Specimens

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Background: Measurement of 5-hydroxyindole-3-acetic acid (5HIAA) and vanillylmandelic acid (VMA) in urine is useful for diagnosis and treatment of neuroblastic and carcinoid tumors. However, there is insufficient information in the literature regarding the stability of these two analytes at typical clinical lab storage conditions. The aim of this study was to determine short and long term stability of 5HIAA and VMA in native and pH-adjusted specimens at storage conditions of room temperature (RT), 4°C, and -20°C. **Method:** Leftover patient urine specimens with clinical orders for either 5HIAA or/and VMA were selected based on their original testing results. There were three groups for 5HIAA: 3 patient samples in the low range (<2.0 ug/mL), 5 in mid-range (2.0-8.0 ug/mL), and 2 in high range (> 8.0 ug/mL). There were two groups for VMA: 4 patient samples in the low range (<2.0 ug/mL) and 6 in mid-range (2.0-8.0 ug/mL). Half of each patient specimen was acidified with 12 mol/L HCl to pH 2-3. The native urine (n=10) and the acidified urine samples (n=10) were aliquoted and stored in triplicate at RT, 4°C, and -20°C for the time periods (0, 7, 14, 28, 90 and 180 days). The aliquots were analyzed by an established HPLC-MS/MS method when the specific time point was reached. All the results at different time points of different storage conditions were compared with the results of original native urine specimen at time 0. The analyte was considered stable for the specific time point at the specific storage condition if the triplicate mean was within 80% of the original result at time 0. **Results:** 5HIAA was not stable for 7 days at RT in either acidified or native urine. At RT, VMA was stable for up to 7 days in both acidified and native urine. 5HIAA was not stable at 4°C for 14 days in either acidified or native urine. VMA was stable at 4°C for up to 28 days in both acidified and native urine samples. Both 5HIAA and VMA were stable at -20°C for 90 days except acidified specimens for 5HIAA, which was not stable even for 14 days. **Conclusion:** 5HIAA stability was not conclusive for storage at RT or 4°C. 5HIAA was stable at -20°C in native urine for 90 days, while was not stable in the acidified urine. VMA was found stable for 7 days, 28 days, and 180 days at RT, 4°C, and -20°C, respectively in either acidified or native urine samples. Acidification of urine samples was shown to have little impact on VMA stability, while caused decreased stability for 5HIAA.

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The new BD Barricor™ tube does not introduce interferences in the measurement of testosterone by liquid chromatography tandem mass spectrometry.

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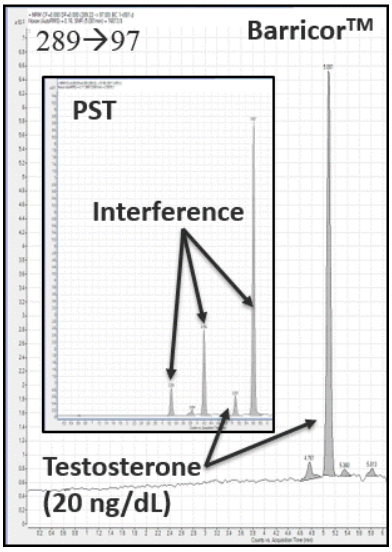
The objective of our study was to see if the new Barricor™ tube (BC) could be used for analysis of testosterone by liquid chromatography tandem mass spectrometry (LCMS). Gel-based separator tubes introduce an isobaric substance that interferes with LCMS analysis of testosterone; this requires labs to extend their chromatography (limiting throughput) or draw an alternative tube (causing an additional blood draw). Since the BC uses an elastomer instead of a thixotropic polyester gel, it might offer a separator tube that yields samples amenable to testosterone analysis by LCMS. To investigate, samples were obtained in lithium heparin, plasma separator tubes (PST) and the BC. Consistent with literature reports, the PST showed intense, isobaric interferences which eluted near testosterone (289->97). These interferences dwarfed the true testosterone signal at lower levels, which is especially problematic as it is at these levels that analysis by LCMS is most important. In contrast, the BC and lithium heparin tube were free of this interference. Furthermore, relative to the lithium heparin tube the baseline noise in the BC was substantially reduced. These results show that the BC can be used for testosterone analysis by LCMS. This will allow labs to request and process a single tube when there are orders for automated chemistry testing and LCMS testing of testosterone.



