Tuesday, August 2, 2016

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

Factors Affecting Test Results

A-239

Nonlinearity of assay results within the presumed linear primary measurement range: examples from analysis of CAP linearity survey data

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

Background: We recently described nonlinearity within the primary measurement range (PRM, undiluted specimen measurement range) of a lipase assay as the cause of inaccuracy in lipase results among samples undergoing automated repeat-on-dilution [PMID: 26474511]. Nonlinearity of this assay was also evident in interlaboratory data reported in the CAP linearity survey by the changing slope (Δresult/Δdilution) across sample dilution series results. As a follow-up to these observations, our objective in this study was to examine CAP linearity survey results for other assays in use at our laboratory (Roche Cobas c500 assays) to determine whether nonlinearity in the primary measurement range could be a factor affecting overall accuracy of assay results. Methods: Primary data were from the CAP June 2015 survey, LN2/LN2VB-A, Chemistry/Lipid/Enzyme Calibration Verification/Linearity, for examination of interlaboratory results for serial sample dilution measurements. Candidate assays for evaluation were those displaying a characteristic pattern in the bias plot for samples within the PRM, in which there was a concentration-dependent, non-random bias of sample dilution series results forming a smooth, inverted "U" shape relative to the central, zero-bias line (the line based on the assumption of linearity of results). Dilution series results data (y) within the PRM were then evaluated according to curve fitting of the form $y = F(x) = A(1\text{-}\exp(\text{-}x/x0))$, where x was the dilution factor, and A and $x0$ were fitted constants. Such curves $F(x)$ show a progressive variation in slope; assays were considered to be nonlinear if $F(x)$ was a better fit to y than that of linear correlation, and if the slopes ($F'(x)$) between limits of the PRM differed by more than 15%. Results: Three Roche Cobas c500 assays (albumin, creatinine, lactate dehydrogenase) were found to be nonlinear by the above criteria. Changes in slope across the PRM were -21% (albumin), -41% (creatinine), and -18% (lactate dehydrogenase). Revised bias plots based on $F(x)$ eliminated the inverted "U" pattern and its associated residuals. Despite curvature, however, linear correlation coefficients for dilution series data within the PRM were all high ($r > 0.99$), and all biases of data within the PRM were well within CAP-defined acceptable limits when linearity was assumed. However, in the CAP survey, each assay showed a disjunction in bias of results when comparing those for samples from within the PRM to those for samples from outside of the PRM (viz., from samples secondarily measured by automated repeat-on-dilution). Conclusions: Certain assays, for which calibrations are based on a linearization of the PRM response vs. concentration curve, exhibit instead functional non-linear characteristics within the PRM. The assumption of linearity leads to an inherent, predictable pattern in bias plots. Bias could be eliminated were calibration based instead on a nonlinear curve. Biases in results for these assays due to the assumption of linearity within the PRM are unlikely to be clinically significant, however. Nonetheless, as a technical note, laboratory directors should be aware that nonlinearity within the PRM for these assays can cause a disjunction of sample dilution series results across the upper limit of the PRM, as observed in CAP linearity surveys.

A-240

Effect of Intravenous Immunoglobulin on Hepatitis B Serology Testing

R. Hawkins. *Tan Tock Seng Hospital, Singapore, Singapore*

Background: Intravenous immunoglobulin (Ig) is used in a variety of conditions, including immunodeficiency, ITP and Kawasaki disease. With a half-life of 40 days, its in vitro effect on serology tests may be overlooked, leading to false positive results and misdiagnosis. This study examined the effect of 3 different pharmacological concentrations of Intragam P (Singapore) on anti-HBe, anti-HBs, HBeAg, HBsAg, anti-HBc-IgM and anti-HBc-total assay results on 2 different immunoassay systems (Roche e601 and Abbott Architect). **Methods:** Three different concentrations of Intragam P (stock concentration 60 g/L) were prepared in saline to simulate the range of 0.6-2 g/kg used in clinical practice, and IgG were measured (Beckman Coulter DxC-800). Aliquots were then analysed for the following assays: anti-HBe, anti-HBs, anti-HBc-total (Roche e601 and Abbott Architect); HBeAg, HBsAg, anti-HBc-IgM (Roche e601 only). **Results:** The final samples had IgG concentrations of 7.7 g/L (sample A), 17g/L (B) and 26 g/L (C). The serology results were: HBeAg, HBsAg, anti-HBc-IgM all non-reactive (A,B,C); anti-HBc-total non reactive with (A) but reactive with (B,C) with both Roche and Abbott assays; anti-HBe non-reactive with both (A,B) but reactive with (C) with both Roche and Abbott assays. The anti-HBs (IU/L) concentrations with the Roche and Abbott assays were: (A) 71.51, 36.28, (B) 318.1, 210 and (C) 551.4, 369.6 respectively. **Conclusions:** Pharmacological concentrations of Intragam P can give false positive results for anti-HBc-total, anti-HBe and anti-HBs with the Roche e601 and Abbott Architect assays. HBeAg, HBsAg and anti-HBc-IgM are unaffected by the presence of Intragam P. Clinicians should ideally delay hepatitis serology testing 5-6 months following Intragam P administration to avoid false positive results.

A-241

Smartphones Can Monitor Medical Center Pneumatic Tube System Parameters

G. R. Mullins, J. H. Harrison, D. E. Bruns. *University of Virginia, Charlottesville, VA*

Background:

The pneumatic tube system (PTS) has become a common means of patient sample transportation in medical centers. Although convenient and efficient, excessive acceleration force and time/distance traveled in the PTS have been correlated with increased sample hemolysis. As a result, regular monitoring and adjustment of pneumatic tube forces has been recommended to ensure sample integrity, but we have not found a fast and cost-effective way to do so. The purpose of this study was to assess the utility of smartphones to monitor acceleration and transport times in a hospital PTS.

Methods:

Two smartphones (iPhones) were sent through the PTS from two different hospital locations. Each smartphone used two apps as data-loggers; each app recorded force of acceleration vs time. To relate the data to sample integrity, blood was collected into Li-heparin-containing tubes from 5 volunteers in triplicate. One sample was transported by hand, and the others were transported through the two PTS routes. After transport, the hemolysis (H) index and plasma lactate dehydrogenase (LD) were measured in all samples (Abbott Architect). We then used a smartphone to illuminate a filled sample tube in a carrier and a second to make an audiovisual recording of the heparinized blood sample in the tube during transport through the PTS.

Results:

The smartphones showed a significant difference in duration of forceful acceleration during transport through the two PTS routes. Smartphones sent through both routes experienced acceleration forces exceeding 8 g (78 m/s^2), but route 1 generated forces in transit for roughly 150 seconds, versus 250 seconds for route 2. These data were consistent between the two smartphones and between the two apps used. The increased duration in Route 2 correlated with significant increases in the H index and LD. The mean H indexes were 13 (SD 10) and 36 (SD 18) and for route 1 and route 2 samples, respectively ($p<0.05$), and 4 (SD 3) for hand-delivered samples. Plasma LD was higher by a mean of 26% in samples from route 2 compared to route 1 or hand-delivered samples ($p<0.005$). Consistent with these data the video demonstrated extreme turbulence resulting in a foamy appearance of the blood sample, with large and small air pockets, during transit through the PTS.

Conclusion:

Our data demonstrate that smartphones can be used to quickly and economically monitor PTS parameters that affect integrity of patient samples. This method could be used to regularly evaluate PTSs to estimate the risk of sample hemolysis, particularly in new or altered routes or those servicing patient populations at higher risk of sample hemolysis.

A-242

The Preparation and Validation of Cystatin C Calibrators Traceable to ERM-DA471/IFCC

J. Gong, Y. Li, Q. Gao. *Beijing Strong Biotechnologies. Inc, Beijing, China*

BACKGROUND

Cystatin C has been an important biomarker to access kidney function for a few years. However, many calibrators in China market lacked the traceability document until recently. Here we produced recombinant cystatin C in E.coli, and the recombinant cystatin C was prepared and validated as calibrators in Gcell cystatin C assays, which were traceable to ERM-DA471.

METHODS

Gene cloning and protein purification: Recombinant human cystatin C was produced by expression in *Escherichia.coli.* and purified from cell extract.

Calibrators preparation: The recombinant cystatin C was diluted to approximate 8mg/L, 4mg/L, 2mg/L, 1mg/L ,0.5 mg/L and 0mg/L using 0.1mol/L KCl, 0.1% BSA, pH 7.0.

Value assignment: The values and uncertainty of the calibrators were assigned according to (1).

Commutability: the commutability of the calibrators was assessed by comparing the results of both Dako cystatin C assay kit and BSBE cystatin C assay kit to a set of 40 human serum samples.

RESULTS

DNA sequencing and N-terminal sequencing: The cystatin C gene sequence was validated by DNA sequencing and the N-terminal of the recombinant protein was identified as natural cystatin C. SDS-PAG showed that the molecular weight of the recombinant cystatin C is about 13kDa and that no other protein contaminants were observed.

Table 1. Value assignment and uncertainty of all levels calibrators

Commutability: The commutability of the six levels of calibrators was demonstrated by applying both the Dako cystatin C assay kits and the Gcell cystatin C assays kits to to a set of 40 serum samples, as there was no significant difference between those two assays.

CONCLUSIONS

We produced the recombinant cystatin C with amino acids sequences identical to natural cystatin C. This recombinant protein can be used as raw material for calibrators in clinical cystatin C assays, which could be traceable to international reference material ERM-DA471/IFCC.

REFERENCE

(1) S. Blirup-Jensen, Clin. Chem. Lab. Med. 46(2008)1470-9

Value assignment and uncertainty of all levels calibrators			
Levels	**Related Standard Uncertainty (%)**	**Extended Uncertainty (K=2)**	**Value (mg/L)**
level 1	490.3	-0.02	0.00±0.02
level 2	2.02	0.02	0.50±0.02
level 3	2.24	0.05	0.99±0.05
level 4	1.24	0.05	2.01±0.05
level 5	1.01	0.08	4.01±0.05
level 6	1.01	0.16	8.03±0.27

A-243

Assessment of Heterophilic Antibody Interference in the ADVIA Chemistry Hemoglobin A1c_3 Assay

P. Datta, S. Patel, J. Dai. *Siemens Healthcare Diagnostics, Newark, DE*

Background: The measurement of HbA1c, the glucose adduct of HbA1, in whole blood is used to monitor long-term care in diabetic patients. The ADVIA® Chemistry Hemoglobin A1c_3 assay, available on ADVIA Clinical Chemistry Systems (1200/1800/2400/XPT) from Siemens Healthcare, automatically pretreats a whole-blood sample and then measures HbA1c in the lysate immunoturbidimetrically. The assay also measures total hemoglobin (Hb) concentration in the same lysate and expresses the result as HbA1c% (or in HbA1c mmol/mol of Hb). Heterophilic antibodies (for example, human anti-mouse antibody [HAMA] and rheumatoid factors [RF]) are endogenous antibodies that could potentially interfere in the immunoreaction, resulting in erroneous results. Since the immunoassay component of this assay uses monoclonal murine antibodies in the reagent, it is important to know if HAMA or RF interferes with the assay.

Methods: The ADVIA Chemistry Hemoglobin A1c_3 assay pretreats a whole-blood specimen with a denaturant reagent that contains surfactants (to lyse the red blood cells) and porcine pepsin (to fragment resultant proteins). The lysate is then reacted with Reagent 1, which contains HbA1c haptens attached to a polymer as an agglutinator and a specific endopeptidase to cleave the HbA1c moiety containing five amino acid residues from the rest of the peptide. After the R1 reaction, Reagent 2, containing murine monoclonal anti-HbA1c antibody coupled to latex particles, is added. The resulting turbidity is measured at 694 nm. Sample HbA1c concentration is inversely proportional to the observed turbidity. To assess the interference of anti-mouse antibodies, we spiked serum with different levels of goat anti-mouse antibody, a quantifiable model for HAMA, and assayed the samples with and without denaturant treatment for the HbA1c portion of the Hemoglobin A1c_3 assay. In addition, we assayed 5 serum samples containing HAMA, 10 serum samples containing RF, and 5 normal serum samples with no RF present using the ADVIA Chemistry Hemoglobin A1c_3 assay. All samples were obtained from a commercial vendor.

Results: When goat anti-mouse antibodies were spiked in human serum and analyzed with the Hemoglobin A1c_3 assay following a denaturant pretreatment as specified in the assay instructions, the Hemoglobin A1c_3 results were undetectable as expected. However, if the samples were analyzed without the denaturant pretreatment, the Hemoglobin A1c_3 results (HbA1c) increased from 0-1.10 µmol/L as the goat anti-mouse antibody concentration increased from 0-750 µg/mL.

