Tuesday, July 29, 2014

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

Factors Affecting Test Results

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Thrombin-Mediated Degradation of Parathyroid Hormone in Rapid Serum Tubes

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Background: Measurement of parathyroid hormone (PTH) is important for the clinical assessment of parathyroid disease and calcium homeostasis. Previous studies have demonstrated decreased stability of PTH in serum versus plasma specimens, although the precise mechanism for this difference has not been established. If thrombin activation during clot formation is responsible, the effect should be exacerbated in tubes containing additional thrombin. Exogenous thrombin is a constituent of Vacutainer Rapid Serum Tubes™ (RST; BD Diagnostics, Franklin Lakes, NJ), which include bovine thrombin to induce accelerated clot formation. As a known thrombin cleavage site exists in the 84 amino acid human PTH polypeptide, we hypothesized that *ex vivo* PTH cleavage in RST tubes might occur. Such a possibility has analytical and clinical implications, as intact PTH diagnostic tests are sandwich immunoassays that use paired capture/detection antibodies to the N- and C-terminal regions of PTH.

Methods: 1) *Screening Study*: Initial experiments were conducted to determine whether measurement of analytes with potential thrombin cleavage sites would be affected by RST tubes. Aliquots from previously collected serum specimens were incubated for 24 hrs in either an RST or a Serum Separator Tube™ (SST) before analysis on a cobas e602 immunoanalyzer (Roche Diagnostics, Indianapolis, IN). A subset of replicate experiments was performed on an ARCHITECT $i1000_{SR}$ (Abbott Diagnostics, Lake Forest, IL). 2) *Fresh Collections*: To verify findings in freshly collected specimens, three tubes of fresh blood were drawn from each of 10 healthy normal donors. Specifically, we collected one Plasma Separator Tube™ (PST), one SST, and one RST from each donor. After clotting and centrifugation, intact PTH assays on the cobas e602 were performed at multiple time points on aliquots held at ambient temperature or 4°C. Confirmatory experiments were conducted using exogenous thrombin and the direct thrombin inhibitor hirudin.

Results: In screening studies, PTH results were lower after specimen incubation in RSTs versus SSTs. These findings were confirmed in additional time-course experiments and on a separate immunoassay platform. In fresh collections, RST and SST specimens stored at room temperature also showed a decrease in PTH results (versus PST specimens) beginning at our earliest measurement time-point (approximately 1 hr post-collection). The magnitude of this decrease was more prominent in RSTs versus SSTs. A similar (but smaller and slower) decrease in PTH results in serum specimens was seen in aliquots stored at 4°C. Further studies confirmed that the decrease in measured PTH was thrombin-mediated, as it was blocked by hirudin.

Conclusion: The present study demonstrates that thrombin is responsible for the decrease in PTH results observed in RSTs. It is presumed that endogenous human thrombin, activated during clot formation, may be responsible for the smaller decreases observed in SSTs. As there is an incomplete understanding of which additional polypeptides may possess bovine thrombin cleavage sites, these studies provide a simplified screening strategy that laboratories can use when evaluating RSTs for assays at their own institutions.

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The effect of different protease inhibitors in blood samples taken for parathormone, insulin, and prolactin analysis.

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Background: Proteolytic degradation by proteases can affect peptide hormone levels, which becomes especially important when there is a lag time between sampling and analysis. This should be taken into consideration when the samples are conveyed from peripheral to central laboratories. Five group of proteases (serine, cysteine, acid, metallo, and threonine) exist with various mechanisms of action. In our study we aimed to evaluate the protective role of different protease inhibitors on the degradation of parathormone (PTH), insulin, and prolactin in blood samples.

Methods: Blood samples (n=10) were collected from healthy volunteers into vacutainer tubes with gel seperator (Becton Dickinson, NJ, USA) with a) no additive, b), 1% protease inhibitory coctail (PIC) (Sigma) which inhibits serine, cysteine, and acid proteases, and aminopeptidases added immediately after blood sampling, c) PIC added after centrifugation (30 min after sampling) d) aprotinin which inhibits serine proteases (500 KIU/mL) (Sigma) added immediately after blood sampling and e) K2 EDTA (1.8 g/L). The samples were allowed to clot for 30 min and centrifuged at 1300xg for 10 min. Then, each batch of sample either stored at 4°C or at room temperature (RT) until analyzed at 6, 24, 48, and 72 hours and compared against baseline values. Insulin, PTH, and prolactin levels were measured with electrochemiluminescence immunoassay in modular E170 (Roche Diagnostics, Germany) analyzer. The desirable bias values were taken from the Westgard QC database.

Results: All parameters remained within desirable bias limits when stored at 4°C until 72 hours. PTH exceeded desirable bias limits when stored at RT longer than 24 hours. PIC addition before or after centrifugation inhibited protease associated PTH degradation. Since the PIC amount was less when added after centrifugation, a more economic application became possible. Insulin stored at RT decreased higher than desirable bias limits after storing longer than 6 hours and only EDTA preserved insulin at RT. Addition of PIC before centrifugation led to hemolysis which enhanced the insulin degradation through proteases. Prolactin remained to be stable under each condition.

Conclusion: These results shows that when the samples are conveyed between different locations, the preservation of peptide hormones should be kept in mind. Although this can be achieved with various protease inhibitors, storing the samples at 4 ºC from sampling until analysis, will work equally well.

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Pre-analytical factors effecting Alzheimer's disease biomarker stability in CSF

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Background: Evaluation of cerebrospinal fluid (CSF) biomarkers in Alzheimer's disease (AD) is becoming increasingly important to improve the reliability of ante-mortem disease diagnosis to ensure proper patient management. Development of highly precise assays for $amyloid_{1-42}$ (Aβ42) and tau protein has allowed investigators to better characterize pre-analytical sample handling factors that may affect clinical interpretation. A prospective collection study was designed to model different CSF handling scenarios for storage conditions at the clinical site, followed by shipping to and then storage and handling at the testing site. **Objective:** Model CSF handling scenarios to identify conditions that would not significantly impact the determination of Aβ42 and tau protein. **Methods:** CSF was prospectively collected from 46 healthy individuals under an IRB approved protocol. A 10mL CSF aliquot was collected by lumbar puncture in the L3/L4 or L4/L5 interstitial space. One milliliter aliquots were stored and shipped from the collection site at: -80°C, -20°C, 4°C and 25°C. Using an immunometic chemiluminescent assay, 6 randomly selected patient samples were used to investigate storage, shipping and handling conditions, including thaw conditions, post thaw handling, and freeze/thaw cycles. Additional studies examined the influence of varying the storage tube manufacturer and the tube type and lot for a single manufacturer. **Results:** When stored at -20°C and 25°C, Aβ42 values were from 5 to 20% lower compared to the control stored at -80°C. For aliquots stored at ambient temperature for 48 hours, the Aβ42 values were 40% lower. There was little to no effect on tau concentrations across the range of storage temperatures. Aliquots initially stored at -80°C or ambient temperature showed the greatest decreases in measured Aβ42 levels (10 to 15%) following a second freeze/thaw cycle, whereas no loss of tau protein was observed following a freeze/thaw cycle. Thawing CSF samples at ambient temperature versus a water bath did not affect concentrations of either biomarker. Results were consistent between patients. Storing samples for 2h at ambient temperature post thaw resulted in less than 10% loss for either marker. The Eppendorf Lobind® tubes allowed transfer of CSF and storage at 4°C for up to six days without significant loss of either Aβ42 or tau protein biomarkers. No significant difference in either Aβ42 or tau protein concentrations was observed between different lots and sizes of these tubes. **Conclusion:** The measured level of Aβ42 was significantly affected by CSF storage, shipping and handling conditions, although there was no appreciable impact on tau. The utility of these markers in routine testing can be improved by using conditions at the collection and testing sites that minimize analyte losses. Immediate storage of freshly collected CSF at -80°C and shipment on dry ice gave the best Aβ42 recovery. These conditions will also preserve tau protein. Also, it was found that use of Lobind® tubes did not affect the measurement of either marker.

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Using Factorial Design-of-Experiments (DOE) to Investigate Interactions among Pre-Analytical & Analytical Factors in the Laboratory

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Loss of cannabinoids from solution during processing and analysis has been shown. Roth et al (1996) showed the impact of storage and kinetic conditions on THC-COOH loss due to analyte binding to surfaces. Stout, Horn, and Lesser (2000) show the contribution of storage temperature to THC-COOH loss in urine specimens. In addition to the factors commonly investigated, we investigate the interactive effects of storage, sample and processing factors on THC-COOH loss. The first behavior investigated is a consistent low bias of 10-12%; the second behavior studied is a less frequent, 30-60% loss of analyte encountered in some patient samples. Erratic losses of 30-60% was observed in less than 0.2% of specimens tested. Both pre-analytical as well as analytic factors, and the interactions amongst these factors, were theorized causes of these observed losses. In order to understand the causes of the bias as well as the outliers in measured concentrations, six handling, storage, and processing factors were investigated.

Methods The investigation of factor effects and interactive effects on the THC-COOH loss during storage, handling, and processing conditions is studied using a sequence of investigative studies. The impact of dilution method on THC-COOH loss was investigated across four analytical chemists. Each chemist prepared replicate dilutions by two different methods on a non-glucuronidated solution fortified to 100 ng/mL concentration. The first method prepared a dilution at a 2 mL exact volume; the second method removed 500 mL of excess solution after dilution preparation. Graphical analysis of variance (ANOVA) was used to investigate the impact of the dilution method on percent loss.

The outlier losses in THC-COOH observed during urinalysis by GCMS were studied using a blocked, fractional factorial design of experiment (DOE). These six factirs were: initial specimen state (thawed vs. never frozen), storage conditions (light vs. dark), vortexing, pipette transfer, glucuronidated vs. non-glucuronidated specimens, and temperature (42ºF vs. Room Temperature). 64 samples were tested across all levels of the factors. Impact on THC-COOH concentrations were evaluated through a least squares screening analysis that included both main effects and all pair-wise interactions.

Conclusions 1. The dilution method had a statistically significant effect on % loss. The dilution preparations that required removal of excess solution resulted in 40-50% loss of THC-COOH. This effect reproduced across all four chemists who performed dilutions.