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Blood collection practices in the Emergency Department: Association with sample hemolysis rate

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Background:

Hemolysis is the major reason for rendering samples unsuitable for biochemical analysis. Although hemolysed hemolyzed samples are received into the laboratory from all locations within the hospital, the majority originates at the emergency Emergency departmentDepartment (ED). This project iIn collaboration with Center for Disease Control (CDC) we examined blood collection practices in effort to evaluate the effectiveness of evidence based LMBP WG recommended practices to reduce hemolysis rate in the Emergency DepartmentED.

Methods:

During the first level of this projectInitially, we collected baseline data on current blood sample collection practices in use at a large teaching county hospital (Parkland Memorial Hospital) accompanied with the baseline hemolysis rate. PHemolysis preventing measurement of potassium levels wasis used to calculate hemolysis rates.as an indicator for both denominator (total number of samples) as well as the number of rejected samples due to hemolysis because of its exquisite sensitivity associated with hemolysis. For the practices, the bBaseline data were collected from 120 patients admitted to the emergency departmentED. using tTwo approaches were used,; direct observations (n = 59) of the practices in use to draw blood specimen and retrograderetrospective interview sessions (n = 61) with the sample collection staff forassociated with specimens identified hemolyzed specimens by the laboratory as hemolyzed. A Survey Monkey toolsurvey was developed with specific observation parameters was developed; the technique of sample collection (straight needle or existing intravenousI/V line), needle size, use of saline lock, use of syringe, and syringe size, and use of J-loop, and direct use of a vacutainer collection device. Collection practice variables as well as sample quality outcomes were analyzed.

Results:

The institutional Hhemolysis rate of was 6.7%. Collection practices were varied with many different options, 25 different combinations were observed. Among hemolyzed samples 79 % were collected using existing intravenous lines with 9 different combination of practices. Among the non-hemolysed hemolyzed collections, only 6% used the existing intravenous lines to collect blood specimens.

Conclusion:

There was no standardized blood collection practice in the emergency departmentED with various options for needle size, and collection techniques. Although there was no particular practice responsible for hemolysis, the use of intravenous lines was commonly associated with hemolysed hemolyzed samples.

As next stepsNext, we will be implementing recommended practices (straight needle) to draw blood as well as other identified practices identified to reduce the hemolysis rate.

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Validation of Lipemia, Icterus, and Hemolysis Interference for Common Chemistry Analytes using the Beckman AU 5800

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Background: Interfering substances can be a significant source of error in clinical laboratory measurements and may lead to inappropriate follow-up testing, incorrect diagnoses, or adversely affect treatment decisions. Hemolysis, icterus, and lipemia account for the most common interferences in clinical samples and are automatically assessed on most high-volume chemistry analyzers. Because interferences may be method or analyzer dependent, it is important to verify manufacturer interference claims when validating new instrumentation and developing interpretive comments for affected test results. The approach and level of detail provided to the consumer may vary by manufacturer. The objective of this study was to evaluate the presence, direction, and degree of interference from hemoglobin, bilirubin, Intralipid, and human lipids on 41 common chemistry assays on the Beckman AU 5800 chemistry analyzer.

Methods: Hemolysate, Unconjugated Bilirubin, and Triglyceride-rich lipoprotein interferents were purchased from Sun Diagnostics (New Gloucester, ME). Intralipid was acquired from the hospital pharmacy. Pooled patient samples were spiked with varying concentrations of the interferents corresponding with the LIH indices and run on the AU5800. Significant deviation from the original result was assessed for each analyte using CLIA limits (if available) or a difference of 10% as recommended in the AU5800 IFU assay-specific interference criteria.

Results: 22 of 41 analytes tested required appending of a comment due to interference exceeding the defined acceptable limit for that analyte. Interference thresholds were established for 5 analytes.