Conclusion: The data obtained in this study show that HAMA or RF (heterophilic interference) was not observed in the samples tested with the ADVIA Chemistry Hemoglobin A1c_3 assay. *(ADVIA and all associated marks are trademarks of Siemens Healthcare Diagnostics Inc. or its affiliates).

A-244

Updated Ammonia Handling and Storage Requirements Improve Specimen Rejection Rates and Reduce Risk to Patient Safety

M. Kozelkova, N. Arnini, D. Montezuma Calvo, B. Kezlarian, L. Favazza, J. Zajechowski, C. Feldkamp, B. Cook. *Henry Ford Health System, Detroit, MI*

Background: Ammonia (NH_3) is routinely measured in plasma to assess liver function. NH_3 is considered a labile analyte and samples require special handling. Mishandling of the sample or delay in its reaching the laboratory can result in inaccurate NH_3 concentrations. Some studies report that plasma NH_3 is stable for up to four hours when stored at 4 °C (refrigerated) and up to four days when stored at -20 °C (frozen). The current analytical procedure at Henry Ford Hospital (HFH) states that plasma NH_3 is stable for three hours from sample collection if stored refrigerated. Between April 2014 and April 2015, 87% of plasma NH_3 specimens obtained from nursing homes did not meet the laboratory handling and storage requirements. **Objective:** The purpose of this study was to validate a longer stability limit for ammonia in plasma to improve specimen rejection rates and reduce risk to patient safety. **Methods:** To determine the ammonia stability limit, we used venous blood from patients with normal ("normal plasma") and compromised liver function ("abnormal plasma"). Blood was drawn into evacuated tubes with and without gel separator containing sodium heparin (NaHep), lithium heparin (LiHep), or ethylenediaminetetraacetic acid (EDTA) anticoagulants. For each sample type, blood was centrifuged immediately, plasma was removed from collection tube, aliquoted, and stored up to six hours refrigerated and up to 48 hours frozen. The first aliquot was tested immediately (t = 0) and the result obtained was used as a baseline. Subsequent aliquots were tested hourly. Ammonia concentrations were measured using a Beckman Coulter DxC 800 analyzer. **Results:** Linear regression analysis revealed no statistical difference between NH_3 concentrations in "normal plasma" collected into LiHep and EDTA tubes with gel separator. No clinically significant changes in NH_3 concentrations were observed after six hours of refrigerated and after 48 hours of the frozen specimen storage. Linear regression found a modestly increasing trend in HN_3 concentrations in frozen "normal plasma" collected into LiHep and NaHep tubes without gel separator over 48 hours in storage. More testing is required to investigate the cause. NH_3 in "abnormal plasma" (n=4) was also stable six hours refrigerated and 48 hours frozen. **Conclusion:** Special instructions for specimen handling and storage conditions for NH_3 in the HFH analytical procedure and the electronic laboratory user's guide (ELUG) were updated. According to the new procedure, plasma must be transported on ice and arrive at the laboratory within six hours of collection. Changes in the procedure were communicated to nursing homes and hospital staff. It was demonstrated, that by freezing plasma at the collection site, the specimen rejection criteria can be relaxed and the risk of obtaining inaccurate NH_3 concentrations is minimized. By using gel separator tubes, the need for manual separation of blood plasma from cells can be eliminated, which can significantly reduce time in the laboratory for specimen processing.

A-245

In vitro effect of two types of medical contrast media on routine chemistry results by three automated chemistry analyzers

J. Rim, Y. Park, J. Yim, S. Lee, J. Kim. *Severance Hospital, Yonsei University College of Medicine, Seoul, Korea, Republic of*

Background: Medical contrast has been elucidated as one of intefering analytical error sources which physicians face sometimes in daily practice. Various types of medical contrast media have been evaluated for their interference on laboratory results. Here we present the results of medical contrast media interference on the routine chemistry laboratory results performed by three most commonly used automated chemistry analyzers in Korea.

Methods: Ten levels of pooled serum were used in the study. Two types of medical contrast media [i.e. Iopamiro (iopamidol) and Omnipaque (iohexol)], which are infused most commonly in computed tomography and magnetic resonance imaging, were evaluated. To evaluate dose dependent effect of contrast media, Iopamiro and Omnipaque were spiked separately into aliquots of serum to make final concentrations of 1.8%, 3.6%, 5.5%, 7.3%, and 9.1%. To compensate the dilutional effects, negative controls with residual volume of distilled water were analyzed concomitantly. 28 analytes included in the routine chemistry panel were measured by Hitachi 7600 analyzer (Hitachi High-Technologies Corporation, Tokyo, Japan), AU5800 analyzer (Beckman Coulter Inc., CA, USA), and Cobas 6000/c501 analyzer (Roche Diagnostics, IN, USA). We calculated the percentage difference between the samples and the control, and examined dose-dependent trends to determine true interfering effects of contrast media.

Results: For the evaluation of dilutional recovery, the expected values and actual measurement of negative controls in each pair did not show a difference of more than 10%. Different levels of pooled serum specimens showed various trends by two kinds of medical contrast media in three analyzers. All percentage difference values in pair of contrasts and analyzers were less than 10% except serum iron in Hitachi 7600 analyzer and Cobas 6000/c501 analyzer.

Conclusion: Our study suggests that interference from organic iodine contrast media is minimal and does not affect overall routine chemistry lab results significantly except serum iron in specific analyzers. Based on these results, we can apply more flexible medical evaluation process for patients requiring both laboratory tests and imaging studies.

A-246

Pseudohypercreatinemia due to monoclonal IgM kappa

M. R. McGill, M. G. Scott. *Washington University School of Medicine, St. Louis, MO*

Background: Plasma creatinine (PCr) is an important marker of glomerular filtration. However, misleading results can occur due to interfering substances. Enzymatic creatinine methods have been developed to replace the traditional picric acid method and are thought to be more specific, but these too are subject to interference. It has been suggested in previous reports that paraproteins can cause falsely elevated PCr by enzymatic methods, but this has not been thoroughly investigated. We present the case of a patient with a monoclonal gammopathy of undetermined significance (MGUS) who was found to have elevated PCr during evaluation for lung transplantation. We sought to conclusively demonstrate interference in enzymatic creatinine measurement by the patient's paraprotein. **Methods:** The Roche Creatinine Plus enzymatic assay method and the Roche picric acid (Jaffe) method were used to measure PCr. Glomerular filtration rate was measured by iothalamate clearance. Cystatin C was measured at the Mayo Clinic. Plasma proteins were removed by size exclusion filtration with molecular weight cutoff >30 kD. Protein electrophoresis and immunofixation were performed using the Helena SPIFE ImmunoFix method. Serum immunoglobulins were isolated by 40% saturated ammonium sulfate precipitation followed by dialysis with 0.9% saline. Non-IgM immunoglobulins were removed from plasma by adsorption with Protein G sepharose beads. **Results:** Comparison of results from the Roche enzymatic assay (1.51 mg/dL) in our laboratory and from the Jaffe method (0.84 mg/dL) using the same patient sample revealed a discrepancy. All other measures of renal function, including glomerular filtration rate (74 mL/min/1.73m^2) and Cystatin C (0.82 mg/L), were normal, suggesting interference in the enzymatic method. Serum immunofixation revealed an IgM kappa (IgMk) paraprotein. To determine if this was responsible for the interference, we filtered the patient's serum to remove large proteins. Creatinine concentration in the filtrate was 0.7 mg/dL by the enzymatic method. We then isolated the immunoglobulin fraction of the patient's serum and spiked it into four control patient plasma samples with normal PCr values. The patient's Ig fraction increased PCr in these samples by 0.58-0.62 mg/dL. Furthermore, removal of non-IgM immunoglobulins from the index patient's plasma did not reduce the interference, indicating it was not due to an endogenous IgG. Finally, comparison of enzymatic and Jaffe method results in samples from other patients with a monoclonal IgMk revealed that not all patients with an IgM paraprotein have falsely elevated PCr. **Conclusion:** These data show that this patient's PCr was falsely elevated in the Roche enzymatic method due to her IgMk paraprotein. The clinical team proceeded with lung transplantation. Altogether, these data definitively show that some paraproteins can interfere in enzymatic creatinine measurement.

A-247

Interference of multiple myeloma-targeted monoclonal antibody therapeutics with immunofixation electrophoresis: an emerging challenge

A. B. Karger[1], D. Peterson[2], A. A. Killeen[1]. *[1]University of Minnesota, Minneapolis, MN, [2]University of Minnesota Medical Center, Minneapolis, MN*

Background: Since the first monoclonal antibody therapeutic was approved in the mid-1980s, there has been steady growth in this class of biopharmaceutical, with estimates projecting over 70 monoclonal antibody therapies in use by 2020. These drugs were first reported to interfere with serum protein electrophoresis tests in 2010, detectable as a monoclonal protein by both immunofixation and capillary electrophoresis. However none of the drugs at that time were specifically targeted treatments for multiple myeloma, and thus interference was not commonly encountered. Now, with the recent approval of two multiple myeloma-targeted monoclonal antibody therapies in November of 2015 (daratumumab and elotuzumab), laboratories should anticipate an increased rate of interference of these drugs with immunofixation and protein electrophoresis. Methods: Immunofixation was performed on the Sebia Hydrasis instrument, using reagents and materials from a Sebia Hydragel kit. Waste serum was obtained from a patient with a history of kappa light chain restricted multiple myeloma on daratumumab treatment, to determine the degree of potential interference with the Sebia immunofixation assay. Results: Immunofixation demonstrated bands in the IgG and kappa lanes, migrating at the cathodal end of the gamma region, as has been previously reported for daratumumab interference. This result confirms that a standard dosing regimen for daratumumab is sufficient to cause a clearly visible monoclonal protein by immunofixation electrophoresis.

Conclusion: Both daratumumab and elotuzumab are IgG-kappa monoclonal proteins. Therefore, in patients with IgG-kappa myeloma, co-migration of the therapeutic antibody with the patient's M-protein could lead to misdiagnosis of treatment-resistant or recurrent disease. In myeloma patients with non-IgG kappa M-proteins, this interference could lead to misdiagnosis of a new clone. With the recent FDA approval of two multiple myeloma-targeted monoclonal antibody therapeutics, laboratories need to develop proactive strategies to address the anticipated increase in interference with immunofixation and protein electrophoresis assays due to these drugs.

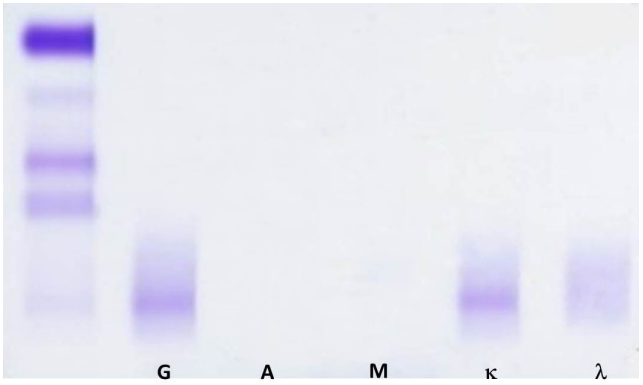


Figure 1: Detection of daratumumab by immunofixation electrophoresis in the serum of a multiple myeloma patient being treated with this IgG-kappa monoclonal antibody therapeutic.

A-248

Evaluation of Automating pH Verification Prior to Urine Chemistry Testing

W. E. Owen[1], L. K. Pace[2], R. W. Greer[2], J. R. Genzen[3]. *[1]ARUP Institute for Clinical and Experimental Pathology, Salt Lake City, UT, [2]ARUP Laboratories Inc., Salt Lake City, UT, [3]Department of Pathology, University of Utah Health Sciences Center, Salt Lake City, UT*

Objectives: Stability and/or solubility of urine analytes can be affected by hydrogen ion concentration (pH). As such, urine pH adjustment post-collection may be required when clinical testing cannot be conducted immediately. This is particularly important for specimens transported to regional or national reference laboratories. While assay-specific pH requirements and instructions are available from most laboratories, there has been little published work describing how frequently these requirements are followed. The workflow impact of pH screening (usually conducted with manual pH test strips upon specimen receipt in the laboratory) has not previously been described. The objectives of this study were to: 1) evaluate what percent of specimens were received outside pH recommendations for a select set of pH-sensitive analytes, 2) conduct a performance evaluation of an automated urine pH assay, and 3) assess the cost and workflow implications of fully automating pH assessment, including the use of IT middleware rules to automatically apply disclaimers when pH is outside of recommended limits.