2. The experiment showed three factors and two interaction effects as statistically significant. Non-glucuronidated specimen exhibited greater losses across the 64 samples with a maximal loss of 40%. Non-glucuronidated samples lost 20-30% more THC-COOH than did the gluceronidated samples. Transfers had the greatest effect on non-glucuronidated samples and had an additive effect with vortexing. Exact-volume pipetting reduced the impact of vortexing.

The control of kinetic and handling conditions is essential in order to reduce the loss of THC-COOH during normal processing of urine specimens. The relationships amongst the sample handling factors with state of conjugation provide insight into causes of THC-COOH loss in GC-MS analysis.

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Developing a Cutoff for Urinalysis of Bloody Urine

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Background: When bloody urine is submitted to the lab for testing, the most common procedure is to spin it down and test the clear supernatant. Clarity of supernatant is visually determined by technologist which caries a subjective bias. Additionally, plasma contents may significantly alter the results of testing, despite passing a visual inspection. In our project we were looking for objective criteria that define bloody urine which is acceptable for laboratory testing.

Methods: Both, hemolyzed whole blood (n=11) and fresh, non-hemolyzed blood where mixed with urine at known decreasing concentrations. After spinning down, the supernatants were tested by dipstick and by chemistry analyzer. The precipitants were submitted for microscopic examination.

Results: We found that blood has a significant impact on urinalysis even when it accounts for 0.8% of the urine volume. In this mixture, dipstick showed (+3) blood concentration, and the total protein as well as albumin level went up by 448% and 2240% respectively, when compared to the concentration in the urine. Significant differences were also noted in microscopic examination. Similarly, the mixture of hemolyzed whole blood and urine at 0.8% concentration showed an increase of 1762% in the total protein and 4560% in the albumin. The mixture of urine with blood at 0.08% concentration showed (+2) blood concentration by dipstick and an increased concentration of total protein by 160% and albumin by 700%. Microscopic evaluation was affected to a lower extent and the rest of the analytes tested within clinically acceptable ranges.

Conclusion: We have demonstrated that dipstick can be used as a method to evaluate acceptability of bloody urine for laboratory testing. A blood concentration of (+2), which correspond to 0.08% of blood volume within the urine, may consist of acceptable cut off for most of the analytes with exception of total protein, albumin and RBC/WBC on microscopic evaluation. If the blood concentration within the blood/urine mixture exceeds 0.08% the specimen is not acceptable for the majority of testing such as total protein, albumin, creatinine, specific gravity, and microscopic analysis.

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The Effect of Hemolysis and Lipemia on 23 Analyte Values Measured on an Abbott c16000 Chemistry Analyzer

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Background: Medical laboratory test values may become erroneously elevated or decreased due to interfering substances or endogenous contamination such as hemolysis, icterus, or lipemia (HIL). Spectrophotometric-based assays are of particular concern as these can be affected by interferents that absorb incident light or inhibit transmitted light. The objective of this study was to evaluate the effects of hemolysis and lipemia on several analytes as determined on an Abbott c16000 chemistry analyzer with particular attention as to how interference could affect low, medium, or high baseline analyte values and how any observed changes could alter the clinical treatment of a patient.

Methods: Hemolysate was prepared from discarded whole blood specimens and Intralipid was obtained from the pharmacy. For each of 23 analytes to be measured, residual serum or plasma samples containing low, medium, and high concentrations of analyte were pooled to obtain a sufficient volume at a desired concentration. Hemolysate or Intralipid was added to these samples prior to analysis to obtain a final concentration of 65, 320, 1180 mg/dL hemoglobin (Hgb) or 50, 250, 1000 mg/dL triglyceride, respectively. Analyte concentrations and indices for hemolysis and lipemia were compared to unmodified specimens.

Results: Of the 23 analytes tested, 18 were partially or significantly altered by the presence of hemolysate or lipemia. Deviation from baseline values was caused either from increased analyte in hemolysate or Intralipid; or by interference of the spectral quantification of the analytes. Results of significant clinical interest are summarized below:

Interferent	Measured Analyte	Significance of Interference
Hemolysate	LDH	+ 1.2 U/L for every 1 mg/dL of Hgb
	AST	+ 5.7% of the Hgb value (U/L AST, mg/dL Hgb)
	Mg	+ 0.3-0.4 mg/dL per 100 mg/dL Hgb
	K	+ 0.3 mmol/L per 100 mg/dL of Hgb
	P	+ 0.2 mg/dL per 100 mg/dL of Hgb
	Total protein	+ 0.3 g/dL per 100 mg/dL Hgb
	Lactic acid	+ 1.0 mmol/L per 1000 mg/dL of Hgb
	Direct bilirubin	Both Hgb and analyte concentration dependent
	Triglycerides	+ 5 mg/dL per 100 mg/dL of Hgb
Intralipid	Mg	+ 0.1 mg/dL per 100 mg/dL of Intralipid
	Total Protein	+ 1 g/dL per 300 mg/dL of Intralipid
	Lactic acid	+ 1.1 mmol/L per 1000 mg/dL of Intralipid
	Direct bilirubin	Both lipid and analyte concentration dependent
	Triglycerides	Directly proportional

Conclusion: Our results provide a basis for predicting changes in analyte concentrations coordinate with hemolysis and lipemia indices on an Abbott c16000 chemistry analyzer, thereby enabling laboratory personnel to (1) assess the quality of the sample; (2) mitigate inaccurate test results; and (3) provide clinicians direction in interpreting results of specimens harboring interferents.

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How to reduce TAT delays in a large Molecular Biology Laboratory

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Background: Quality can be defined as the ability of a product or service to satisfy the needs and expectations of the customer. Turnaround time (TAT) is one of the signs

of laboratory service and is often used as indicator of laboratory performance. To improve the service's quality, the TAT should be constantly monitored and the outliers should have their causes investigated to avoid further delays.

Objective: The aim of this study was to measure and to reduce TAT delay in a large Molecular Biology laboratory.

Methods: Statistical study was performed during the period from January to December of 2013. In this period, the total number of tests reported and the number of tests release with delay were verified, based on reports obtained from our internal Laboratory Information System (LIS -Motion). TAT goals were determined according to the complexity of each test. For tests based on manual procedures; the goal was defined as 95% of the tests released monthly within the TAT established. For automated tests, the goal was defined as 98%. From July to December, the following actions were taken: meeting with the team to present the TAT data and mapping the process to identify the main causes of delays, such as pre-analytical (errors in patients input information, lack of documents needed for consistency analysis of the results) and analytical causes (routine days not well established, lack of trained labor, turnover). Additionally, some improvement alterations in the routine were done: training, define routines priority, changing platform from manual to automated. The data was monitored monthly using histograms for each test involved.

Results: Every month an average of 27.000 results are reported at DASA's Molecular Biology laboratory. From January to June the average percentage of delays was 0,89%, according to a report generated by internal management system (Motion). In the second semester, (July to December) the percentage of TAT delays was 0,49%. We observed that changing manual tests to automated platforms and team training were the most significant factors for reducing TAT delays.

Conclusion: In our experience, we were able to reduce the laboratory TAT delays in 45% by implementing strategies to improve the process and making people committed to the service's quality.

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Bias, imprecision and uncertainty evaluation for some immunoassays

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Background: Bias (B), imprecision (I) and uncertainty (U) are important parameters in evaluating the analytical performance of a laboratory. Internal quality control (IQC) data can be used as an indicator of imprecision while external quality assessment (EQA) data can be used to detect biases. We can calculate the uncertainty of a measurement, using these data and other uncertainty resources. The purpose of this study was to evaluate the analytical performance of immunoassays in our core laboratory according to Fraser criteria.

Methods: B, I and U of 12 immunoassays were calculated according to EURACHEM/CITAC Guide, by using the IQC and EQA data of the period between January 2013 and December 2013. Cortisol, Estradiol, FSH, hCG, Insulin, LH, Prolactin, Parathormone, FreeT3, FreeT4, TSH and Testosterone assays were used for evaluation of analytical performance of immunoassay autoanalyzers - ADVIA Centaur XP (Siemens Healthcare Diagnostics Inc., USA). Desirable specifications for allowable total error (TE), I and B were used in evaluation according to Fraser criteria.

Results: Cortisol, estradiol, insülin, LH, prolactin, and TSH met the desirable specifications for B, I and TE. hCG, FreeT4 and testosterone performance were the worst of all and met none of the desirable specifications. Additionally, FSH and free T3 had imprecision problem while parathormone had inaccuracy problem (Table 1). **Conclusion:** Immunoassays are difficult tests to achieve standardization. There are many factors affecting the immunoassays and the amount of measurand is very small, so accuracy and precision are very important. When it is not possible to meet the optimum and desired specifications, minimum specifications may be considered. Uncertainty does not mean error or doubt about the measurement, but it is about the confidence of the result of measurements. Each laboratory should make this evaluation to determine whether there are systematic or random errors.

Table 1: Evaluation of each immunoassay according to Fraser criteria.

	Calculated Uncertainty	Comment (TE)	Calculated Bias	Comment (B)	Calculated Imprecision	Comment (I)
Cortisol	27,84	PASS	10,52	PASS	9,11	PASS
Estradiol	17,31	PASS	6,16	PASS	6,08	PASS
FSH	18,37	FAIL	7,37	PASS	5,48	FAIL
hCG	38,01	FAIL	16,20	FAIL	9,94	FAIL
Insulin	23,73	PASS	9,55	PASS	7,04	PASS
LH	16,78	PASS	6,78	PASS	4,95	PASS
Prolactin	14,83	PASS	5,70	PASS	4,74	PASS
PTH	28,95	PASS	12,64	FAIL	7,04	PASS
Free T3	12,72	FAIL	3,65	PASS	5,20	FAIL
Free T4	22,10	FAIL	8,13	FAIL	7,48	FAIL
TSH	16,19	PASS	5,36	PASS	6,07	PASS
Testosterone	32,29	FAIL	12,56	FAIL	10,14	FAIL

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Sample recollection, an experience in a hospital where samples are collected by nurses, São Paulo, Brazil.