Conclusions: This study validated the degree of lipemia, icterus, and hemolysis interference outlined in the Beckman AU5800 IFUs for 41 chemistry analytes as well as established the directionality of the interference. Once defined, interpretive comments were developed to guide clinical decisions using affected test results.

LIPID	mg/dL		BILIRUBIN	mg/dL		HEMOLYSIS	mg/dL	
Ammonia	>40	Inc	Alk. Phos.	>20	Inc	Albumin	>300	Inc
AST	>300	Inc	Ammonia	>20	Inc	Ammonia	>50	Inc
T. Bilirubin	>300	Inc	Magnesium	>20	Inc	AST	>50	Inc
						BHB	>50	Inc
Prealbumin	>200	Dec	Lactate	>2.5	Dec	Potassium	>50	Inc
Ferritin	>300	Dec	Tot. Chol	>5.0	Dec	Iron	>50	Inc
ALT	>300	Dec	Lipase	>10	Dec	LDH	>50	Inc
Iron	>300	Dec	Creatinine	>20	Dec	Magnesium	>100	Inc
			Amylase	>20	Dec	Phosphorus	>300	Inc
						D. Bilirubin	>50	Dec
						Haptoglobin	>50	Dec
						Amylase	>200	Dec
						Alk. Phos.	>300	Dec
						GGT	>300	Dec

Table 1: Degree and direction of interference determined via spiking with Intralipid, Triglyceride-rich lipoproteins, Bilirubin, or Hemolysate for chemistry analytes using the Beckman AU 5800. (Inc= increase, Dec = decrease)

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Evaluation of Biotin Interference on Abbott ARCHITECT Assays

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Background and Relevance:

The use of biotin as a supplement has increased significantly in recent years and many health care professionals do not realize their patients are taking high doses. The increase has resulted in an increased prevalence of people being exposed to levels much higher than the recommended daily dose and as a consequence, inaccurate lab results for assays that utilize the free capture biotin-streptavidin methodology. The purpose of this study was to identify any Abbott assays that may be susceptible to biotin interference based on assay design and then evaluate the performance of these assays with high concentrations of biotin. After a comprehensive review of Abbott's current on market ARCHITECT clinical chemistry and immunoassay methods, no assays were identified that utilize the free capture biotin-streptavidin; however, 5 assays were identified for subsequent interference testing as they contain streptavidin or biotin in the assay design.

Materials and Methods: For each of 5 ARCHITECT assays: (Methotrexate; Active B12; Vitamin D; 2nd Generation Testosterone; Anti-CCP), sample pools were created and spiked with concentrations of biotin between 30 – 1000 ng/mL. For Active B12, a single sample in the normal range was utilized. For the other assays, two sample pools were used for testing with one negative/near the lower end of the assay or medical decision point and one positive/near the upper end of the assay or medical decision point. The biotin spiked samples were tested against a control sample preparation (no biotin) to determine if there was a statistical difference between the untreated and biotin containing specimens.

Results:

Table 1 ARCHITECT Assays

Assay	Concentration of untreated Sample pool	Specimen	% Difference From Untreated Sample Pools (95% Confidence)
Active B12 (LN 3P24)	87.91 U/mL	Normal	-3.51 to 3.70
Anti-CCP (LN 1P65)	0.66 U/mL	Low (Negative)	-3.59 to 7.40
	10.70 U/mL	High (Positive)	-0.15 to 3.80
Methotrexate (LN 2P49)	0.051 umol/L	Low	-3.80 to 3.71
	0.877 umol/L	High	0.36 to 6.15
Testosterone – 2nd Gen (LN 2P13)	6.92 nmol/L	Low	-4.59 to 0.52
	21.36 nmol/L	High	-4.84 to 1.15
Vitamin D LN 3L52	25.76 ng/mL	Low	-0.14 to 8.02
	35.16 ng/mL	High	-0.35 to 7.32

Conclusions: Five ARCHITECT assays potentially susceptible to biotin interference, based on assay design, were tested at increasingly high concentrations of biotin. No Abbott ARCHITECT assay evaluated as part of this study yielded result variability due to biotin interference at concentrations up to 1000ng/mL.