Methods: Using IRB-approved protocols, de-identified retrospective data from clinical urine specimens was reviewed for 6 analytes: calcium (Ca^{2+}), citric acid (CIT), magnesium (Mg^{2+}), myoglobin (MYOG), inorganic phosphate (PHOS), and uric acid (UA). Performance characterization of an automated pH assay (SVT pH; Roche Diagnostics) on a cobas c502 chemistry analyzer was conducted, assessing imprecision, linearity, accuracy, and reference interval verification. Comparison studies were completed with a small volume pH electrode (InLab Micro / SevenEasy pH meter; Mettler-Toledo) as well as manual pH test strips (pH Test 0-14, BDH).

Results: The percent of clinical urine specimens received with pH outside of laboratory recommended limits was: Ca^{2+}, 13.7%; CIT, 3.8%; Mg^{2+}, 6.5%; MYOG, 43.6%; PHOS, 7.4%; and UA, 7.4%. The automated SVT pH assay demonstrated excellent precision with %CV's of 1.1% at pH 2.8, 0.8% at pH 6.3, and 0.6% at pH 11.4. The SVT pH assay was linear between pH 3 and 11 (slope 0.977). Non-linearity was observed below pH 3. Accuracy studies showed comparability (Deming regression, slope 0.837; r=0.98; bias 0.4%) to pH electrode measurements, although overall positive bias was observed below pH 2.5. Using pH electrode results as a target, both the SVT pH assay and pH test strips produced results within acceptable total allowable error (<20%). Previously published urine pH reference intervals were verified using the SVT pH assay. Workflow and financial analyses demonstrated that a switch to automated pH testing would save 538 hrs of manual work per year for these 6 orderables at approximately equivalent cost to manual pH test strip measurements. Creation of middleware rules to automatically apply pH disclaimers, however, would enable overall cost-savings with automated testing.

Conclusion: Automated pH testing has the potential to improve workflow efficiency in a reference laboratory setting. In combination with middleware rules, it can reduce overall cost of testing. At urine pH ranges relevant to clinical processes, automated testing demonstrated results comparable to pH test strips and pH electrode measurements. Additional educational efforts are needed to improve adherence to requirements for post-collection pH adjustment, particularly for MYOG and Ca^{2+}.

A-249

Commutability - Is it important and can patient samples be noncommutable? Use of CLSI EP14-A3

J. Budd, S. Dayal, S. Kuklok. *Beckman Coulter, Chaska, MN*

It is common practice for manufacturers to use patient samples as secondary reference standards within a calibrator traceability scheme or as trueness controls. It has been well established that the commutability of reference material received from external sources must be ensured before being used for such purposes*. However, little work has been published that shows whether internally collected/created patient samples do or do not demonstrate commutability. Often such samples need to be either diluted or spiked with analyte in order to achieve desired measurand concentrations. It is understandable that such modified samples may demonstrate non-commutability. However, interferences or other sample specific characteristics may make specimens drawn from certain subjects behave differently than specimens form other subjects.

A study was conducted over multiple immunoassays to determine if secondary reference material created from unpooled plasma and serum units were commutable. Given that most immunoassays do not have a reference measurement procedure (RMP) available, most comparisons between measurement procedures (MP) were between commercially available MPs. For those with an RMP the RMP was also used in the commutability determination. At least 20 additional neat patient specimens covering the measuring interval of each assay were included in the study to create the patient specimen distribution. The analysis techniques described in CLSI EP14-A3 were used to make the commutability determinations.

A number of different non-commutability behaviors were seen in these studies. All of these behaviors will be demonstrated vis the commutability plots recommended in EP14-A3. Some non-commutability was seen in diluted samples, but the question remains whether this was due to the difference in behavior between the two MPs used with respect to the specific diluent being used. Some relationships required a data transformations (per EP14-A3) in order to show a consistent variability across the measuring interval. Some relationships showed high variability in neat patient specimens that could be used to single out individual patient specimens that should be excluded from the secondary reference material panel. In summary, commutability analyses as recommended in EP14-A3 can be used to identify non-commutable patient specimens.

*Miller WG, Myers GL, Rej R, Why Commutability Matters, Clin Chem, 52:2006, p553-4.

A-250

Interferences on the results of the dipstick urinalysis by Vitamine C and fluorescein sodium

S. Chang[1], W. Lee[2], A. Lee[3], C. Jeon[3], Y. Kim[4]. *[1]Daegu Fatima Hospital, Daegu, Korea, Republic of, [2]Keimyung University School of Medicine, Daegu, Korea, Republic of, [3]College of Medicine, Catholic University of Daegu, Daegu, Korea, Republic of, [4]Yeungnam University College of Medicine, Daegu, Korea, Republic of*

Background : Dipstick urinalysis is a basic tool for testing of pH, glucose, protein, hemoglobin, leukocyte esterase and so on in urine. It is known that many materials in urine interfere the results of the dipstick urinalysis. Vitamine C is one of the important interfering materials which show false negative results and commonly used vitamine dietary supplement in Korea. Fluorescein sodium is the dye used to examine the circulation of the retina and excreted through the urine causing yellow-green appearance. This study is to analyze the interference effects on the results of the dipstick urinalysis by vitamine C and fluorescein sodium.

Methods : Study was done on the random urine specimens from 4 general hospitals in Daegu, Korea from July to November, 2015. Dipstick urinalysis was tested by Uriscan Pro III and Uriscan 11 strip(YD Diagnostics,Korea). Urine sediments were tested by the microscopic exam and Sysmex UF-1000i(Sysmex Co.,Japan).

Results : 1,110 of 5,006(18.1%) random urine specimens were positive for vitamine C. When we added vitamine C to the urine specimens for the final concentration of 12.5, 25, 50 100 and 200 mg/dl in vitro, the interferences by vitamine C were not seen on the protein and nitrite positive urine specimens but the result values of the glucose, hemoglobin and leukocyte esterase decreased as the concentration of vitamine C increased. In case of in vivo interferences by vitamine C, 74 of 175(42.3%) vitamine C positive urine specimens which have above 180 mg/dl blood glucose showed negative results on glucose of the dipstick urinalysis. 507 of 1,507(33.6%) and 503 of 1,507(33.4%) of vitamine C positive urine specimens showed more than 1 level lower results of hemoglobin and leukocyte esterase compared to results of the urine sediments. 118 of 164(72%) of vitamine positive urine specimens which have positive bacteria results of the urine sediment showed negative results on nitrite of the dipstick urinalysis. Fluorescein sodium showed false negative results on the hemoglobin and leukocyte esterase of the dipstick urinalysis.

Conclusion : Interferences by vitamine C were seen on more than 30% of the total results of the glucose, hemoglobin and leukocyte esterase. It will be helpful to measure vitamine C together on the same urine specimens for the accurate interpretation of the false negative results of the dipstick urinalysis by vitamine C. More studies are needed for a variety of the interference materials on the urinalysis results.

A-252

Carryover from HbEE Samples Increase the Percentage of HbA2 Quantified with the Bio-Rad Variant II Beta Thalassemia Short Program HPLC Method

B. Landin, L. Fredriksson, C. Sisowath. *Karolinska University Laboratory, Stockholm, Sweden*

Background: High-performance liquid chromatography (HPLC) is frequently used for screening and diagnosis of hemoglobinopathies. HPLC can be used for provisional identification of hemoglobin variants and quantification of both hemoglobin variants and normally occurring hemoglobin fractions, i.e. HbA2. A stable assessment of HbA2 is of great importance since HbA2 is a cornerstone in the diagnosis of beta thalassemia trait. The percentage of hemoglobin variants usually range from approx 15-90% of total hemoglobin but also minor fractions might be of clinical importance, like the delta globin variant HbA2' and low expression variants like Hb Constant Spring. Since we had occasionally seen indications of carryover from HbSS and HbCC samples in subsequent HPLC chromatograms and since HbA2 and HbE coelute in the HPLC method we use, we wanted to evaluate whether carryover from HbEE samples significantly affected the quantification of HbA2.

Methods: We use one Variant™ II instrument dedicated for the Beta Thalassemia Short Program from Bio-Rad for our ordinary work flow. Our HbA2 quantification method is accredited according to ISO15189. The instrument was subjected to annual service according to the manufacturer´s maintenance program. 4 different lots of reagents (columns and buffer) were used during the 2 year study period. During the time period 2014 - 2015 we regularly reanalyzed all samples injected immediately following a HbEE sample. Reanalysis was performed on the same day or the day after initial analysis.

Results: During the study period we analyzed approx 4 800 patient samples and 37 of these samples were initially injected following a sample from a patient homozygous for HbE (HbEE). In all cases, the HbA2 result obtained at reanalysis was lower than the first result. The difference ranged from 0.1 to 0.5% HbA2 (mean ± SD being 0.3 ± 0.11%). The difference between the mean of the first and second result for the same sample was highly significant ($p<0.0001$, Student´s paired *t*-test). All reruns were performed in random order, i.e. anywhere in the injection series. Control samples were analyzed both in the beginning and end of the series and also as every 10th sample. During the study period three different lots of control material were used at each level and imprecision ranged from 2.0-2.3% (CV% coefficient of variation) at 2.8% HbA2 level and from 1.1-1.5% at 5.7% HbA2 levels. There was no consistent pattern showing differences between results of control samples analyzed in different injection positions. The same carryover effect as was seen for patient samples was found for control samples injected immediately after HbEE samples. Samples from HbE heterozygotes (HbAE) normally contain only approx 20-25% of total hemoglobin as HbE. Also when HbA2 results in samples analyzed immediately following HbAE samples were evaluated a slight carryover effect could be noted.

Conclusion: Each HPLC method used for analyzing hemoglobin fractions should be evaluated to find out whether carryover might be a problem. For users of the Bio-Rad Variant II Beta Thalassemia Short Program samples injected immediately after samples from patients likely to be homozygous for HbE (HbEE) should be routinely reanalyzed to avoid falsely increased HbA2 results.

A-253

Effects of Hemoglobin(Hb)J-Bangkok, HbE, HbG-Taipei and HbH Traits on Measurements of Glycated Hb(HbA_1c) by IE-HPLC

X. Zhang, D. Wen, S. Xu, M. Suo. *Zhongshan Hospital Affiliated to Sun Yat-sen University, Zhongshan city,Guangdong Province, China*

Background: Glycated Hb(HbA_1c) is a marker of long-term glycemic control and diagnosis in patients with.diabetes. HbJ-Bangkok, HbE, HbG-Taipei and HbH are very common Hb variants in southern China.We investigated the accuracy of HbA_1c measurement in the presence of HbJ-Bangkok, HbE, HbG-Taipei and HbH traits.

Methods: Methodology comparison. Collected five patients whose IE-HPLC HbA1c measurement results were inconsistent with their average FBG, performed Hb electrophoresis analysis of whole blood samples using Hb capillary electrophoresis, sequenced Hb genes using dideoxy-mediated chain termination, and measured HbA1c with borate affinity HPLC and turbidimetric inhibition immunoassay (TINIA)

Results: Two patients were HbJ-Bangkok; their Hb genotypes and HbJ-Bangkok content were $\beta^{41-42}/\beta^{J\ Bangkok}$ and $\beta^{N}/\beta^{J\ Bangkok}$ and 93.9% and 52.4%, respectively. The remaining three patients were HbE (β^{N}/β^{E} Hb genotype, 23.6% HbE content), HbG -Taipei ($\beta^{N}/\beta^{G\ Taipei}$ Hb genotype, 39.4% HbG-Taipei content), and α-thalassemia HbH (6.1% HbH content, 2.8% Hb Bart's content). There was interference in both IE-HPLC and TINIA HbA1c determination in the patient with β-thalassemia with HbJ-Bangkok; in the remaining four patients, there was interference in IE-HPLC HbA1c determination but not in that of TINIA. For all five patients, there was no interference in AE-HPLC HbA1c determination.**Conclusion:** The HbJ-Bangkok, HbE, and HbG-Taipei variants and α-thalassemia HbH disease cause varying degrees of interference in IE-HPLC HbA1c detection. For such patients, we suggest using other methods free from such interference to detect HbA1c, or using other indicators to monitor blood glucose levels.

A-254

Evaluation of plasma ACTH stability using the Roche Elecsys Immunoassay

J. P. Theobald, A. Algeciras-Schimnich. *Mayo Clinic, Rochester, MN*

INTRODUCTION: Adrenocorticotropic hormone (ACTH) measurement is useful in the evaluation of hypothalamic-pituitary-adrenal disorders. ACTH is labile in blood, due to proteolytic degradation, and care should be taken during the preanalytical phase to prevent in vitro degradation. Our current collection instructions indicate to collect the sample in a chilled EDTA tube, keep tube on ice after draw, and spin down immediately in a refrigerated (4°C) centrifuge. Often, the laboratory receives questions regarding sample handling requirements (need for chilled tube, refrigerated centrifuge) as well as inquiries about delayed centrifugation and separation from cells. Our laboratory does not have data to support the various scenarios encountered at the collection site in order to determine the acceptability of the specimens collected outside the current protocol.