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Background In recent years, there has been increasing interest in quality improvement and patient safety activities in healthcare. The clinical laboratory has a leader in the field of healthcare quality management with a focus on analytical quality born of its scientific background and was one of the first areas to use quantitative statistical control methods. However laboratories are now being asked to widen their focus to consider activities outside their immediate control. Accreditation agencies are increasingly requiring laboratories to go beyond analytical quality and take responsibility for the pre- and post-analytical (or extra-analytical) phases where most errors arise. Blood sample collections are performed by venipuncture for the implementation of this procedure it is required that the professional is qualified, have technical training and practical experience in nursing. During the analytical process it is identified that the sample cannot be analyzed due same pre-analytical interference, the most frequent causes are inadequate, insufficient material, hemolysis and clotted. Consistent final result does depend only by the analytical process, a well collected sample is also important. When the samples are collected by nurses it is expected a high number of recollection because nurses are more used to the patient care, laboratory practices is not part of the experience.

Methods: The laboratory as an outsource service follows the number of recollection monthly and to keep the total number of blood recollection according with standards recommendations of 2,0% is challenging once the procedure is hold by the nurse team. All recollection we analyzed considering only reasons related with the technics applied during the procedure. The causes of recollection are, inadequate material, insuficiente, hemolysis and clotted samples.

Results: In 2013 the laboratory of a private hospital received 414.902 solicitation of laboratorial tests, 0,7% of this total were recollected, 45% were from hemolysis, 38% clotted, 12% insuficiente material and 3% inadequate material. 91.428 patients were attended in this period.

Conclusion: Nurses that Work in the laboratory are more used to blood collection than the team that work with patient care, so the laboratory must follow the procedure done by the hospital and is responsible for improving these number of recollection by stablishing a training program , and also avaliate if the program is effective because the main reason of following these data is to reduce the impact to the patient.

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Stability and clinical usefulness of thyroid hormones, TSH, GH, IGF1, BP3, SDHEA, and cortisol results in serum frozen at -20°C after long- term storage.

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Background: Stored samples are commonly used. It would be necessary to verify if samples stored for a given time at -20°C in a serum bank maintain stability for subsequent clinical interpretation.

We believe that the definition of stability should be different for long-term or short-term storage conditions.

The concept of uncertainty (U) is useful for long-term storage conditions because it considers all sources of error in a result. Error may be associated with method precision (CVa%: analytical coefficient of variation) and with method accuracy (bias). Collaborative Peer Group data are used to calculate the bias of the results. Therefore

U is calculated according to the following formula: $U= 2\sqrt{(CVa)^2 + bias_{media}\%^2 + (CV_{group}/\sqrt{n_{lab}})^2}$. Where CV_{group} is group coefficient of variation and n_{lab} number of laboratories in the Peer group.

The reference change value (RCV: $Zx\sqrt{2} \times \sqrt{(CVa2 + CVi2)}$ Where Cvi is biological coefficient of variation) shows a significant change if two consecutive results are outside this range, which may be interpreted as a change in patient status.

Objective: To assess stability and/or clinical usefulness of the results of thyroid hormones, TSH, GH, IGF1, BP3, SDHEA, and cortisol in serum stored at -20°C for 8 months.

Materials: Statistically representative numbers of sera from pediatric patients were evaluated according the following process T0: measured between 1 and 2 hours post-extraction. T1: after 8-month storage at -20°C. .

Results were considered stable and/or clinically useful if T1 was within the 95%CI for U and RCV, respectively.

Results:

Analyte (n)	U % (95%CIT_1)	RCV % (95%CIT_1)
TSH (40)	11.81 (NS)	58.6 (CU)
T3 (40)	14.39 (S)	34.7 (CU)
T4 (40)	12.18 (NS)	28.3 (CU)
fT4 (40)	7.75 (NS)	34.0 (CU)
GH (27)	15.82 (NS)	20.0 (NCU)
IGF1 (27)	15.59 (NS)	20.8 (NCU)
BP3 (27)	There is not peer group	23.0 (CU)
CORTISOL (21)	16.42 (NS)	30.0 (CU)
SDHEA (27)	17.27 (NS)	28.6(CU)

Stable: S

Not Stable: NS

Clinically Useful: CU

Not Clinically Useful: NCU

Conclusions: Under these storage conditions only T3 remained stable and the results could be used for reference range and/or diagnosis-related group values. GH and IGF1 were not stable, and their results were not clinically useful. The remaining results were not stable but were clinically useful.

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Factual or fictitious hypocalcemia?

M. Chen, B. C. Handy, E. A. Wagar, Q. Meng. *MD Anderson Cancer Center, Houston, TX*

Background: Calcium plays very important physiological functions. Measurement of serum calcium helps to identify many clinical disorders. Accurate results play a pivotal role in patient management. Unrecognized hypocalcemic emergencies can lead to significant morbidity or death. On the other hand, misdiagnosed hypocalcemia can result in inappropriate management and significant impact on patient care. Spurious hypocalcemia is not uncommon in clinical laboratory practice, and distinguishing false hypocalcemia from true hypocalcemia is essential. We report here two typical cases of hypocalcemia.

Methods: Total calcium and ionized calcium were measured from the original sample and redrawn blood samples to verify the results.

Results: Case 1 was a 19-year-old man with acute leukemia who underwent leukophoresis in which acid-citrate-dextrose formula A (ACD-A) was used as the anticoagulant. His initial blood chemistry results were as follows: serum calcium 7.9 mg/dL (normal range 8.4-10.2), ionized calcium 1 mmol/L (1.13-1.32), serum magnesium 2.0 mg/dL (1.8-2.9), serum phosphorus 8.3 mg/dL (2.8-4.6), blood urea nitrogen 25 mg/dL (8-20), and serum creatinine 1.26 mg/dL (0.70-1.30). The ionized calcium measured by point of care testing was 1.04 mmol/L (1.12-1.32). Repeat measurements from specimens collected next day showed consistently low total and ionized calcium: serum calcium 6.6 mg/dL (8.4-10.2) and ionized calcium 0.84 mmol/L (1.13-1.32) on chemistry analyzer. Case 2 was a 10-year-old boy with multiple endocrine neoplasia type 2 whose calcium level was initially unmeasurable. Calcium measurement was repeated several times from this sample and the results were the same. Magnesium and iron also were reported as undetectable from this sample, while potassium level was greater than 10.0 mmol/L. Specimen was recollected into a serum separator tube, and analysis of this sample revealed a calcium level of 9.4 mg/dL and potassium level of 4.5 mmol/L.

Conclusion: The low total and ionized calcium results in Case 1 are suggestive of factual hypocalcemia. The low calcium is presumed to be due to the chelation of calcium by citrate in the ACD-A during dialysis. Citrate-induced hypocalcemia is a major side effect of dialysis. Case 2 is clearly a fictitious hypocalcemia caused by EDTA contamination due to the wrong order of draw. Spurious hypocalcemia needs to be immediately recognized and appropriately interpreted in order to avoid misdiagnosis and unnecessary intervention.

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Laboratory investigation of spurious hyperkalemia

M. Chen, B. C. Handy, E. A. Wagar, Q. Meng. *MD Anderson Cancer Center, Houston, TX*

Background: Pseudohyperkalemia is a laboratory artifact that is induced during the process of specimen collection, transportation, and preparation. The most common causes for spurious hyperkalemia include *in vitro* hemolysis, excessive tourniquet time or fist clenching during phlebotomy, contamination with potassium ethylenediaminetetra-acetic acid (K_2EDTA), and specimens collected from patients with hematological disorders such as leukocytosis and thrombocytosis. Factitious hyperkalemia occurs frequently in laboratory practice. Here we report two representative cases that presented with plasma potassium greater than 10.0 mmol/L, illustrating the investigation process for hyperkalemia.

Methods: Various types of peripheral blood specimens were collected. Repeat measurements of potassium were performed on different analyzers including Vitros Fusion 5.1, blood gas analyzer IL Premier 3000, and POCT. The results of these analyses were compared.

Results: Case 1 was an 84-year-old woman with chronic lymphocytic leukemia (leukocytes 181,300/μL). Her plasma potassium levels were persistently elevated (>10.0 mmol/L). Repeated measurements of potassium on Vitros Fusion 5.1 showed a potassium level >10.0 mmol/L in lithium-heparin plasma with or without gel but a potassium level of 3.6 mmol/L in serum. A sample collected into a lithium-heparin balanced syringe and analyzed by blood gas analyzer IL Premier 3000 showed a potassium level of 3.6 mmol/L, while a sample collected simultaneously in lithium-heparin showed a level of 11.1 mmol/L on Premier 3000. The patient's potassium level as determined by POCT was 2.6 mmol/L. Case 2 was a 10-year-old boy with multiple endocrine neoplasia type 2 who presented with a potassium level greater than 10.0 mmol/L. Surprisingly, the levels of calcium, magnesium, and iron in this sample were undetectable. In another specimen collected separately in a serum separator tube, the potassium was reported as 4.5 mmol/L and the calcium 9.4 mg/dL.

Conclusions: Severe hyperkalemia is a potentially life-threatening condition; immediate recognition and appropriate interpretation are critical. Case 1 is clearly a pseudohyperkalemia attributed to heparin-induced cell lysis in leukocytosis, whereas case 2 is confirmed due to contamination of K_2EDTA during specimen collection not following the order of draw. Venous blood collected in a lithium-heparin balanced syringe and analyzed by blood gas analyzer is recommended for potassium measurement in patients with leukocytosis. Standard operating procedures and order of draw should be followed for specimen collection. An investigation algorithm needs to be developed and the laboratory should follow the algorithm when pseudohyperkalemia is suspected.

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Case Report: Multiple False Electrolyte Abnormalities In A Patient with Primary Biliary Cirrhosis Due To Extreme Hypercholesterolemia

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Background: Primary biliary cirrhosis (PBC) is an inflammatory, likely autoimmune, liver disease marked by the destruction of intrahepatic bile ducts. Common features of PBC include hyperbilirubinemia, pruritus, and elevation of plasma lipids, especially total cholesterol (TC). 96% of symptomatic patients are reported to have TC concentrations greater than 200 mg/dL, with an average of 377 mg/dL and a range of 77 mg/dL to 1035 mg/dL. We report a case of PBC where the finding of hyponatremia led to the discovery of a plasma TC level > 2000 mg/dL.