OBJECTIVE: To investigate time and temperature effects on plasma ACTH concentration.

METHODS: ACTH was measured using the Roche Elecsys immunoassay as per manufacturer's instructions. The current collection instructions indicated as "baseline" (sample collection in a chilled tube, keep tube on ice, and spin down immediately in a 4°C centrifuge) were compared to the following conditions: condition A: immediate centrifugation versus delayed centrifugation; condition B: chilled tube and centrifuge versus ambient tube and centrifuge; and condition C: specimen stability at ambient and refrigerated temperatures after centrifugation and sample aliquot. For each condition samples from 10 healthy volunteers were drawn between 8-10 am in K_2-EDTA tubes (Becton Dickinson). All samples, including the baseline sample, were stored at -80ºC prior to testing and then thawed and tested on the same day once the study was complete. For condition A, 3 scenarios were evaluated: current protocol, collection tubes in the refrigerator for 2 or 4 hours prior to centrifugation. For condition B, 2 scenarios were evaluated: current protocol and ambient temperature sample collection and centrifugation. For condition C, ACTH stability was analyzed at ambient and refrigerated temperature after 2, 4, 8, 12, 24 and 48 hours of collection.

RESULTS: For condition A, the average % differences from baseline ACTH concentrations were 3.4% (range -1.5-14.9%) and 2.1% (range -1.4-15.6%) when samples were kept refrigerated prior to centrifugation for 2 and 4 hours, respectively. For condition B, collection and processing of the samples at ambient temperature showed an average % difference from baseline of 1.6% (range -1.4-15.3%). For condition C, the average % difference from baseline was <10% at 12 hours ambient and 24 hours refrigerated.

CONCLUSIONS: ACTH stability is not affected if the samples are collected and centrifuged at ambient temperature. A 4 hour delay in sample centrifugation does not affect ACTH concentration. Once specimens are spun and aliquoted, the acceptable ACTH stability is up to 12 hours at ambient temperature and up to 24 hours refrigerated. These less stringent collection parameters would benefit laboratories that don't have access to refrigerated centrifuges for sample processing.

A-257

Validation of reference intervals for common biochemistry analytes in a multi-ethnic population

M. A. Hey, P. Heng, A. Omar, M. Wong, M. Wong. *Khoo Teck Puat Hospital, Singapore, Singapore*

BACKGROUND: Reference intervals are essential for interpretation of clinical laboratory test results in patient care. It can be expensive and logistically difficult for every clinical laboratory to establish reference intervals for all tests performed within the laboratory. Reference intervals provided by manufacturers are thus usually adopted in place of laboratory-established reference intervals. However, manufacturer-

established intervals are often based on Caucasian populations. The Department of Laboratory Medicine of Khoo Teck Puat Hospital (KTPH) conducted a study to validate in-use reference intervals for common clinical biochemistry laboratory tests.

MATERIALS AND METHODS: Serum samples (n=540) were obtained from healthy volunteers (age 21 - 70 years) during the annual hospital staff health screening over three years. Samples were analysed on the Roche Cobas c501 chemistry analyser (Roche Diagnostics, Switzerland) for 24 tests: sodium, potassium, chloride, bicarbonate, urea, creatinine, total protein, albumin, alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), total bilirubin, direct bilirubin, gamma-glutamyl transferase (GGT), creatine kinase (CK), lactate dehydrogenase (LDH), iron, transferrin, calcium, phosphate, magnesium, C-reactive protein (CRP), amylase and uric acid. The study was approved by the Ethics Board. Results were plotted in histograms. Reference intervals were obtained using parametric analysis for analytes that follow Gaussian probability distribution and non-parametric analysis for analytes that demonstrated non-Gaussian distribution. Study-derived reference intervals were compared with existing reference intervals in the laboratory.

RESULTS: Our results showed that 18 out of the 24 analyte reference intervals in the study were generally similar to the in-use reference intervals; the latter were validated using volunteers recruited from staff screening exercises conducted in another facility between 2009 and 2011. However, there were 6 analytes whose reference intervals were different from the in-use intervals. The analytes included: (1) CRP which had a 97.5 percentile cut-off of 9.0mg/L in the study group against the recommended cut-off of 5 mg/L; (2) Amylase, whose 97.5 percentile value was 138 U/L vs the current cut-off of 100U/L; (3) CK, whose range was 24 to 200U/L vs the study-derived range of 51 to 371 U/L. Current upper limits of reference intervals for intracellular enzymes like ALT, AST and GGT were also notably lower than the study-derived intervals even after correcting for suspected transient illness.

CONCLUSION: Validation of manufacturer-provided reference intervals and previously validated-data is important to ensure reference intervals remain relevant to the population served by the laboratory, to ensure appropriate care has been prescribed based on the laboratory results. Further studies may be required to ensure data can be translated from a group of healthy, physically-active volunteers to a population with heterogeneous levels of activity as certain analytes like serum CK concentrations can be also affected by strenuous physical activity.

A-258

Method-to-Method Variability in Urine Albumin Measurements

A. E. Merrill[1], J. Khan[1], J. A. Dickerson[2], D. Holmes[3], N. Harris[4], D. N. Greene[1]. *[1]University of Washington, Seattle, WA, [2]Seattle Children's Hospital, Seattle, WA, [3]St. Paul's Hospital, Vancouver, BC, Canada, [4]University of Florida, Gainesville, FL*

Background: Urine albumin (uALB) is a useful marker in screening, diagnosis, and treatment of renal microvascular disease. Beckman Coulter recently re-formulated their uALB reagent for the AU series of instruments to increase the analytical measurement range (AMR) of the assay. We aimed to determine the performance characteristics of the re-formulated AU reagent. Additionally, we evaluated the susceptibility of six uALB reagents across five commonly used analyzers to the high-dose hook effect.

Methods: Characterization of the re-formulated uALB reagent was performed on the Beckman Coulter AU680 (Brea, CA). Intra-assay precision included consecutive measurements (n=20) of two concentrations of BioRad QC material (Hercules, CA); inter-assay precision entailed analysis of each concentration (n=1/day) for 20 days. Linearity within the manufacturer's claimed AMR (0.7–45 mg/dL) was confirmed using a residual high urine sample diluted with albumin-free urine. A data-driven approach was utilized to determine the necessary reportable range. On-board and manual dilutions were validated to support this range. Inter-assay variability was assessed using 78 residual urine specimens. Specimens with sufficient volume (n=74) were also analyzed using the Siemens Vista. The hook effect was evaluated on the AU680, Roche Cobas (Indianapolis, IN), Siemens Vista (Malvern, PA), Siemens BNII (Malvern, PA), and Siemens DCA Vantage (Malvern, PA). Serum was spiked into albumin-free urine to generate a range of albumin concentrations (n=12; 0–4,500 mg/dL). Each sample was measured neat and on dilution, where applicable.

Results: Despite similar imprecision, the QC means of re-formulated reagent demonstrated a significant positive bias (~50%) compared to the production reagent. Linearity within the manufacturer's claimed AMR was confirmed (slope=1.017; y-intercept=-0.774 mg/dL), along with the accuracy of 10X and 51X dilutions to extend the technical range. Patient sample comparison demonstrated a positive bias using the re-formulated reagent (n=78; concentration range=0.7–1156 mg/dL; slope=1.110, y-intercept=0.185 mg/dL; average bias=10.6% or 13.5mg/dL), which paralleled the differences in QC material mean. When compared to the Siemens Vista, average negative biases of 10.7% and 21.2% were observed with the re-formulated and original AU reagents, respectively. Based on neat measurements of the hook effect samples, uALB exceeding 3,000 mg/dL may be erroneously depressed into the AMR of both AU reagents. The DCA Vantage assay "hooked" at even lower levels of uALB (>500 mg/dL), while the Vista, Cobas, and BNII assays were unaffected.

Conclusion: The re-formulated AU uALB reagent met the manufacturer claimed performance characteristics. A striking observation was the ~11% bias between the two AU reagents. uALB assays are clearly not standardized, yet clinical guidelines dictate result interpretation. To illustrate the potential implications, 4% of the patient samples used for method comparison would be interpreted as moderately increased albuminuria (30–300 mg/g creatinine) using the original AU reagent and severely increased albuminuria (>300 mg/g creatinine) using the re-formulated AU reagent. As these levels are typically monitored longitudinally, this could result in the appearance of disease progression and lead to potentially inappropriate changes in clinical management. While the hook effect also could obscure uALB interpretation, the DCA Vantage is the only instrument of those tested that may be affected at physiologic concentrations.

A-259

Critically Low Carbon Dioxide in Patients with Severe Hypertriglyceridemia

J. R. Wiencek[1], C. Bowman[2], B. Adams[3], C. Sussman[2], G. Sephel[4], M. F. Linton[5], J. Colby[1], J. H. Nichols[1], A. Woodworth[1]. *[1]Vanderbilt University School of Medicine, Nashville, TN, [2]Vanderbilt University, Department of Medicine, Nashville, TN, [3]Vanderbilt University, Diagnostic Laboratories, Nashville, TN, [4]Veterans Affairs Tennessee Valley Healthcare System, Nashville, TN, [5]Vanderbilt Lipid Clinic, Nashville, TN*

Background: Hemolysis, icterus and lipemia (HIL) are common interferences in clinical laboratory testing. These interferents may absorb or scatter light in spectrophotometric methods, which can lead to erroneous results. Assay manufacturers are required to document HIL thresholds through interference studies. Unlike hemoglobin and bilirubin there is no single chemical substance available that completely mimics the heterogeneity of a lipemic sample. In an attempt to simulate lipemia, manufactures will often use Intralipid a sterile, nonpyrognic fat emulsion. The range of lipid particle sizes for Intralipid is 200-600nm and does not cover the large lipid particle sizes of chylomicrons (70-1000nm). In this study, we identified a patient with repeated critically low carbon dioxide (CO_2) concentrations on the Abbott Architect CO_2 assay with no clinical signs of hypocapnia. The patient had marked mixed hyperlipidemia (hypertriglyceridemia and hypercholesterolemia) with acidemia. Therefore, an interference was suspected with the Abbott CO_2 assay.

Objective: To investigate the performance of the Abbott CO_2 assay in the presence of increasing amounts of hyperlipidemic samples.

Methods: The effect of lipemia on the Abbott CO_2 assay was assessed by performing mixing experiments and historic data review. Residual plasma from the hyperlipidemic and normolipidemic patients' plasma specimens collected for routine patient care were utilized. Hyperlipidemic specimens from a patient with severe hypertriglyceridemia (triglycerides >3,500 mg/dL) were titrated into a normal plasma pool. Samples with increasing lipid concentrations of 0, 5, 15, 25, 35, 50, and 100% were analyzed by either the Abbott Architect or Ortho Vitros enzymatic CO_2 assays. Linear regression analysis was used to obtain expected CO_2 concentrations for each of the samples analyzed. The expected and observed CO_2 concentrations were compared and percent bias calculated. Laboratory information system (LIS) records for a 6-month time period were reviewed. Patients >18 years with simultaneous CO_2 and triglyceride measurements on the Abbott Architect were evaluated (n=22,595).

Results: The package insert for the Abbott CO_2 assay reports 0 and 2% bias with 1000 and 2000 mg/dL of Intralipid respectively. Whereas the package insert for the Ortho Vitros CO_2 assay notes no significant interference with triglyceride concentrations up to 900 mg/dL. In mixing experiments, with triglyceride concentrations of approximately 1000 mg/dL, 2000 mg/dL and 3500 mg/dL gave negative CO_2 biases of 23%, 42% and 65%, respectively on the Abbott Architect. Measurement of CO_2 on the Ortho Vitros for the same lipemic specimens demonstrated negative biases of 6%, 23% and 36% respectively. Systematic review of LIS records for the past 6-months demonstrated that all patients (n=26) with triglycerides >1500 mg/dL in this time period had low CO_2 concentrations (4-22 mmol/L).

Conclusion: The use of Intralipid does not adequately mimic all the potential interferences within a hyperlipidemic sample. Patients with severe hypertriglyceridemia will have falsely low CO_2 results when determined by the Abbott or the Ortho Vitros CO_2 assays. In patients with hypertriglyceridemia, the

use of clinical symptoms, lipemic indices and other laboratory results should be used in the context of potentially low CO_2 concentrations in order to avoid unnecessary hospital admissions.

A-260

Investigation of biotin interference in common thyroid function tests using the Roche Elecsys® immunoassay system

B. M. Katzman, C. Rosemark, B. K. Hendrix, D. R. Block, N. A. Baumann. *Mayo Clinic, Rochester, MN*

Background: Many FDA-cleared automated immunoassays used in clinical laboratories allow for the rapid measurement of serum analytes through biotin-streptavidin interactions. Depending on the nature of the assay, sufficient biotin concentrations in serum can lead to falsely increased (competitive assay) or falsely decreased (sandwich assay) results. Biotin is a water-soluble B-complex vitamin found in small amounts in numerous foods, but is also commonly found in both therapeutic preparations and over-the-counter multi-vitamins and hair, skin, and nail supplements. Our core laboratory has recently identified several Mayo Clinic patients with erroneous thyroid function test results obtained on the Roche Cobas® e 601 immunoassay platform due to biotin supplement consumption. In one case, the patient was scheduled for radioiodine thyroid ablation due to erroneous thyroid function test results. However, this serious patient safety incident was averted due to additional testing performed in the laboratory and diligent communication of the interference to the clinician.

Objectives: The aims of our study were to characterize the extent of biotin-mediated interference with Roche Elecsys® thyroid assays and to develop a troubleshooting protocol for depleting endogenous biotin from serum samples using streptavidin agarose.

Methods: Biotin interference studies were performed by adding biotin (Sigma-Aldrich) in varying concentrations to a residual waste serum pool. Serum was mixed with biotin stock solution (9:1, high biotin pool) or saline (9:1, zero pool). The zero biotin and high biotin serum pools were mixed to create five samples with varying biotin concentrations (ng/mL) for each assay (free thyroxine (FT4) and thyroid stimulating hormone (TSH), 0-102 ng/mL; total triiodothyronine (T3), 0-51 ng/mL; total thyroxine (T4), 0-408 ng/mL). Biotin interference studies were performed for the following analyte concentrations: FT4 (0.8, 1.5 ng/dL), T4 (5.6, 8.4 mcg/dL), T3 (54, 129 ng/dL), and TSH (2.5, 6.5 mIU/L). Samples were measured in triplicate using a Roche Cobas® e 602. The results were analyzed using EP evaluator to determine the biotin interference threshold (+/-10% considered clinically significant bias). Biotin depletion studies were conducted by treating biotin-spiked serum (1000 ng/mL biotin) with varying volumes of Pierce™ streptavidin agarose (Thermo Fisher Scientific Inc.), incubating for 1 hour ambient, centrifuging and analyzing supernatant.

Results: The following experimentally derived biotin interference thresholds were obtained (Roche package insert cut-off): FT4 = 61 (25) ng/mL, T4 = 348 (100) ng/mL, T3 = 19 (10) ng/mL, TSH = 30 (20) ng/mL. Treatment of 0.45 mL biotin-spiked serum with 0.05 mL streptavidin agarose beads for 1 hour effectively removed biotin and eliminated interference.

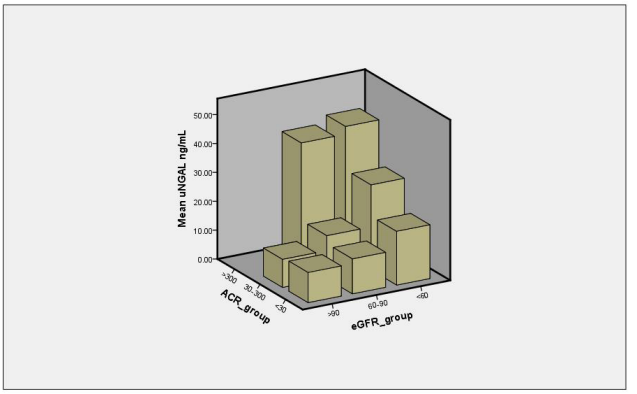
Conclusions: The experimental biotin spiking studies corroborated the Roche package insert thresholds for biotin interference and showed that the FT4, T3, and TSH assays are most sensitive to biotin interference. By treating the samples with streptavidin agarose, biotin interference was eliminated. This treatment protocol may be helpful when investigating cases of potential biotin interference. However, because biotin interference can mimic results found in biochemical hyperthyroidism (elevated FT4 and decreased TSH), identifying samples with interference may be difficult. This highlights the importance of laboratory, physician, and patient awareness of potential biotin interference in immunoassays.

A-261

Factors affecting urinary Neutrophil Gelatinase Associated Lipocalin levels in stable patients with Cardiovascular Disease without Acute Kidney Injury

K. Makris[1], K. Pirgakis[2], N. Kafkas[3], A. Haliassos[4]. *[1]Clinical Biochemistry Department, KAT General Hospital, Athens, Greece, [2]Vascular Surgery Department, KAT General Hospital, Athens, Greece, [3]Cardiology Department, KAT General Hospital, Athens, Greece, [4]Diamedica SA, Athens, Greece*

Several low-molecular weight proteins (LMWp) have been proposed as biomarkers for the early detection of acute kidney injury (AKI). Among them is neutrophil gelatinase-associated lipocalin (NGAL), produced by neutrophils and released in the circulation. Systemic-NGAL is freely filtered by the glomerulus and reabsorbed by megalin-facilitated endocytosis in proximal tubules. AKI will reduce reabsorption of systemic-NGAL, and stimulate tubular-epithelium to release NGAL increasing its urinary levels. However, filtered albumin is also reabsorbed by the same receptor. Independently of tubular-injury, competition for receptor-mediated transport between albumin and other LMWp could account for increased levels in the presence of proteinuria. Moreover chronic kidney disease (CKD) reduces filtration of NGAL. The objective of this study is to evaluate the relationship between uNGAL with urinary-albumin and GFR in patients with stable Cardiovascular Disease without AKI. We enrolled 226 patients with cardiovascular diseases. Urinary NGAL was measured with an ELISA (Bioporto, Denmark). Serum cystatine-C (sCysC) and creatinine (sCr) were measured on Architect 8200 analyzer (Abbott, USA). GFR was calculated with the CKD-EPI equation using both sCr and sCysC measurements (eGFR). Urinary-albumin and urinary-creatinine were measured on the same analyzer and the albumin-to-creatinine ratio (ACR) was calculated. We observed a negative, significant non-linear relationship between uNGAL and eGFR (r=-0.604) and a positive, significant, non-linear relationship (r=0.496) between uNGAL and ACR. Multivariate regression analysis revealed that both eGFR, and albuminuria affect baseline uNGAL levels. Our result show (figure) that levels of uNGAL increase significantly as GFR drops and albuminuria increases (MANOVA). In conclusion, albuminuria and CKD may increase the threshold for detection of AKI by increasing the excretion of LMWp. The presence of high uNGAL along with low eGFR and high ACR might also indicate progressive kidney disease. We consider that baseline measurements of uNGAL levels along with the estimation of GFR and ACR could help in risk-stratification of patients.



A-262

Decreased ascorbic acid interference by auto-oxidation of ascorbic acid in glucose, total cholesterol, and triglyceride measurement

J. Yim, J. Cho, S. Ahn, S. Lee, J. Kim. *Severance hospital, Seoul, Korea, Republic of*

Background: We studied the effects of ascorbic acid interference and its auto-oxidation with the lapse of time on the measurements of glucose, total cholesterol, triglyceride and uric acid assay. **Method:** Five samples of healthy volunteers within the reference interval of glucose level (80-100 mg/dL) was used to observe for potential ascorbic acid interference. We spiked three different levels of ascorbic acid to five EDTA whole blood samples making final ascorbic acid concentration of 3, 15, and 30 mg/dL in each samples, followed by centrifugation and serial analysis on each time for each plasma sample. For control, normal saline was spiked instead of ascorbic acid solution. Each spiked volume was less than 10% of the total volume, according to

recommendations by the International Federation of Clinical Chemistry in guidelines for the evaluation of drug effects in clinical chemistry. Measurements of glucose, total cholesterol and triglyceride were perfomed both by the reagent containing ascorbate oxidase (Sekisui Medical Co., LTD., Tokyo, Japan) and the reagent not containing ascorbate oxidase (Roche Diagnostics GmbH, Mannheim, German). On the other hand, both manufacture's uric acid assays contained the ascobate oxidase. **Results:** Ascorbic acid at concentration 3 mg/dL did not interfere the measurement of cholesterol significantly (less than 5%). However, negative interferences in the measurement of cholesterol were observed in the presence of 15 mg/dL ascorbic acid when the reagent without ascorbate oxidase was used. All five cholesterol results showed significant negative biases (from -24% to -39%) when compared with controls at 1 hour after adding ascorbic acid. More negative interferences (from -45% to -75%) were shown at the level of ascorbic acid 30 mg/dL. Whereas no significant difference between the results of control and test samples was observed in measurements using the reagents containing ascorbate oxidase, which converts ascorbic acid to dehydroascorbic acid preventing ascorbic acid interference. When we repeated cholesterol assay with the same samples at 6 hours, the negative biases were less than 10% at the level of ascorbic aicd 15 mg/dL. However, persistent negative interference (around 15%) was observed up to 12 hour in the ascorbic aicd concentration 30 mg/dL. The similar pattern of interference by ascorbic acid on measurement of glucose and triglyceridewas observed. On the other hand, all the results of both uric acid assays were not showed significant difference over time. **Conclusion:** In case of using the reagents not containing ascorbate oxidase, we recommend to repeat the test after more than 12 hours when negative interference by ascorbic acid is suspected in measurements of total cholesterol, glucose, and triglyceride.

A-263

A Negative Interference Observed in an Enzymatic Creatinine Assay Due to Dopamine and Dobutamine

J. C. Seegmiller, A. K. Saenger. *University of Minnesota, Minneapolis, MN*

Background: Measurement of serum creatinine is important for assessing renal function by estimating glomerular filtration rate (eGFR). The present study was designed after an inpatient with renal failure at our institution had a significant decrease in serum creatinine that could not be explained by medical intervention. The patient's previous creatinine was 4.25 mg/dL and the questionable result drawn 6 hours later was 2.07 mg/dL (reference interval: 0.52-1.04 mg/dL). All creatinine measurements were conducted on the Siemens Dimension Vista 1500 using an enzymatic creatininase method. After further investigation it was noted that three specimens with a questionable result were collected from a peripherally inserted central catheter (PICC) line; simultaneous arterial line samples were consistent with previous high creatinine results and the patient's clinical condition. Review of the patient's medical history revealed infusion of catecholamines (dopamine and dobutamine) during the time period the questionably low creatinine results from specimens collected from the PICC line were reported. Our objective was to investigate the extent to which catecholamines interfered with the Siemens creatinine method.

Methods: Three serum pools were made with varying concentrations of creatinine: 0.69 mg/dL (Pool 1), 3.32 mg/dL (Pool 2) and 6.89 mg/dL (Pool 3). An aliquot from each pool was spiked with dopamine (Pools 1 and 3) or dobutamine (Pool 2) to achieve a catecholamine concentration of 10 mcg/mL. Each spiked aliquot was serially diluted (x2, x4, x8, x16, x32, x64, x128) to observe the creatinine results in the presence of dopamine/dobutamine. All dilution aliquots were analyzed using the Vista enzymatic creatinine (IDMS traceable, peroxide detection) and the Abbott iSTAT enzymatic creatinine (non-IDMS traceable; sarcosine oxidase detection) methods.

Results: The patient's Vista enzymatic creatinine results demonstrate a significant decrease and deviation from previous results (difference:-2.18 mg/dL). The next draw hours later remained substantially lower (difference: -1.99 mg/dL) compared to baseline. Creatinine increased significantly to 4.95 mg/dL 4 hours later. Simultaneously paired arterial line and PICC line specimens were collected with the next draw (difference: 1.86 mg/dL). Additional specimens were collected by venipuncture and all samples were analyzed on the Vista and iSTAT. Absolute difference in creatinine across time points was minimal with the iSTAT (0.9 mg/dL) compared to the Vista (2.93 mg/dL). Serial dilutions of the 3 pools demonstrate a significant decrease in the creatinine concentrations at 10 mcg/mL of dopamine or dobutamine. Pool 1 had a decrease in creatinine of 48% and Pool 3 showed a decrease of 26% when spiked with dopamine; Pool 2 (dobutamine) also demonstrated a 22% decrease. In all cases, negative interference was minimized at higher dilutions such that the interfering substance was likely diluted out. iSTAT creatinine results were less susceptible to catecholamine interference at 10 mcg/mL or less.

Conclusions: Dopamine and dobutamine are routinely administered in intensive care settings. Significant false negative results were observed with the Siemens Vista enzymatic creatinine method (peroxidase detection mechanism) and related to PICC line draws. Enzymatic creatinine methods using sarcosine oxidase appear less susceptible to this interference.