Methods: All measurements were made on patient plasma utilizing a Roche cobas® c501 instrument using manufacturer-supplied reagents and instructions unless otherwise specified.

Case Report: A 43 year-old female with PBC was admitted to the hospital after outpatient laboratory tests showed hyponatremia. Her complaints on admission included blurry vision, nausea, and significant pruritus. Physical exam was remarkable for scleral icterus; no xanthomas were noted. Relevant abnormal laboratory results

were: sodium, 121 mmol/L (reference range: 135-145); potassium, 3.0 mmol/L (3.6-5.0); chloride, 87 mmol/L (98-109); and total bilirubin, 10.0 mg/dL (0.2-1.3). She was started on intravenous (IV) fluids (0.9% sodium chloride). Subsequently, a lipid panel was ordered. Her previous lipid panel from 1.5 years ago showed a plasma TC concentration of 322 mg/dL (120-199). Her current lipid panel showed a TC concentration of 2156 mg/dL; triglycerides, 226 mg/dL (50-150); and HDL, 37 mg/dL (45-65). The LDL was unreportable. This was the highest total cholesterol value measured by our laboratory. An investigation took place to determine if this was an erroneous result. There was no evidence of contamination. The sample appearance was clear and not grossly viscous or lipemic. The hemolysis index of the sample was 3, icterus index, 11, and lipemic index, 73. No significant interference is expected below indices of 700, 14, and 2000, respectively, per the manufacturer. Furthermore, serial dilution of the specimen indicated no interferences. A second sample from the patient was obtained and showed a TC value of 2415 mg/dL; triglycerides, 299 mg/dL; and HDL, 42 mg/dL (LDL, unreportable). Treating these as accurate values, we investigated whether the patient's sodium concentration was falsely low due to hyperlipidemia. Direct ion-selective electrode measurement of the initial sample on a Radiometer ABL825 FLEX® analyzer showed a sodium concentration of 141 mmol/L (137-145); potassium, 4.4 mmol/L (3.6-5.5); and chloride, 105 mmol/L (101-111). Serum lipoprotein electrophoresis done at a reference laboratory confirmed the elevated TC level at 2295 mg/dL and found the major component to be lipoprotein X.

Conclusion: This case is important due to the degree of hypercholesterolemia, lack of lipemic sample appearance, and its link to multiple false electrolyte abnormalities. To our knowledge, there is only one other report of a PBC patient with a cholesterol level > 2000 mg/dL, and it, too, was associated with pseudohyponatremia significant enough to prompt clinical action. In this case, the clinical team stopped treatment with IV fluids upon learning of the patient's hyperlipidemia, and the patient was eventually discharged.

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Biological Variation as a goal for comparability of thyroid stimulating and free-thyroxine hormones measurements in patients

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Background: Laboratory testing of serum thyroid stimulating hormone (TSH) and free-thyroxine (FT4) are essential for the assessment of thyroid function. However, changes of methodologies could limit the application of clinical guidelines depending on the standardization status. The IFCC Working Group for Standardization of Thyroid Function Tests reports no significant differences in healthy individuals within TSH assays (when calibrators are traceable to WHO international standards). They used 10% as harmonization goal that is considered the current state-of-the-art of immunoassay comparability. The same occurs with FT4 quantification if methodologies are traceable to reference method.

Objective: To assess the comparability of patients' results between two TSH and FT4 immunoassays using Biological Variation (BV) criteria as comparability goal.

Material and methods: Following CLSI EP9-A2IR protocol, TSH and FT4 from 40 randomly selected patient serum samples were performed in two analysers, Elecsys (Roche) and Advia Centauro XP (Siemens) by duplicate. Both TSH methods were traceable to second and third WHO international standards (81/565 and 80/558) respectively. FT4 methods were traceable to different standards: Elecsys was traceable to the reference measurement procedure based on equilibrium dialysis and Centauro was traceable to internal standard using United States Pharmacopoeia material. Analytical imprecision of all methods were within desirable limits based on BV. BV desirable bias was set as analytical goal: TSH 7.82 % and FT4 3.34 %. Confidence interval at 95% (CI) of the predicted difference was compared to laboratory allowable bias at medical decision levels (MDL).

Results: Table shows the main results of the study.

TEST Units (Assayed range)	MDL	Difference	CI	Allowable BV desirable bias	Goal achieved
TSH µIU/mL	0.27	0.05	-0.10 to 0.19	0.02	Not
(0,37-4,71)	4.20	-0.71	-0.86 to -0.55	0.33	Not
FT4 ng/dL	0.90	0.1	0.06 to 0.13	0.03	Not
(0,87-1,63)	1.80	-0,18	-0.24 to -0.13	0.06	Not

Conclusions: Despite standardization efforts, applying biological variation criteria, comparability of TSH and FT4 results could not be guaranteed in patients. When a change of methodology occurs, laboratory should always perform studies in order to alert clinicians about the possible non interchangeability of patients' results with possible impact in their follow-up.

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PATHFAST™ Presepsin (sCD14-ST) Sample Matrix Evaluation

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Background Soluble CD14 is released from monocytes during activation by TLR4-specific inflammatory reaction against infectious agents yielding presepsin (sCD14-ST). Presepsin demonstrated powerful diagnostic and prognostic information in critical ill patients with infectious-inflammatory diseases. The objective of our study was to examine the suitability of different sample types for presepsin determination.#

Methods Whole blood samples were collected from 20 patients in serum, lithium heparin, K2 EDTA and Na3 citrate blood collection tubes (S-Monovette, Sarstedt, Germany). The patients were hospitalized with different degrees of infectious-inflammatory conditions at the intensive care unit (ICU).Serum and plasma samples were prepared from each whole blood sample by centrifugation (20 minutes at 2500 x g) and separation of plasma/serum within 2 hours after blood drawing. The native serum and plasma samples were measured using the PATHFAST™ Presepsin assay. For estimation of the inflammatory status C-reactive protein (CRP) values were determined in the heparin plasma samples using the cobas CRP assay (Roche Diagnostics).

Results No considerable differences of the presepsin concentrations measured in the different sample matrices were observed. The samples spanned a significant portion of the measurement range of the test. EDTA plasma samples ranged from 161 to 7400 pg/ml. The measurement of presepsin in the different sample matrices revealed CVs between 2.11 and 12.34 % showing a high concordance between the samples. The results are summarized in Tab. 1.

Tab. 1: Presepsin concentrations (ng/L) measured by using PATHFAST™ Presepsin in different sample matrices

	N	Mean	Median	Minimum	Maximum	IQR
Serum	20	1715	618	169	7251	273-2453
Heparin plasma	20	1749	635	182	7335	277-2610
Na-citrate plasma	20	1614	559	194	6997	265-2225
EDTA plasma.	20	1715	632	161	7400	223-2373

Conclusion The results demonstrated excellent comparability between the different sample matrices across the full measurement range suggesting that PATHFAST™ Presepsin may be useful for risk stratification of critical ill patients from inflammatory diseases in the emergency room and point-of-care setting.

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Reference Intervals of Many Common Chemistry Tests are Affected by Advancing Age in Adults

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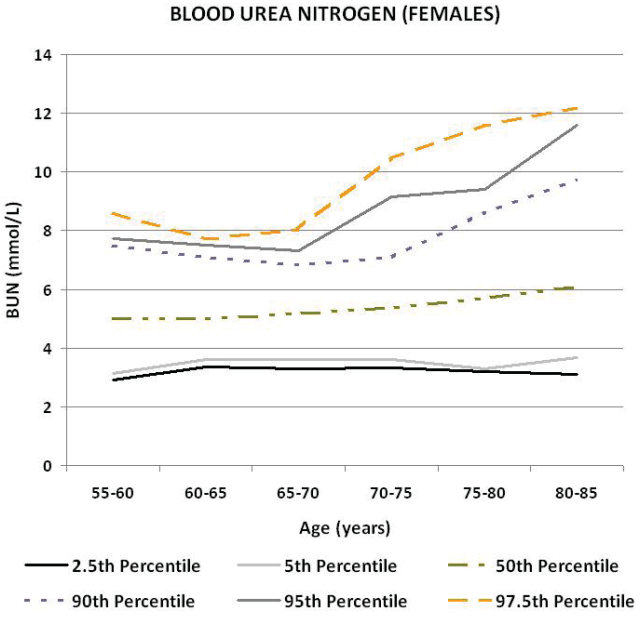
Objectives: More than 40 million Americans are over 65 years of age and their number will grow rapidly over the next 40 years. Current laboratory reference intervals have age-specific ranges for some tests but only for infants, children, and non-senior adults. Not enough is known about the effect of aging on many tests and geriatric reference intervals are not widely available. This study aims to determine the effect of advanced age in adults on chemistry reference intervals.

Methods: We compiled the results of common chemistry tests and pertinent demographic data of 55 to 85 year old US volunteers sampled in 4 cycles of the US National Health and Nutrition Examination Survey (2005-2006, 2007-2008, 2009-2010, 2011-2012). Reference interval diagrams were constructed with the 97.5, 95, 90, 50, 5, and 2.5th percentiles plotted against age intervals: 55-60 years (n=64 males, n=45 females), 60.1-65 years (53 M, 72 F), 65.1 to 70 years (50 M, 63 F), 70.1 to 75 years (50 M, 65 F), 75.1 to 80 years (51 M, 57 F), and 80.1 to 85 years (94 M, 83 F).

Only non-Hispanic whites with a waist circumference less than 105 cm (male) or 100 cm (female) were included in this study.

Results: The Figure shows a very interesting reference interval diagram. The increase in BUN with age is clearly apparent. We observed similar patterns for creatinine, potassium, osmolality, and total bilirubin. In contrast, we observed inverse relationships with age for albumin, ALT, and cholesterol. We found only minimal age-dependent relationships for ALP, AST, calcium, chloride, globulins, total CO2, iron, LD, sodium, phosphorus, and total protein.

Conclusions: Advancing age in the adult population affects many common chemistry tests. Our results suggest that it will be necessary to include age-appropriate reference intervals when reporting test results for geriatric patients.