A-264

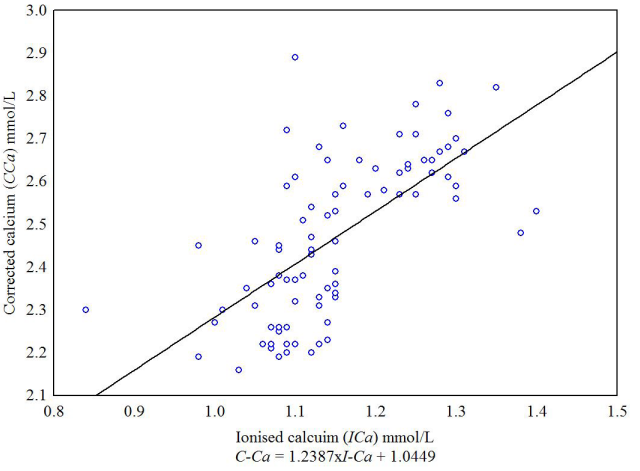
Validation of the Calcium Correction formula using serum total calcium, albumin, corrected calcium and ionized calcium

O. Kiabilua[1], S. Fuma[2], T. S. Pillay[2]. *[1]University of Pretoria/NHLS Tshwane Academic Division, Pretoria, South Africa, [2]University of Pretoria/NHLS Tshwane Academic Division, Pretoria, South Africa*

Background: The correction of serum calcium has been traditionally based on a historical equation which has not been fully validated. This study assessed the correlation between serum corrected calcium with serum ionized calcium values in an academic laboratory. Corrected calcium is derived from Payne's formula and consists of adjusting the serum total calcium levels based on the serum albumin level to reflect the "true" calcium status .The corrected calcium equation has been widely used in clinical laboratory but recent evidence indicates that some modification may be required based on the local context.

Methods: This was a prospective study using samples received at a laboratory for the measurement of serum calcium and ionized calcium. Serum albumin is measured by Bromocresol purple (BCP) in a Beckman DXC automated analyzer and the total calcium is determined by ion selective electrode method in a Beckman DXC automated analyzer. Ionized calcium is directly measured by OptiLion analyzer. The results of corrected calcium and ionized calcium will be grouped based on the different albumin cut-off levels of below 30 g/l, between 30-34 g/l, within albumin reference intervals (35-52 g/l) and greater than albumin reference intervals (> 52 g/l), respectively. Corrected calcium was then compared with ionized calcium across all levels of albumin. In total, 252 patient results were analysed.

Results: The graph illustrates the concordance between corrected calcium and ionized calcium.



For albumin levels < 30g/L; only 71% of results were in agreement; With albumin levels of 35-52 g/L only 14% of results were in agreement. Payne's formula overestimated hypercalcemia in 43% of patients and underestimated hypocalcemia in 72%.

Conclusion:

Calcium adjusted for albumin using Payne's formula fails to assess "true" calcemia. Payne's correction formulae should be abandoned in favor of uncorrected calcium in the routine laboratory. In cases of doubt , ionized calcium should be measured directly.

A-265

N-acetyl Cysteine Interferes with the Trinder Reaction Based Assays and Beyond

Y. Wang, S. Clapps, B. D. Horne, E. Champion-Lyons, Y. Zhu. *Medical University of South Carolina, Charleston, SC*

Background: N-Acetyl-L-cysteine (NAC) is a sulfhydryl-containing drug that is used to treat acetaminophen poisoning and other diseases. NAC has been recognized as a

source of interference in multiple laboratory tests that are based on (triglycerides, total cholesterol, LDL, HDL, lactate, uric acid, and lipase) or not based on (creatinine, total protein, albumin, AST, ALT, ALP, total bilirubin, and glucose) the Trinder reaction, respectively. However, different assay platforms, NAC dosages, and interference criteria were employed in these studies, and therefore the effect of NAC interference on these analytes remains inconclusive. This study aims to systematically determine the clinically relevant interference of NAC in the aforementioned assays as well as insulin immunoassay performed on the Abbott Architect platforms.

Methods: Excess patient serum containing 5 different physiopathological concentrations of analytes was spiked with 5 therapeutic doses of NAC (50 - 2500 mg/L). Serum without NAC was used as a control. Analytes were measured either immediately post-NAC addition or after up to 48 hr storage at 4 °C. Samples from 6 different acetaminophen-overdosed patients collected pre- and post- NAC infusion were also assayed. The interference was defined as % difference in NAC-containing samples from the control. A bias greater than the allowable total error of each analyte according to CLSI's recommendation was considered as clinically relevant. Additionally, the inference data were subjected to multiple regression analysis to predict the true analyte value in NAC-containing samples.

Results: NAC dose-dependently reduced the analyte measurements with a maximum of 5-95% reduction among different analytes. The magnitude of interference also varied with analyte concentrations. Clinically relevant interferences were observed for triglycerides, total cholesterol, lactate, insulin, uric acid, and lipase with NAC at concentration as low as 250 - 1250 mg/L, respectively. Statistically significant but non-clinically relevant negative bias for LDL, HDL, albumin, AST, creatinine, and ALP and positive bias for total protein were also observed in the presence of NAC. In general, NAC interference was gradually attenuated by prolonged storage except that a negative bias in insulin immunoassay increased with storage time and lasted for up to 48 hrs. A significant decrease of total cholesterol and uric acid was also observed in post-NAC treatment samples from acetaminophen-overdosed patients. Additionally, the predicted analyte concentrations in NAC-containing samples using multiple variable regression model showed significant Pearson correlation with the measured values ($P<0.05$).

Conclusion: Our results indicate that therapeutic dosages of NAC can cause clinically relevant negative interference in several assays involving the Trinder reaction on the Abbott Architect platforms. The magnitude of interference can be markedly different, depending on the concentrations of NAC and analyte as well as the storage time. Additionally, this study reports for the first time the significantly negative interference of NAC in insulin immunoassay, which might be attributed to the NAC binding to insulin causing impaired antibody-insulin recognition. Therefore, particular caution should be taken when clinicians interpret these assays' results of patients receiving NAC treatment. Finally, this study demonstrates the possibility of using multiple variable regression test to predict the analytes' concentration in NAC-containing specimens.

A-266

The Effects of Hemoglobin E variant on Hemoglobin A1c measurement

B. Pratumvinit[1], K. Reesukumal[1], S. Hanyongyuth[1], S. Wangchaijaroenkit[1], T. P. Loh[2]. *[1]Department of Clinical Pathology, Faculty of Medicine Siriraj Hospital, Bangkok, Thailand, [2]Departments of Laboratory Medicine, National University Hospital, Singapore, Singapore*

Background: Hemoglobin A1c is a marker of long-term glycemic control in diabetes patients and is linked to the risk for developing diabetic complications. Hemoglobin E is one of the most common hemoglobin variant in the world, especially in South-East Asia region. The aim of this study is to compare the HbA1c values of non-diabetic subjects with normal hemoglobin typing and heterozygous hemoglobin E variant, using two laboratory methods, which are immunoassay and high-performance liquid chromatography (HPLC)

Methods: The leftover blood samples of healthy, non-diabetic subjects, who attended the Siriraj Hospital for health screening and did not have impaired fasting glucose or diabetes (fasting plasma glucose <100 mg/dL). These subjects must not have abnormal liver or kidney function tests, as defined by having raised liver enzymes (AST/ALT) and reduced estimated glomerular filtration rate (eGFR <60 mL/min/1.73 m^2). These blood samples will be subjected to analyse using the Roche Integra 800 (immunoassay) and the Bio-rad D-10 (HPLC) analyzers.

Results: The mean±SD HbA1c values in the homozygous A (n=80) measured by the immunoassay method were 5.52±0.28 (range 4.9-6.3%), and 5.42±0.32 (range 4.7-6.4%) by the HPLC method. In hemoglobin E heterozygote, the mean±SD HbA1c values were 5.37±0.33 (range 4.4-6.6%) by immunoassay, and 5.35±0.33 (range 4.5-6.1%) by HPLC. By using unpaired t-test, HbA1c values were significantly different between homozygous A and hemoglobin E heterozygote analyzed by immunoassay method (P =.001), but not by HPLC (P=.115)

Conclusion: In normal fasting plasma glucose subjects, HbA1c values in hemoglobin E heterozygote subjects were significantly lower than homozygous A subjects by using immunoassay method. The interpretation of HbA1c values in the presence of hemoglobin E variant should be performed with caution.

A-267

Efficacy of Calcium Correction in a Cancer Population

B. Carroll, S. Richardson, M. Pessin, L. Ramanathan. *Memorial Sloan-Kettering Cancer Center, New York, NY*

Background: Plasma calcium exists in three forms with approximately 15% bound to anions, 40% bound to albumin and 45% circulating as free ionized calcium. Methods that measure total calcium are affected by protein concentration fluctuations, while measurements of ionized calcium are unaffected. Studies on calcium correction for albumin levels have been extensively studied in hemodialysis patients and the critically ill, but few have investigated the utility of these equations in cancer patients. The objective of this study was to investigate the usefulness of calcium correction for patients with hypoalbunemia and the resulting potential impacts on laboratory operations.

Methods: A retrospective analysis was carried out to determine the potential value of calcium correction on cancer patients at Memorial Sloan-Kettering Cancer Center from January 2013 to December 2015. Using the hospital database information, the following data was obtained: (1) total number of critically low calcium values (<6.5 mg/dL) out of total calcium results, (2) total number of critically low calcium values with corresponding low albumin levels (<4.0 g/dL), (3) number of critical ionized calcium values (<3.3mg/dL) and (4) average time spent making critical calcium callbacks. To correct for serum albumin concentration the Payne formula was utilized:

Corrected calcium = measured Ca (mg/dL) + 0.8 * (4.0 g/dL - patient albumin (g/dL))

Results: Over a three-year period, 706,552 calcium levels were analyzed of which 1,761 (0.25%) were critically low. Of these critically low calcium results, 97.4% had corresponding low albumin levels. Using the Payne formula to correct the calcium level, the number of critically low calcium values dropped to 14% of the uncorrected critical values (248 or 0.04% of the total calcium measurements). Next, to evaluate the accuracy of calcium correction in estimating actual critical calcium events in cancer patients with hypoalbunemia, we examined those critically low calcium results that also had an ionized calcium result within an hour. Within these paired results, there were a total of 554 critically low calcium results, and of those, 99% had albumin levels below 4.0 g/dL. Importantly, within these paired results, only three had corresponding critically low ionized calcium results. To determine the potential impact that correcting calcium in patients with hypoalbunemia could have on workflow and staffing within the laboratory, the amount of time technologists spend on repeat testing of critical value specimens and critical value callbacks was estimated. On average, a total of 15 minutes was spent on each critically low calcium result, which adds up to a total of 440 hours over the three-year period. If low calcium values would have been corrected, a total of 378 hours of technologist time could have been saved over three years through the reduction of repeat critical value testing and critical callbacks.

Conclusion: In cancer patients with hypoalbunemia, correcting calcium results using the Payne formula maybe a better indicator of calcium homeostasis than measured concentrations alone. This correction has the potential to provide healthcare professionals with more accurate patient results and reduce technologist time spent responding to these clinically insignificant low calcium values.

A-268

Identification and Quality Control of FFPE and cfDNA Samples using Agena Bioscience ExomeQC Panel

R. Birse, P. Hunt, D. Irwin. *Agena Bioscience, San Diego, CA*

Background: Highly-annotated, well-sourced bio-specimens, are essential in elucidating the molecular mechanisms for initiation and progression of cancer along with being a highly valuable resource to validate panels of actionable mutations for clinical utilization. Formalin-Fixed Paraffin-Embedded (FFPE) tissue archives are most commonly used for oncology research, validation studies and diagnostic testing. Accurate detection of mutations is often problematic in FFPE tissues since they tend to have highly variable levels of fragmentation, which frequently limits the number of DNA template, formalin introduced sequence artifacts and the presence of PCR

inhibitors. Lack of consistency between the various protocols for sample handling and extraction for DNA is one of the major obstacles in translating biomarker analysis to clinical practice. **Methods:** Pre-analytical protocols for FFPE samples generally assess either sample identity (ID) or nucleic acid template quality (QC). Often samples are assumed to be poor quality when low call rates are observed. To address these QC and ID issues, we have designed the ExomeQC panel, a single reaction panel that will perform DNA quality assessment and template copy number enumeration across a broad dynamic range of 100-100,000 copies (0.3-300ng) as well as monitoring sample fragment size over a 100-500bp range. This is performed by competitive PCR of selected known housekeeper genes derived from cancer studies, which are largely devoid of polymorphisms and somatic mutations, and have minimal copy number variation in germline and somatic tissues. To assess the intact, amplifiable template copy number across a size range of 100-200-300-400-500 nucleotides we have 5 assays at each amplicon size, with 200, 1000, 3000, 10000 or 100,000 of input competitive template copies. Sample ID is enabled via 21 highly polymorphic exonic SNPs along with 3 XY paralogues; while localized and historical identity matching and quality assessment is enabled via a completely automated software solution. **Results:** The ExomeQC panel was applied to archived FFPE (n=48) samples to assess amplifiable DNA copy number and template fragmentation. Pre-analytical utility was determined via comparison to control samples and where available downstream analytical performance from these samples. **Conclusion:** The ExomeQC panel provides a unified resource for pre-analytical identity authentication and quality assessment of potentially degraded clinical samples.