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Keeping Bacteria Under Control to Minimize Impact on Assays and Maximize Analyzer Uptime

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Low bacteria count in pure water is particularly critical in clinical analyzers, because bacteria can generate numerous interferences in biochemistry and immunochemistry assays. The objective here is to describe several of those issues on assays and analyzer maintenance, and provide solutions to avoid bacterial contamination in water supplying the analyzer.

Typical impacts of bacterial contamination on assays include unstable calibrations, high absorbance of blanks, reference drifts, and errors on mean patient values. Those effects are observed, for instance, on the Arsenazo calcium assay, the potassium potentiometric assay, as well as immunoassays involving alkaline phosphatase (*e.g.* CTNI, fluorescence 6-MUP- and AMPPD-based assays). Those effects generated by the typical bacteria strains identified in clinical analyzers (*Ralstonia pickettii*, *Sphingomonas paucimobilis*, *Caulobacter crescentus*) result from proteins and small organic acids released by bacteria.

Bacteria also have an effect on maintenance of the analyzer: an incomplete rinsing generates interferences with the 340 nm detection of assays using NAD/NADH chemistry and the frequency of sanitization can be significantly increased. Maintaining low bacteria level in the analyzer and the water purification system supplying the instrument can reduce downtime and minimize the risks of false results.

Some of the issues mentioned above and resulting from poor design can be avoided by selecting key purification technologies, such as ultrafiltration and germicidal 254 nm UV lamps. A reduction of two logs is observed on bacteria levels of water treated by UV mercury lamps (*ca* 100 cfu/mL to < 1 cfu/mL, using CLSI® recommended bacteria culture procedure). Blank variation in the CTNI assay was shown to decrease over a 30 day period, from a range of 18 to 52 mAU to a range of 20 to 26 mAU by adding an ultrafiltration step to the water treatment process. The ultrafiltration removes the alkaline phosphatase that is released by bacteria, and interferes with the biochemical cascade of the CTNI assay. The stability of the blank ensured a much higher reproducibility of the results and reduced the need for frequent calibrations of the assay.

Recommendations for the maintenance of the water purification systems are described to ensure a consistent supply of clean water to feed clinical analyzers, in-line with CLSI C3-A4 guideline.

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Comparison of RBC hemolysis according to plasma and serum separator tubes among outpatient specimens

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Background: In our laboratory, we use plasma separation tubes (PST) for chemistry analysis to obtain outpatient results rapidly. If lactate dehydrogenase (LD) measurement is required, serum separation tubes (SST) are used. PST can be used immediately after centrifugation with no need for clotting, and a greater volume of blood can be obtained. Although many studies have compared the results of blood chemistry analysis using PST and SST, there has been no evaluation of hemolysis using these types of tube. Herein, we compare the hemolytic index obtained using PST and SST, and apply this to quality control in the laboratory.

Methods: We analyzed the hemoglobin index of outpatients visiting the Asan Medical Center (Seoul, Korea) from January to December 2012. The hemolytic index, a quantitative serum index, was scored from 0 to 10 according to the concentration of hemoglobin (0-5.0 g/L) using the Toshiba-200FR automated instrument (Toshiba Medical Systems Co., Tokyo, Japan). Hemolytic index was classified by sample tube type (PST or SST), and significant hemolysis was defined as a hemolytic index of 2 or greater. In cases of significant hemolysis, electronic medical records were reviewed to identify the cause.

Results: Significant hemolysis was found in 0.66% (1,128 of 171,519) of the total specimens, 0.68% (1,051 of 154,886) of PST specimens, and 0.46% (77 of 16,633) of SST specimens. The mean hemolytic index in PST was 0.18 (SD: 0.43), which was significantly greater than that in SST (0.14, SD: 0.37) (P<0.001). The proportion of significant hemolysis was also higher in PST than in SST (P=0.001). The cause of significant hemolysis was identified in 48.1% (543 of 1,128) of the specimens; the causes of in vivo hemolysis were chemotherapy and prosthetic valve. The remaining hemolysis specimens (51.9%, 585 of 1,128) may have been due to complex sampling errors. Hemolysis of unknown cause was particularly common in pediatric samples.

Conclusion: The incidence of hemolysis was slightly higher using PST compared to SST, although both were <1%. We conclude that PSTs are thought to be more useful than SST in outpatient testing because they offer more rapid turnaround and can contain a greater sample volume in our laboratory.

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The effect of storage at room temperature on the measures of lactate and pyruvate in cerebrospinal fluid with and without blood contamination

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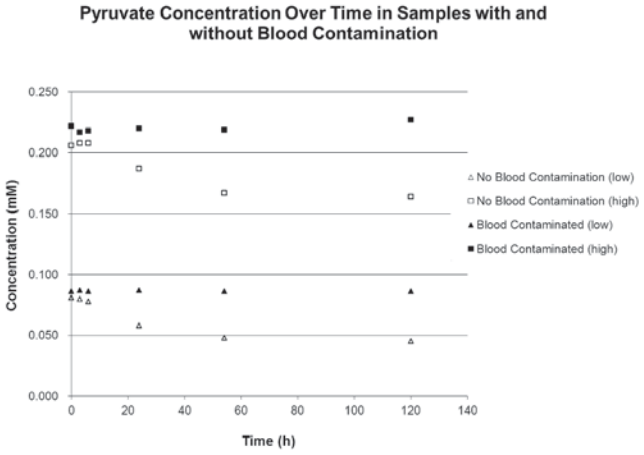
Background: Measurement of pyruvate and lactate, intermediates of carbohydrate metabolism, in cerebrospinal fluid (CSF) is important in evaluating disorders of the central nervous system. However, the stability data of these analytes in CSF is scarce in literature, especially for pyruvate. Objective: To assess the in-vitro stability of pyruvate and lactate at room temperature (RT) in CSF specimens with and without blood contamination. Method: Blood contaminated and non-contaminated CSF specimens were collected for this study. Separate pools were made for contaminated and non-contaminated samples. Unspiked pools were used for low concentration levels (0.09mM pyruvate and 1.9mM lactate) and high levels were spiked at 0.22mM pyruvate, 3.5mM lactate. Pools were stored at RT over 120h while duplicate samples were aliquoted at each of 6 time points (0h, 3h, 6h, 24h, 52h, 120h). Samples were deproteinized (12% trichloroacetic acid) and analyzed by enzymatic assays for both lactate and pyruvate. Results: Pyruvate concentrations were constant up to 6h in non-contaminated CSF samples at both concentrations (<5% decrease). The concentrations of pyruvate progressively decreased after 6h, reducing up to 45% after 120h and showing a greater instability at the low concentration level. In contaminated samples (hemolytic index=38), pyruvate concentrations showed no significant change through 120h (<3% decrease) at both high and low levels. At low concentration,

lactate concentration was constant through 48h in both contaminated and non-contaminated samples. High lactate concentration remained constant through 6h. Conclusion: Pyruvate and lactate at physiological concentrations in CSF specimens with and without blood contamination remain constant at RT up to 6h.



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Performance Evaluation of Olympus AU2700 Plus by Six-Sigma Using Three Different Internal Quality Control Materials

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Background: The analytical performances of the instruments in the medical laboratories should be satisfactory. Six-sigma level is a measurement of quality in the evaluation and comparison of performance. We evaluated the sigma levels of the parameters in our comprehensive metabolic panel in a single instrument by using different internal quality control (QC) materials.

Methods: The chemistry instrument evaluated was Olympus AU2700 Plus (Beckman-Coulter, Brea, CA, USA) and the QC materials provided were Beckman Coulter (Beckman-Coulter, Brea, CA, USA), BioRad (Bio-Rad Laboratories, Hercules, CA, USA) and Randox (Randox Laboratories, North Ireland, UK). Our comprehensive panel included glucose, creatinine, uric acid, total bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total cholesterol, triglyceride, calcium, potassium, magnesium, amylase, creatine kinase, gamma-glutamyl transferase (GGT), uric acid, total bilirubin, direct bilirubin, lactate dehydrogenase (LDH), sodium, chloride, albumin and total protein. We measured internal QC materials at two levels for 20 days consecutively. If a measurement was outside two standard deviation range then a rerun preformed. Analytical reproducibility assessed using the CLSI EP-5 protocol. CLIA criteria were the basis for the allowed total error values except for two parameters, which are GGT and direct bilirubin.

Results: Our data is expressed in the table with two cutoff values: six-sigma of more than six and less than three.

Conclusion: Health services aim zero error but being under the influence of many variables it is difficult to achieve this goal. Six-sigma methodology is useful in the planning of laboratory quality control process. The sigma level calculated for each test may provide insight about the quality. We found that the calculated sigma levels using different QC materials are similar in some parameters but different in others. We support the idea of using appropriate internal quality control material for each test and this procedure can guide in reducing the cost of poor quality.

Table 1: Calculated six-sigma values of three different QC materials.