A-269

Sample Rejection in Clinical Chemistry Assay; Causes and Trends in a Named Laboratory: A pilot Study

M. T. Longe, A. Onyeaghala, O. Famuyiwa. *University College Hospital, Ibadan, Nigeria*

Background: Laboratory operations play a crucial role in patients' management thereby improving treatment and prognosis. Delivering quality laboratory data within an acceptable turnaround time depends on the use of right sample, collected at the right time, into an appropriate sample container which is delivered to the laboratory within the acceptable time frame. Previous studies on determinates of laboratory quality have indicated that pre-analytical issues accounted for the highest number of errors; and premised on this information, proper control of these errors could be of significant importance in improving the quality of data generated from the laboratory. Data detailing major contributors to pre-analytical issues in Nigerian laboratories are limited. This pilot study was conducted to determine the causes and trends of samples rejected for analysis in a clinical chemistry laboratory.

Methods: This study was conducted in one of the laboratories within the University College Hospital, Ibadan, Nigeria. Records detailing samples meant for clinical chemistry assay, collected for the period of two years, (2012-2014) were examined retrospectively and reasons for the rejection which were documented in the sample rejection log were reviewed. The data collected was analyzed using simple descriptive statistics.

Results: For the period under review, out of the total number of 92,374 samples received in the laboratory for clinical chemistry assay, 177 samples were found to be rejected representing 0.2 % of the samples collected. Major reasons for samples rejected in the laboratory were: Insufficient sample, 58 (33%); collection into wrong samples, 39 (22%); Clotted samples, 33 (19%); fasting samples collected at the wrong time, 16 (9%); wrong labeling of sample, 13 (7%); overnight samples sent to the laboratory, 10 (6%); unlabelled samples, 4 (2%) and haemolysis, 2 (1%)

Conclusion: From this study, it was evident that among all other factors observed, collection of insufficient sample and collection into wrong sample bottles and anticoagulants were major reasons contributing to samples rejected by the laboratory. Although the percentage of samples rejected was small compared to the total samples received, yet appropriate education and development of necessary quality documents could be of high significance in reducing these trends with a view to improving quality data, turnaround time, patient management and prognosis.

A-270

A survey on Indian patients about understanding of fasting requirements before fasting glucose measurements.

A. Hazra[1], S. Mandal[2], J. Chakraborty[3]. *[1]Christian Medical College (CMC), Vellore, tamil Nadu, India, India, [2]AIIMS Jodhpur, Jodhpur, Rajasthan, India, India, [3]ESI PGIMSR Maniktala, Kolkata, WB, India, India*

Background: Fasting is an important controllable preanalytic variable. Fasting glucose testing needs 8 to 14 hours of fasting as per WHO guidelines. Hours of fasting, water intake, sudden change in smoking or exercise, medication withholding or intake etc. affect the fasting test results. Counselling to generate patient awareness regarding above factors is important. **Methods:** In order to understand the ground reality whether patients are being explained about need for fasting, its meaning and associated requirements a survey was undertaken in the outpatient phlebotomy section of a subsidized care government hospital in Kolkata, WB, India. Exclusion criteria were health professional subjects e.g. technician, nurse etc. The questionnaire was framed in local native language (Bengali). Information collected were age, sex, perception of hours of fasting required, and perception about whether to restrict water intake, beverage, light breakfast, religious eatables (e.g. Prasad for Hindu or Sehir for Ramadan etc.), smoking, drinking, medication, exercise during the fasting and their behavior in previous three days regarding excessive exercise, change in food quantity, whether they were instructed, who instructed, was it verbal or in writing, what was the actual number of hours they fasted, whether they actually took some edibles in the morning, what food or beverage they ate last etc. **Results:** Ninety people were given questionnaire, 72 (80%) consented for the study, 42 female and 30 male. The result of the survey was very striking and only some highlights are given. Fasting duration varied from 2 hours to 16 hours. A staggering 83% of the participants perceived that nobody explained to them anything about the nature of the fasting. Of the rest 14% were explained by the doctor and 3% knew from health professional relatives. The cursory verbal instruction by doctor seemed to make either minor or no improvement or even paradoxic worsening in compliance. 69% of the uninstructed subgroup were not aware that drinking water was allowed at all, in the instructed group 57% were unaware. 13% of the uninstructed subgroup thought that drinking tea, coffee etc was allowable in the morning, a much larger proportion of 29% in the instructed subgroups also had the same concept. Even more paradoxical results were apparent in perception about snacks- the 14% of the instructed and 3% of the uninstructed thought that light morning snacks could be allowed. Only 3% of uninstructed thought religious food could be taken in morning, while a staggering 29% of the so called instructed group thought the same was allowable. **Conclusion:** Counselling for awareness about fasting and understanding of its nature need corrective interventions in the present setting. Doctors were overburdened with patients and yet the instructed minority received it from doctors. But the instructions were neither thorough nor effective, did not touch upon locally relevant religious confusion about fasting, was often misunderstood and rather gave a false confidence in wrong assumptions. It could be improved by introducing distribution of printed leaflets, posters and also training the nurses, phlebotomists and social workers who could also use audio-visual aids in addition to verbal reminders.

A-271

Evaluating microtainer neonatal whole blood minimum volume requirements

J. L. Gifford, A. Thommasen, I. Seiden-Long. *Calgary Laboratory Services, Calgary, AB, Canada*

Introduction:

While microtainers can potentially reduce the amount of blood drawn from neonates, short draws with microtainers can lead to high rates of recollects. We have re-evaluated our minimum volume requirements with a new wider bore design of BD Microtainers® (cat# BD365967). Our study asked two questions. First, when transferring separated plasma/serum using a micro-pipette, transfer pipette, or pouring, what volumes of plasma/serum can we recover from the new SST with different initial volumes of whole blood with neonatal hematocrit? Second, how many chemistry tests can be performed with different volumes of whole blood with neonatal hematocrit?

Methods:

To answer these questions, we adjusted adult whole blood to resemble that of a neonate (adjusted hematocrit: 0.61 ± 0.02 L/L) and aliquoted set volumes of the adjusted whole blood into BD Microtainer® serum separator tubes (SSTs). After centrifugation, the volume of separated plasma was measured following transfer by a micro-pipette, transfer pipette, or pouring. Using false bottom tubes (Beckman

Coulter, cat# 448774), we investigated the maximum number of tests a Roche Cobas 6000 analyzer can perform for each transfer method.

Results:

Total theoretical plasma volume in a tube was estimated as: 1-hematocrit x total blood volume. Of the different transfer methods evaluated, the micro-pipette is the most effective at transferring plasma with a mean ± avg deviation recovery of 68% ± 3% of total theoretical serum volume, but at higher volumes (300 µL, 400 µL), pouring off the separated serum is nearly as effective (67% ± 1.5%). Transferring using a plastic transfer pipette is the least effective method with recoveries of 56% ± 1.2%. Of the different whole blood/transferring methods we examined, whole blood volumes of 300 µL yielded 80 - 82 µL of plasma, which was enough for the Cobas 6000 to measure serum indices plus four tests requiring 2 - 6 µL sample/test. Lower volumes of 150 µL and 200 µL yielded 31 - 47 µL plasma. For the whole blood volumes below 300 µL, there was not enough plasma to measure serum indices (requires 6 µL) plus one chemistry test. In our study, we found that the required sample volume underestimated the total volume required for testing and that a significant volume of plasma is lost per test run (10.1 ± 0.4 µL/test) as part of the sampling process on this analyzer type and this was consistent across the run volumes.

Conclusions:

For neonates, a minimum volume of 300 µL should be drawn into the BD Microtainers®. Pouring or using a micro-pipette can transfer enough serum for 3 - 4 general chemistry tests. However, additional volume is required for each test in addition to the stated volume that is used in the reaction itself. Labs are cautioned not to set minimum volume requirements by adding up the amount of sample volume for tests as stated in the manufacturer's application on automated analyzers. Neonates require the analysis of multiple chemistry analytes, and our findings will prevent redraws in this group.

A-272

Identification of biotin interference with selected VITROS® biotin-streptavidin-based immunoassays in individuals taking supplements

I. Perjar[1], M. Gearhart[1], C. Hammett-Stabler[2]. *[1]UNC Hospitals, Chapel Hill, NC, [2]University of North Carolina School of Medicine, Chapel Hill, NC*

Background: Biotin is a known interfering compound in immunoassays based on biotin-streptavidin interactions. Serum biotin concentrations in individuals with normal dietary intake of this vitamin typically range between 200-500 pg/mL and are generally not found to cause interference; however, biotin supplementation is not uncommon among patients with clinical biotin deficiency and those taking over-the-counter vitamins for general health and beauty benefits. Biotin concentrations in these individuals can greatly exceed expected reference levels, presenting an opportunity for immunoassay interference.

Objective: In light of recent clinical inquiries regarding the above with assays performed in our laboratory, we sought to determine if this was an issue, and, if so, characterize the magnitude of interference at varying concentrations of biotin.

Methods: Selected immunoassays were evaluated using the VITROS 5600 (Ortho Clinical Diagnostics) chemistry system by adding biotin (Sigma-Aldrich®) to pooled plasma to span a range representative of that found in healthy individuals and those reported in individuals taking supplements. Our laboratory uses a 10% acceptability limit in interference studies.

Results: Excess biotin in pooled plasma resulted in variable but clinically significant interference with assays relying on biotin-streptavidin interactions (table).

Conclusion: Supraphysiologic serum biotin concentrations in individuals taking biotin supplements can result in clinically significant interference with common laboratory assays and must be taken in consideration when evaluating inconsistencies between clinical presentation and laboratory parameters. This is the first report of this phenomenon for the VITROS analyzer. Negative interference was observed for immunometric (sandwich) assays due to displacement of biotinylated antibodies by biotin. The competitive assays exhibited falsely positive results in the presence of excess biotin secondary to competition of free biotin for binding sites on streptavidin. Little is known about the effects of biotin metabolites on chemistry tests. Clinicians and laboratory scientists must be mindful of common potential interfering compounds with analyte quantification.

Assay	Assay type*	Units	Pooled plasma	Pooled plasma with added biotin (pg/mL)					Manufacturer published interference <10% (pg/mL)
				500	5,000	12,500	25,000	50,000	
Troponin I	IM	ng/mL	0.073	0.074	0.056	<0.034	<0.034	<0.034	2,500
TSH	IM	mIU/L	2.96	2.95	2.61	1.25	0.75	0.27	5,000
FSH	IM	mIU/mL	13.3	13.0	11.5	9.0	5.9	<0.5	10,000
LH	IM	mIU/mL	6.8	6.8	6.1	4.7	2.4	<0.5	5,000
Prolactin	IM	ng/mL	19	19	17	15	11	<1.4	10,000
bHCG	IM	mIU/mL	624	631	603	551	355	16.5	10,000
CEA	IM	ng/mL	3.7	3.8	3.7	3.7	3.4	<0.5	10,000
PSA	IM	ng/mL	1.42	1.39	1.3	1.24	1.06	0.16	10,000
Ferritin	IM	ng/mL	418	424	434	419	403	88	10,000
Progesterone	COMP	ng/mL	1.97	1.95	1.99	2.00	2.26	20.7	20,000
Estradiol	COMP	pg/mL	162	155	156	150	402	error	5,000
Testosterone	COMP	ng/dL	98	103	94	85	75	1550	10,000

*IM = immunometric, COMP = competitive

A-273

The Effect of Preservatives on Long-Term Stability of Emerging Urinary Biomarkers of Kidney Injury

C. M. Adamo, A. A. Sethi, T. H. Carlson. *Pacific Biomakers, Seattle, WA*

Relevance

Kidney injury and loss of function can be assessed using several emerging urine biomarkers. The urine of patients who are at risk for developing either acute or chronic loss of kidney function could be frozen for future biomarker measurement alongside newly collected urine. However, since the long-term stability (LTS) of these markers is unknown, a significant change in concentration may not necessarily indicate disease. Therefore, LTS of novel biomarkers is a critical element that needs to be verified to confidently detect risk of disease.

Objective

This study compared the LTS of five emerging urine biomarkers of kidney injury (Cystatin C (CysC), KIM-1, NGAL, osteopontin (OPN), and clusterin) using three processing methods, including a proprietary preservative.

Methods

Ten single-donor urine samples were collected and processed with three different methods: 1) neat and centrifuged (N&C), 2) pretreated with a protease inhibitor cocktail (PIC), or 3) pretreated with protease inhibitor cocktail plus carrier protein (CP+PIC). After processing, aliquots were stored at -70°C until assayed (baseline, 7 mo, 12 mo). Between-run precision (BRP) of each biomarker was determined from QCs. A sample was considered stable if the %Difference from baseline was ≤ 2*CV% of the BRP for the assay, but not to exceed >30%Difference from baseline. The biomarker was defined as stable if at least 4/6 (≥67%) samples had no individual changes exceeding the acceptance criteria, and rendered unstable if it did not meet the acceptance criteria for two consecutive time points.