QC material	Six-sigma >6	Six-sigma <3
Beckman Coulter level 1	Glucose, creatinine, uric acid, total bilirubin, ALT, ALP, total cholesterol, triglyceride, calcium, potassium, magnesium, amylase, creatine kinase, and GGT	Sodium, chloride, and total protein
Beckman Coulter level 2	Creatinine, uric acid, total bilirubin, AST, ALT, ALP, triglyceride, LDH, magnesium, amylase, and creatine kinase	Total protein, sodium, chloride, and GGT
BioRad level 1	Uric acid, total bilirubin, triglyceride, calcium, potassium, magnesium, and amylase	ALT, LDH, and sodium
BioRad level 2	Uric acid, ALP, triglyceride, potassium, chloride, magnesium, amylase, and creatine kinase	Urea, total bilirubin, AST, and sodium
Randox level 1	Uric acid, total bilirubin, total cholesterol, triglyceride, magnesium, amylase, and creatine kinase	Glucose, urea, AST, ALT, LDH, sodium, and chloride
Randox level 2	Albumin, uric acid, ALP, total cholesterol, triglyceride, magnesium, amylase, and creatine kinase	Glucose, LDH, and sodium

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Bilirubin oxidase resolves bilirubin interference in a colorimetric acetaminophen assay

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Background: Acetaminophen is a pain reliever found in over-the-counter and prescription medications. Acetaminophen toxicity is the most common cause of drug overdose and acute hepatic failure in the US. Measurement of acetaminophen levels in blood, once a suspicion of toxic ingestion has been established, is crucial for risk assessment, treatment and management. However, samples with high levels of bilirubin (icteric), which are very common in individuals with drug-induced hepatotoxicity, interfere with acetaminophen measurement in most commercial clinical assays and can lead to false positive results. Bilirubin oxidase (BOx) is an enzyme that catalyzes the oxidation of bilirubin to biliverdin. **Objective:** In this study, our objective was to explore the potential use of BOx as an additive to samples with high bilirubin concentrations to resolve acetaminophen interference. **Methods**: In experiment 1, pooled non-icteric heparinized plasma samples were spiked with acetaminophen and aliquoted. Aliquots were then spiked with acetaminophen-free samples containing different amounts of bilirubin. Acetaminophen and Icteric index (I-index) were measured at baseline and after a 30min incubation step with BOx (1 U/ml) at 37°C. In experiment 2, highly icteric patient samples (n=9, I-index>8) were analyzed before and after incubation with BOx (2 U/ml) at room temperature for 30min and 60min. All samples were analyzed using the Roche Integra 800, for which the manufacturer reports interference to acetaminophen with an I-index>4. Assay limit of detection is 15ug/mL. **Results**: See table. Experiment 1 demonstrated that BOx effectively removes bilirubin without affecting acetaminophen. Experiment 2 demonstrated that under normal lab conditions, BOx may be used to resolve false positive acetaminophen results due to elevated bilirubin. Data for 60min incubation not shown; all acetaminophen levels at 60min <15ug/mL. **Conclusion**: BOx can be used to resolve false positive acetaminophen results due to bilirubin interference. This suggests a potential role for BOx to resolve bilirubin interference in other clinical chemistry assays.

Experiment 1	Before BOx incubation		After BOx incubation (30 min at 37C)		Experiment 2	Before BOx incubation		After BOx incubation (30 min at RT)	
	I-index	Acetaminophen (ug/mL)	I-index	Acetaminophen (ug/mL)		I-index	Acetaminophen (ug/mL)	I-index	Acetaminophen (ug/mL)
Baseline	Non-icteric	22	Non-icteric	21	Patient 1	21.6	<15	8.8	<15
Sample 1	1.4	22	0.5	20	Patient 2	15.8	<15	7.4	<15
Sample 2	2.4	22	0.7	21	Patient 3	9.2	<15	6	<15
Sample 3	4	22	0.9	20	Patient 4	39.5	29	15.2	<15
Sample 4	7.7	23	1.4	19	Patient 5	40.2	24	12.7	<15
					Patient 6	8.9	<15	4.4	<15
					Patient 7	17.8	<15	5.5	<15
					Patient 8	16.4	<15	8.6	<15
					Patient 9	14.7	<15	6.1	<15

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Case Report: Paternity case with three genetic incompatibilities

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Background: The occurrence of germ line mutations at microsatellite loci may cause problems in ascertaining non-fatherhood status in paternity testing. A mutation event of DNA markers caused by loss or gain of repetitive units is quite a common phenomenon in forensic practice and is becoming more frequent due the increased number of paternity tests performed worldwide.

Objective: To describe a case that revealed a potentially erroneous exclusion from paternity due to the presence of three genetic inconsistences between the alleged father and the child.

Methods: The case was performed during the routine of paternity test at DASA. The DNA from mother, child and alleged father were obtained from blood samples in FTA-treated cards. The punching process was automated with BSD600 Duet equipment (Life Technologies, Foster City, CA). A total of 47 loci were analyzed. The STRs (Short Tandem Repeats) markers amplification was performed using AmpFlSTR® Identifiler® Direct PCR Amplification Kit (Life Technologies). Additional STRs were performed using Powerplex 16 HS System (Promega Corporation, Madison, WI), NGM Select (Life Technologies) and others 26 "in house" loci. The detection was performed at 3130*xl* Analyzer (Applied Biosystems, Foster City, CA). Paternity index (PI) was calculated including the mutation rates according to American Association of Blood Banks (AABB) report.

Results: Out of 47 analyzed loci, three genetic inconsistencies between the alleged father and the child were detected: two mutations at STRs D2S1338 e D2S1776 and the presence of null allele at STR TPOX. The inconsistencies at STRs D2S1338 and TPOX were confirmed by 2 different commercial kits, while the STR D2S1776 was confirmed by co-amplification. The combined paternity index in the case was 19.897.397.674.899,85 which corresponded to final probability of paternity value higher than 99.9999%.

Conclusion: The overall number of cases performed in our paternity laboratory, since 2005, is more than 38.000. This is the first case that we have found three genetic inconsistences in a non-exclusion paternity investigation. This case emphasizes the requirement that an exclusion from paternity must be based on calculating the appropriate statistical estimations in every case.

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Identification of key meta data to enable safe accurate and effective transferability of biological variation data.

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Background: Biological variation data (BVD) are reference data with many applications in laboratory medicine. Appropriate transfer of BVD across populations and through time requires the user to have -knowledge of the characteristics of the population from which the data were derived -an understanding of how the data were derived and -an appreciation of the uncertainty that surrounds the reported estimates. As a consequence an estimate of within and between subject biological variations should be transmitted and adopted for use only if accompanied by a set of meta data that sufficiently characterises the BVD in those contexts. The Biological Variation Working Group (BVWG), set up by the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM), have undertaken work to identify a candidate minimum data set (MDS) to accompany published indices of within and between subject biological variations to enable this issue to be addressed.

Methods: The BVWG considered and discussed the content of published literature and web based databases to identify the key meta data to accompany BVD to enable safe accurate and effective transfer and application across populations and through time.

Results: Key meta data were identified under six main headings. Those are, with example subheadings: -

Target - *analyte and measurand, sample matrix, method characteristics.* Population characteristics - *demographics, state of well being, physical/physiological characteristics, medication* Study Characteristics - *study duration and design, power of study to detect BVD indices, model assumptions, and statistical approach.* Data Characteristics - indices of biological variability, confidence intervals, tests for model assumptions. Publication Details - *links to the original publication.* Data rating - *new concept to be developed to indicate the quality of the BV data against a set of key criteria.* **Conclusion:** Published reviews of the literature describing BVD for albuminuria, haemoglobin A1c, C-reactive protein and liver enzymes all indicate a high degree of heterogeneity in the approach to derivation and reporting of BVD. Published BVD are of varying quality, often poorly characterised and consquently applied inappropriately into clinical laboratory practice. This highlights the need for generation of recognised standards for these important data sets.

The working group believe that availability of a standardised minimum data set, as proposed above, will enable users to be more objective in the transfer of published BVD into their local and wider practice. This will prove challenging to deliver, and require mechanisms to facilitate the extraction of meta-data from publications for attachment to the BVD to enable onward transmission and transferability (e.g. incorporation into databases). This will require further development of the concept of a BVD data archetype incorporating internationally accepted coding systems (e.g. SNOMED, LOINC) and vocabularies.

The MDS has been identified as part of a larger programme of work being undertaken by the BVWG aimed at developing a critical appraisal checklist for papers containing BVD. This will enable the creation of the data rating concept included in the MDS.

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Evaluation of Technopath Controls on the ARCHITECT Family of Instruments

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Introduction: Quality controls are important for laboratories to ensure that released results meet the required quality in regard to accuracy and precision. Consolidation of controls is an important trend in laboratories to simplify QC testing. Recently, multi-constituent controls (MCCs) have been introduced by Technopath Manufacturing Ltd (Ballina, Ireland) that cover a wide range of clinical chemistry and immunoassay analytes.

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

Methods: Three European sites (Paris, France; Stuttgart, Germany; Sondrio, Italy) used the three controls for a minimum of thirty days in parallel with the lab's routine QC controls. Testing was performed on the ARCHITECT *c*8000, *c*16000, $i1000_{SR}$ and $i2000_{SR}$ instruments. Data presented here are from the following serum clinical chemistry analytes: (A)ALT, (A)AST, total bilirubin, chloride, total cholesterol, creatinine (enzymatic and picrate), glucose, potassium, total protein, sodium, triglycerides and urea; the following immunoassays: CA 19-9, CEA, total PSA, free T3, free T4, TSH, troponin-I, total beta HCG, testosterone, estradiol and FSH; and on the following urine assays: chloride, creatinine (enzymatic and picrate), glucose, potassium, sodium and urea. The Multichem S Plus and IA Plus are serum based with three control levels; the Multichem U is prepared from human urine with two control

levels. All data were collected via AbbottLink for automated data retrieval. Means, standard deviations and ranges were calculated for all controls. Assay reagent lots and calibrator lots varied across the sites and within the sites.

Results: The results from twelve frequently performed clinical chemistry assays were analyzed. The %CV for the 12 assays with the Multichem S Plus control ranged from 0.42 to 4.71% at the individual sites. The %CV for the 6 assays with the Multichem U control ranged from 0.50 to 5.24% at the individual sites. For both controls, the majority of the CVs were less than 2%. The results from these eleven frequently performed immunoassays were analyzed. The %CV for the 11 assays with the Multichem IA Plus control ranged from 1.82 to 14.94% at the individual sites; however the majority of the CVs were less than 5%. Overall little variation was seen instrument to instrument, site to site or reagent lot to reagent lot.

Conclusions: The Technopath S Plus, IA Plus and U controls demonstrated similar performance to the routine internal laboratory quality controls. The use of these MCCs reduce the number of controls required for the analytical quality control testing of both clinical chemistry and immunoassay analytes with no compromise on quality.

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Equivalency Testing of Serum and Plasma on the Siemens Dimension Vista® 1500 System at University Hospitals (UH) Case Medical Center

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Background: UH Case Medical Center has 1032 beds and processes 6000 samples a day and 5.2 million tests per year. Consistent and rapid turnaround time (TAT) requires efficient processing and analysis of samples. Using plasma will eliminate clotting time and decrease pre-analytical processing aiming to shorten TAT.

Objective: To demonstrate the equivalency between serum (x) and plasma (y) on assays performed on the Dimension Vista System.

Methodology: Serum and lithium heparinized plasma samples were obtained from a single draw in individual patients in the hospitalized population and tested in parallel. Least squares regression analysis was performed on single determinations of serum (x) and corresponding plasma (y) samples. The criteria used for assessing equivalency were a slope of 0.90 to 1.10, an intercept that is clinically insignificant, a correlation coefficient greater than 0.95 and average percent bias (plasma-serum) less than 10% or the CLIA Total Allowable Error, whichever was greater.

Results: 46 assays (n=53-59 serum and corresponding plasma samples) evaluated met the above criteria. Table 1 lists data observed on representative assays of different method principles. The minimum and maximum slope, correlation coefficient and average percent bias observed for 46 assays are 0.92-1.05, 0.976-1.000 and -8.7 to 5.3%, respectively. Intercepts for these assays were clinically insignificant.

Table 1

Assay	Units	Slope	Intercept	Correlation Coefficient	n	Low	High	Average % Bias (plasma-serum)
1. ALB	g/dL	0.995	-0.037	0.977	56	2.1	3.9	-1.7
2. A1AT	mg/dL	0.947	6.875	0.983	57	116	376	-2.0
3. ALTI	U/L	0.977	-0.005	0.998	58	10	168	-2.3
4. ASL	U/mL	0.962	0.855	0.997	59	13	342	-2.9
5. AST	U/L	1.020	-0.780	0.998	58	7	232	-0.3
6. B2MIC	mg/L	0.992	0.014	1.000	58	1.21	33.19	-0.4
7. FERR	ng/mL	0.978	-1.096	0.999	58	21.2	3108	-2.4
8. FT4	ng/mL	0.991	0.013	0.993	58	0.67	1.97	0.1
9. GLU	mg/dL	1.053	-0.010	0.991	53	69	208	5.3
10. hsCRP	mg/L	1.009	0.360	0.997	55	0.16	164.2	1.6
11. IGG	mg/dL	0.991	-4.038	0.997	57	307	2605	-1.4
12. PBNP	pg/mL	0.986	18.027	1.000	56	25	14637	0.1
13. K	mmol/L	0.988	-0.072	0.976	56	3.2	4.6	-3.0

Other assays tested for serum vs. plasma equivalency: 1) ALP, 2) BUN, 3) CRP, 4) CL 5) CHOL, 6) CKMB, 7) C3,
8) C4, 9) CKI ,10) DBI, 11) ECREA, 12) FT3, 13) GGT, 14) HAPT, 15) HDLC, 16) IGA ,17) IGM,18) IRON, 19) LDI, 20) LDLC, 21) LIPL, 22) MYO, 23) PHOS, 24) PREALB, 25) RF, 26) NA, 27) TBI, 28) TIBC, 29) TP, 30) TRF, 31) TRIG, 32) TSH, 33) URCA

Conclusions The observed data on 46 assays support the equivalency of serum and plasma on the Dimension Vista System based on the predefined evaluation parameters to demonstrate serum and plasma equivalency.

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25-Hydroxyvitamin D2: Prevalence and Impact on 25-Hydroxyvitamin D Measurement

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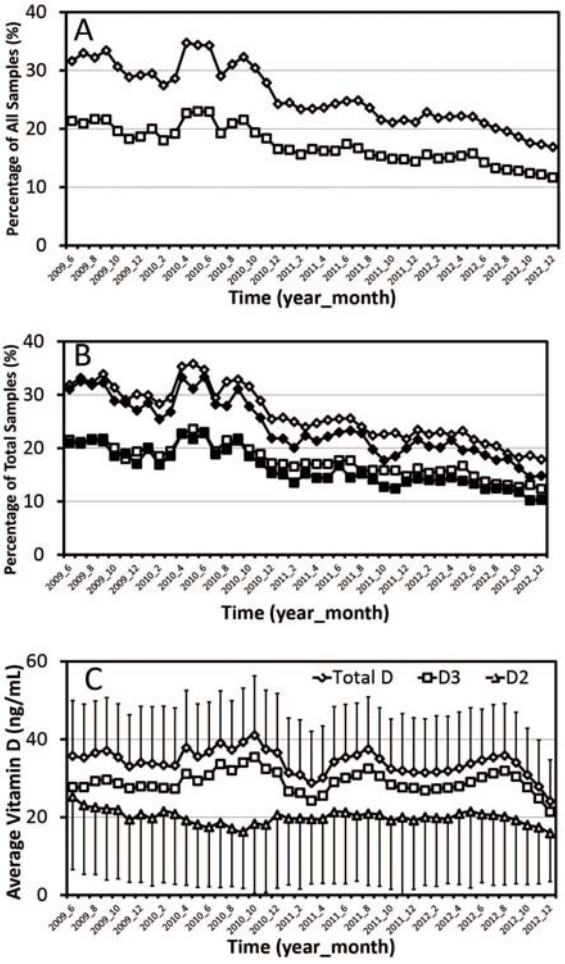
Background: This study is to evaluate the distribution of 25OH-D2/D3 in a general patient population in western New York to provide insights into the current prevalence and the trend of vitamin D2 usage, and its impact on the accuracy of vitamin D measurement.

Methods: 25OH-D2 and 25OH-D3 results measured by in-house LC-MS/MS method at Strong Memorial Hospital at the University of Rochester Medical Center from June 2009 to December 2012 were retrospectively analyzed. The results were retrieved through the laboratory information system using specific parameters to include patients' age, gender, 25OH-D2, 25OH-D3, and total 25OH-D data. All the results during that period of time were included except research samples and the ones with missing data. The mean, standard deviation, overall distribution and monthly prevalence of 25OH-D2, 25OH-D3, and total 25OH-D were calculated using Statistica 10 Software. The significant levels were assessed using two tailed student T test in EXCEL. The limit of quantification (LoQ) for 25OH-D2 and 25OH-D3 were &lt 4ng/mL and &lt 6ng/mL, respectively. Total 25OH-D level of 60 ng/mL was considered potentially harmful.

Results: A total of 266,269 samples from were included for analysis. The percentage of samples with 25OH-D2 levels above assay LoQ decreased from 32% to 17% over the course of the study period. The percentage of samples with 25OH-D2 levels higher than those of 25OH-D3 decreased from 21% to 12% (see figure). Sixty-seven percent of the samples with 25OH-D2 levels above LoQ had serum concentrations of 25OH-D2 higher than those of 25OH-D3.

Conclusion: 25OH-D2, despite its gradual decrease in local prevalence, was still present in 17% of the patients tested in December 2012, many of whom had 25OH-D2 levels higher than those of 25OH-D3. To achieve accurate vitamin D measurement, clinical laboratories should assess the accuracy of their assays for both isomers, and if necessary, determine their local prevalence of 25OH-D2.



A-263

Stability Studies of Hemoglobin A1c (HbA1c) Based on Specimen Storage

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Background: Hemoglobin A1c (HbA1c) is useful for the assessment of glycemic control in patients with known or suspected diabetes and, in some cases, for the diagnosis of diabetes. While freshly collected samples are preferred, in some instances it is necessary to test for HbA1c on stored, whole blood samples. Published studies suggest that both storage time and temperature can affect the HbA1c stability, therefore we investigated the effect of room temperature storage on HbA1c up to seven days, considering variables between specimens including levels of ambient glucose and labile HbA1c.

Methods: Twenty-five patients were included in the study; samples collected from each patient included whole blood (EDTA) and plasma (heparin). Patients were selected to reflect a wide range of HbA1c and glucose values: HbA1c; 4.8-14.7 % and glucose; 95-558 mg/dL. The whole blood samples were stored at room temperature (20°C) then analyzed for HbA1c by HPLC (Variant II Turbo) on days Zero, 4, and 7; whole blood glucose levels were measured on the Abbott i-Stat while baseline plasma glucose levels were also measured on the UniCel DxC800 (Beckman Coulter).

The HbA1c values obtained on days Zero, 4, and 7 were compared by two statistical methods: 1) the means for days 4 and 7 were compared to day Zero levels using the two-tailed paired Student's t-test, and 2) linear regression analysis to assess the degree of change in regression line slope over the incubation period.

Results: The HbA1c values obtained on Day Zero ranged from 4.8 to 14.7% (mean=9.1 +/- 3.6%) representing a wide spectrum of normal and abnormal values; values obtained on Days 4 and 7 ranged from 5.4 to 15.7% (mean=9.1+/- 3.7%) and 5.3 to 15.9% (mean=9.0+/- 3.9%) respectively. The Student's t-test indicates that the Day 4 and Day 7 means are not statistically different from that observed on Day Zero (p=0.86, Day 4; p=0.57,Day 7). Baseline ambient glucose ranged from 95 to 558 mg/dL (mean=217+/-144); baseline labile HbA1c ranged from 1.1 to 6.2% (mean=2.5+/-1.6%). Linear regression analysis of the Days 4 and 7 HbA1c values versus those on Day Zero indicates only a 3 percent proportional difference on Day 4 with an observed slope of 1.03. The latter change could result in an increase of up to 0.18% at the 6.1% cutoff. However, on Day 7, a proportional difference of 8 percent was observed (slope=1.08) suggesting that older stored samples show a consistent positive proportional bias amounting to as much as 0.5% at our current reference range cutoff of 6.1. The Day 4 and 7 regression lines show a good fit (Day 4,Syx=+/-0.37,r^2=0.991; Day 7, Syx=+/-0.74,r^2= 0.967).

Conclusion: We conclude that HbA1c can be performed on whole blood samples stored at room temperature for up to 4 days, irrespective of ambient glucose levels, with no statistically significant change in levels. However increases of up to 3% from baseline should be expected. Test results for HbA1c on whole blood samples stored for 7 days show an unacceptably high degree of proportional error of 8 percent.