Results

uKIM-1, CysC, NGAL and OPN were stable up to at least 12 months, regardless of the processing method. Although clusterin was not stable in N&C urine, as only 60% of the samples met the acceptance criteria, it was stable in PIC- and CP+PIC-preserved urine with over 70% of the samples meeting the acceptance criteria. In N&C urine, KIM-1 showed continuous degradation with a mean %Difference from baseline of -2.9% (ranging from -30.7% to 15.7%) at 7 months to -8.9% (ranging from -61.4% to 25.2%) at 12 months. In contrast, KIM-1 degradation was greatly reduced in preserved urine as evident by the condensed range. The mean %Difference from baseline in PIC-preserved uKIM-1 was 4.8% (ranging from -12.5% to 23.3%) at 7 months and 6.4% (ranging from -28.7% to 30.1%) at 12 months, while CP+PIC-preserved uKIM-1 was 4.2% (ranging from -11.8% to 24.2%) at 7 months and 9.3%

(ranging from -14.3 to 25.1%) at 12 months. A similar trend was seen with CysC and OPN in preserved urine.

Conclusion

While human KIM-1, CysC, NGAL and OPN are stable in N&C urine for up to at least 12 months, the stability of KIM-1, CysC, OPN and clusterin was dramatically improved when preserved, whereas NGAL stability was unaffected by any collection process. The addition of preservative is recommended when samples are stored at -70°C for 12 months or longer prior to analysis.

A-274

Effect of Hemolysis, Icterus and Lipemia Interference on Routine Clinical Chemistry and Therapeutic Drug Monitoring Assays

M. Winik, X. Zhang, K. Bah, M. G. Grace, N. Babic. *Mount Sinai Hospital, New York, NY*

Background: Almost 70% of all medical decisions regarding patient treatment and management are made based on clinical laboratory tests. It is, therefore, crucial that the laboratory provides accurate and reliable results. The majority (46-68%) of laboratory errors are pre-analytical, with ~13% due to specimen integrity, including the presence of endogenous compounds (hemoglobin, bilirubin, lipids) that can interfere with laboratory results. Unless the effect of these interferences is well characterized, the inaccurate result may lead to misdiagnosis or unnecessary treatment, with the potential to cause serious harm to the patient. While most manufacturers provide guidelines on interferences, the cutoffs can be inaccurate, misleading or not provided, resulting in the release of inaccurate results biased positively or negatively by the interferent. In this study, we characterized hemolysis, icterus and lipemia (HIL) interferences on 38 assays. Based on this study, our clinical laboratory practice has been adjusted to provide more appropriate guidance to the clinician.

Methods: For hemolysis interference, the hemolysate was prepared by lysing a unit of type O blood and preparing hemolysate/serum mixtures ranging from hemoglobin levels of <2 mg/dL to >2000 mg/dL. To study icterus interference, bilirubin was purchased from Sigma-Aldrich (St. Louis, MO) and used to spike pooled serum mixtures with bilirubin ranging from 0-80 mg/dL. To assess lipemia interference, specimens were prepared using Intralipid (20%) solution (Baxter, Deerfield, IL). Serum samples were spiked with varying amounts of intralipid to obtain samples with L-index ranging from 0 to 2000. All samples were tested on the Abbott Architect c16000 Clinical Chemistry Analyzer. A subset of hemolyzed specimens was also analyzed on Roche Modular systems.

Results: A total of 38 analytes were tested in our entire study. The magnitude of unacceptable interference was set using analyte specific Total Allowable Error limits as defined by CLIA. We found that hemolysis significantly interferes with the results of 10 analytes, lipemia with 8, and icterus with 5. We modified our practice based on the results of this study by including a comment alerting the clinician about potential interference if the interference results in >10% bias but still below CLIA TAE. In addition, we found that for acetaminophen, AST and ALT vendor claims regarding the magnitude of hemolysis interference may be misleading due to lack of studies covering the entire clinically relevant range.

Conclusion: In conclusion, in order to provide the highest quality patient care, it is very important for the clinical laboratory to fully characterize HIL interferences in-house and not rely completely on manufacturers' guidelines. This will ensure that the laboratory provides clear guidance to the clinician and prevent unnecessary re-draws in challenging, critically ill patients. Most importantly, it will enable the clinician to provide appropriate treatment in a timely manner. Our final analysis of impact of our practice changes indicated that by implementing precise HIL cut-off specific rejection criteria, approximately 400 re-draws per month due to hemolysis and up to 300 re-draws per month due to icterus were prevented.

A-275

Development of a Reporting Algorithm to Facilitate the Release of LDH Results from In Vivo Hemolyzed Samples

Y. Wang, B. Horne, B. Holliday-Hinton, S. Clapps, M. P. Ladson, Y. Zhu. *Medical University of South Carolina, Charleston, SC*

Background: Hemolysis introduces a significantly positive bias in the LDH results and is the most frequent reason for cancellation of the LDH testing in our laboratory. Hemolysis can occur in vitro due to inappropriate blood collection, delivery, processing, and storage or in vivo because of hemolytic diseases. Rejection of in vivo hemolysis samples is considered malpractice. The subsequent follow-up calls and multiple sample recollections often interrupt clinical workflow and increases the turnaround time for patient results. In general, patients with in vivo hemolysis should have decreased haptoglobin and normal potassium concentrations in the absence of any other were comorbidities that change their concentrations. The objective of this study is to establish an algorithm for reporting reliable LDH results and to reduce the incidence of in vivo hemolysis-related cancellation.

Methods: The Abbott ARCHITECT c Systems were used in this study. Hemolysis index (HI) was assessed and expressed in ordinal values 1, 2, 3, and 4. Sixty four serum samples with elevated LDH and HI greater than or equal to 1 were collected and subjected to additional haptoglobin and potassium assays. LDH results were triaged based on HI and a cutoff value for haptoglobin (8 mg/dL) or potassium (5.1 mmol/L), respectively.

Results: The haptoglobin concentrations in 28 out of 64 specimens were less than 8 mg/dL, including 23 patients with sickle cell anemia, 2 other hemolytic anemia, and 3 patients without hemolytic anemia. The remaining 36 specimens had haptoglobin levels of greater than or equal to 8 mg/dL. Among them, 33 samples were from patients with non-hemolytic diseases and only 3 samples were from sickle cell disease patients. The sensitivity, specificity, positive and negative predictive values of haptoglobin in detection of in vivo hemolysis were 89.3%, 91.7%, 89.3%, and 91.7%, respectively. In contrast, only 2 out of 28 patients with hemolytic diseases and 4 out of 36 patients without hemolytic diseases had potassium concentrations greater than 5.1 mmol/L. The sensitivity, specificity, positive and negative predictive values of potassium in detection of in vivo hemolysis were 92.9%, 11.1%, 44.8%, and 66.7%, respectively.

Conclusion: We have developed an algorithm using serum hepatoglobin as a reflexive test for patients with HI greater than or equal to 1 and elevated LDH to identify in vivo hemolysis. Based on the preliminary data, approximately 39.1% unnecessary cancellations of LDH testing can be avoided using this algorithm. However, serum potassium is not a specific marker for identifying in vivo hemolysis.

A-276

Evaluation of the Short and Long Term Stability of Common Clinical Chemistry Analysis for Human Biobanking Specimen

M. Han, N. Lee, H. Lee, S. Song, S. Park. *Seoul National University Hospital, Seoul, Korea, Republic of*

Background: The necessity of biobanking is growing more and more nowadays for clinical research. Since most of the researches with biobanking specimen are for later use, the stability of specimen is the most important factor for maintaining quality of research and biobanking. However, there are few researches on the stability of biobanking specimens, particularly long term stability. This study performs the evaluation of the short term and long term stability of common clinical chemistry tests for biobanking specimen.

Methods: Blood specimens are drawn from healthy volunteers. For short term evaluations, specimens are stored in four different temperatures (20, 4, -20, -70 degree of Celcius), and analytes are measured at basal, 2, 6, 24, 48, 72 hours and 1 week for 28 kinds of analytes including calcium, phosphorus, glucose, urea nitrogen, uric acid, cholesterol, total protein, albumin, total bilirubin, alkaline phosphatase, AST, ALT, gamma GT, creatinine, sodium, potassium, chloride, total carbonates, iron, total iron binding capacity, triglycerides, HDL, LDL, Immunoglobulin G, A, M, C3 and C4. For long term evaluations, specimens are stored in 2 different temperatures (-20 and -70 degree of Celcius), and analytes are measured at 1, 3, 6 months, 1 and 2 years intervals for same analytes as short term evaluations. Percent differences from basal level for each analyte are evaluated.

Results: For short term evaluation, percent changes on all analytes for all temperatures shows less than 10 % from basal levels which are within the range of total error of laboratory tests. However, for long term evaluations, alkaline phosphatase, AST, and ALT shows percent changes greater than 10 % from 6 months storage. Particularly, result changes on -20 degree of Celcius are much greater.

Conclusion: From this study, we can predict how biobanking specimens can be used in clinical researches. Furthermore, alkaline phosphatase, AST, and ALT can be suggested as easily available biomarkers for evaluation of the stability of biobanking specimen.

A-278

Icterus (Total Bilirubin) Measurement Without Reagent and *In-situ* Bias Correction To Chemistry Assays

B. Lee, S. Park. *Samsung Electronics, Suwon, Korea, Republic of*

Background: One of the pre-analytical factors related to sample integrity, "Icterus (Total Bilirubin)", is both a clinical parameter and a major interference to chemical assays. The spectrophotometric interference is due to the fact that the absorption of Total Bilirubin and chromophore typically used in reaction with hydrogen peroxide overlap. Many commercial chemistry analyzers measure Total Bilirubin with reagent assay and add typical additives, such as Bilirubin oxidase, to remove the icteric interference. Our research provides the direct measurement of Total Bilirubin without reagents and its application to remove the icteric interference in the other chemistry assays by *in-situ* correction in a thin film-based clinical chemistry analyzer.

Methods: The thin film-based clinical chemistry analyzer is composed of a spectrophotometer-installed reader and a thin film-based cartridge with one sample injection port and 16 micro-wells connected by microfluidic channels. The dimension of single well is 1.2 x 2.0 x 0.15 mm (length/width/ height) and the volume is 0.32 uL. First, Total Bilirubin value without reagent (TBIL_B) for plasma samples was measured in the blank well by a dose-response curve between the blank-well absorbance for plasma sample and reference TBIL concentration. The blank-well absorbance was calculated by the combination of multiple wavelengths (450, 535, 630, 810 nm). Second, we verified *in-situ* bias correction algorithm of icteric interference in the other chemistry assay by using blood urea nitrogen (BUN) assay and plasma samples (4.9 ~ 88.2 mg/dL). The absorbance of BUN assay was measured in one reagent well and the absorbance of Total Bilirubin was measured in one blank well in the same cartridge simultaneously. The *in-situ* bias correction was done by using the explicit removal of the icteric interference from BUN absorbance, such as OD(BUN assay) - alpha x OD(TBIL_R) and the BUN values were evaluated based on the reference values before and after correction.

Results: For 42 plasma samples (0.17 ~ 10.6 mg/dL), TBIL_B shows the Passing-Boblok regression of Y=1.03X-0.06 (X; reference, Y; our system), and the correlation coefficient of R=0.9981 (P<0.0001). For the case of the narrow TBIL concentration range (0.17 ~ 4.16 mg/dL), the results are Y=1.04X-0.07 and R=0.9947 (P<0.0001). By using BioRad MultiQual controls, the precisions were evaluated as 5.4 %CV for 0.61 mg/dL of TBIL and 2.5 %CV for 7.00 mg/dL. The in-situ bias correction algorithm was verified by 49 plasma samples (4.9 ~ 88.2 mg/dL of BUN measured by Cobas P-modular). The correlation coefficient for non-corrected BUN is R=0.9792 (95%CI : 0.9632 to 0.9883) and, for corrected BUN, R=0.9938 (0.9889 to 0.9965). The ranges of absolute bias based on the reference values are 0.01 to 18.52 for non-corrected BUN, and 0.06 ~ 5.97 for corrected BUN.

Conclusion: This TBIL measurement without reagent can be one of alternative methods in real clinical usage because it is simple and cost-effective. The *in-situ* bias correction algorithm was verified for BUN measurement and it shows significant reduction of absolute bias compared to non-corrected BUN value. This algorithm can be extended to the other chemistry assays, which have highly icteric interference.