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The message content of quality control rules

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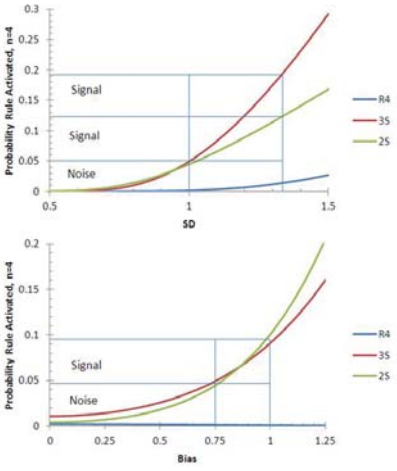
Background: We assessed the ability of three quality control (QC) rules (R_{4S} [R4], 1_{3S} [3S] and 2_{2S} [2S]) to provide signal compared with noise and discriminate between imprecision (random error) and bias. QC rules should identify unacceptable runs and discriminate specifically between bias (B) and random error (standard deviation, SD). Comparing the probability of signal with that of noise quantifies the message content. Appropriate corrective action depends on signal, not noise, and requires discrimination between errors types.

Methods: We calculated the number (*N*) of expected combinations of quality control messages for each rule based on the number of control materials. We calculated baseline probabilities (*P*) of rule activation using the cumulative distribution function (CDF), varying bias (B) from 0 to 1.25 and SD from 0.5 to 1.5, assuming the following allowable errors: **$Error_{Total}$=3, $Error_{Bias}$=0.75; and $Error_{SD}$=1.** ***P(R4)=2{CDF(B,SD,-2)}{1-CDF(B,SD,2)}; P(3S)={CDF(B,SD,-3}+{1-CDF(B,SD,3)}; P(2S)={CDF(B,SD,-2)}^{2}+{1-CDF(B,SD,2)}^{2}. P(Noise)=P(RuleActivation|B=0.75,SD=1); P(Total)=P(rule-activation|B>0.75,SD>1); P(Signal)=P(Total)-P(Noise) P(CombinationActivation)=N*BaselineProbability=N_j*P_j(Signal); j=[R4,3S,2S],i=[SD,Bias].*** **Results:**The number of message combinations (*N*) is *n/2* for R4, *n* for 3S, and *3(n/2-1)+1* for 2S for *n-controls*; for 4-controls: N=2 for R4, N=4 for 3S, and N=4 for 2S. The figure shows the *CombinationActivation* probabilities, for n=4 controls, as a function of bias or SD. Given bias=1 or SD=1.33, *CombinationActivation* probabilities are as follows: P_{R4}(Noise)=0.0012, P_{R4}($Signal_{Random}$)=0.012 (<3% messages), P_{R4}($Signal_{Bias}$)=0.000; P_{3S}(Noise)=0.049 (21% of 3S messages), P_{3S}($Signal_{Random}$)=0.142 (77% of 3S Signals), P_{3S}($Signal_{Bias}$)=0.042; P_{2S}(Noise)=0.045 (25% of 2S messages), P_{2S}($Signal_{Random}$)=0.077 (58% of 2S Signals), P_{2S}($Signal_{Bias}$)=0.056.

Conclusion: The 3S and 2S rules impart significant *noise* (22% of messages). R4 specifically identifies random error but represents < 3% of messages. 3S and 2S discriminate poorly between random and bias errors, with only 77% of messages representing random errors for 3S and 58% for 2S. The quality control rules demonstrate inadequacies in ascertaining run acceptability and discriminating between imprecision and bias.



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Comparison of Uncertainty Values Between Core and Emergency Laboratories for Routine Biochemical Parameters

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Background/Aim: Most regulatory authorities that use International Organization for Standardization (ISO) Standards to assess laboratory competence require an estimate of the uncertainty of measurement of assay test results. According to ISO 15189, the uncertainty of measurement is a parameter associated with the result of a measurement that characterizes the dispersion of the values that could be reasonably attributed to the measurand. ISO 15189 requires that "The laboratory shall determine the uncertainty of results, where relevant and possible". In this study, we aimed to calculate the uncertainty of measurement of our core and emergency laboratory, in terms of routine biochemistry tests.

Materials and Methods: Study was conducted at the Laboratory of Department of Medical Biochemistry in Gulhane School of Medicine, Ankara, Turkey. Internal and External Quality Control (IQC and EQC, respectively) results of two Olympus AU2700 (device 1 and 2 in core laboratory) and one Olympus AU 640 (device 3 in emergency laboratory) autoanalyzers were assessed in the scope of this study. 24 parameters were evaluated between a period of January 2013-December 2013. The calculations were derived from the EURACHEM/CITAC and AACB guides. Then we assessed the analytical performances and uncertainty of measurements according to CLIA, Rilibak, Fraiser and Six Sigma.

Results: Considering the evaluation criteria; the calculated uncertainty according to AACB of three devices met the expectations for in all parameters. However, Cl^- results of device 1 and 3 were determined above CLIA while K^+ results of device 1 and Na^+ results of device 3 were above Rilibak' criteria when calculated according to EURACHEM. Moreover, calculated total error (TE) rates of Na^+, K^+, Cl^-, calcium and total protein were above Fraser' TE rates. Cl^- and urea in all devices; total protein, albumin, Na^+, K^+ and calcium in device 3; Na^+, creatinine and ALP in device 2 were under 3 sigma, according to six sigma.

Conclusion: This study shows that the Core Biochemistry Laboratory of Gulhane School of Medicine Hospital results met the expectations within the appropriate limits of total error defined by CLIA, Rilibak, Fraser in most of the parameters. However, in emergency laboratory, most results were found to be close to the upper limit or above the criteria. As expected, it's quite difficult for emergency laboratories, that work for 24 hours a day and 7 days a week, to meet all criteria due to shift and personnel changes. Establishing uncertainty of measurement guidelines for clinical laboratories can accelerate the pace of understanding both for clinicians and clinical laboratory practitioners.

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Variables impacting the analytic false positive rate for Cardiac Troponin T on the Roche cobas e411

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Background: We have observed rare occurrences of analytic false positive results for cardiac Troponin T (cTnT) using plasma separator tubes (PST) and the STAT Troponin T assay on the Roche cobas e411 immunoassay analyzer. Internal and external (assay manufacturer) discussions identified several potential sources of error: cellular debris, intermittent carryover, electrical supply fluctuation, and inadequate sample centrifugation. PST has historically been used for cTnT testing because it allows for faster processing than serum tubes. Previous studies have demonstrated PST specimens contain more cellular debris. This increases the likelihood of micro-clot formation in the analyzer sample path; which could negatively impact the assay accuracy. The goal of our study was to identify and minimize variables associated with analytic false positive cTnT results while maintaining acceptable turnaround time.

Methods: Our Emergency Department (ED) patients receive a panel consisting of cTnT measurement at 0, 3, and 6 hours after presentation. To capture analytic false positive results, all samples with 0 hour cTnT ≥ 0.04 ng/mL, or 3 or 6 hour samples demonstrating a change of ≥ 0.03 ng/mL from 0 hour value, were repeated. All samples were repeated in this manner between 2007 and 2012. An analytic false positive was defined as a sample that upon repeat analysis demonstrated a change in results of +/- 0.04 ng/mL for cTnT values ≤ 0.10 ng/mL or +/- 20% for values >0.10 ng/mL. Initial attempts to reduce analytic false positives included adjusting centrifugation times from 3 to 5 minutes; and manufacturer-performed instrument and measuring cell performance verification when false positives were noted. To address relatively higher concentrations of cellular debris in PST samples, a rapid clot serum tube (RST) was implemented mid-2012. RST tubes fully clot within 5 minutes, drastically reducing the clot time (up to 30 minutes) of traditional serum tubes. Finally, the electrical power source for the analyzer was stabilized and a redesigned measuring cell was installed, both at the recommendation of the manufacturer.

Results: Neither increasing centrifugation time, performance verification after false positives, nor installation of the redesigned measuring cell significantly impacted the rate of false positives observed (1 in 2200). A slight decrease in analytic false positives was observed when switching from PST to RST (1 in 2200 vs. 1 in 2375, respectively). The most significant decrease in false positive rate was observed when the electrical power was stabilized while using RST. Under these conditions false positive rate decreased to approximately 1 in 5000. In order to determine if this decrease would also be observed with PST; RST vs. PST was re-evaluated with electrical stabilization. RST continued to show a decreased false positive rate.

Conclusion: The combination of electrical power supply stabilization and implementation of RST decreased the rate of analytic false positive cTnT results on the cobas e411 to approximately 1 in 5000. This facilitated rapid analysis of cTnT samples while eliminating the need to repeat positive results for patients presenting to the ED.

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Effects of 49 Different Rare Hb Variants on HbA1c Measurement in Seven Methods

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Background: Hemoglobin A1c (HbA1c) is recommended for routine monitoring of long-term glycemic control in patients with diabetes and for use in diabetes diagnosis. Previous studies have shown interference from the four most common heterozygous Hb variants (HbAS, HbAE, HbAC, and HbAD) with some HbA1c assay methods. Here we examine analytical interference from 49 different less common variants with 7 different HbA1c methods using various method principles (ion-exchange HPLC, boronate affinity HPLC, immunoassay, and enzymatic).

Methods: Hb variants were screened using the Bio-Rad Variant II beta thal short program, confirmed by alkaline and acid electrophoresis, and identified by sequence analysis. The Trinity ultra2 boronate affinity HPLC method and Roche Tina-quant immunoassay were used as primary and secondary comparative methods, respectively, since these methods are least likely to show interference from Hb variants. Other methods included were the Tosoh G7 and G8, Bio-Rad D-10 and Variant II Turbo, and Diazyme Enzymatic. Identified variant samples (n=88) and a group of non-variant (AA) samples (n=92) were analyzed by each method; there were multiple samples for some variants. Samples with HbF >10% (based on G8 %HbF) were excluded. To eliminate any inherent calibration bias, results for each method were adjusted using regression verses the ultra2 with AA samples. When results from the two comparative methods matched within 10%, all other methods' calibration-adjusted results were compared and judged to be acceptable if within 10% of the ultra2 result. For five variants (one sample each), results from the two comparative methods were discordant and this could not be explained by an amino acid substitution that would affect binding of the Tina-quant antibody; these results were excluded from further evaluation.

Results: Almost all variant samples were recognized as such by the ion-exchange HPLC methods by the presence of abnormal peaks or results outside the reportable range. For most variants (80%), interference (>10% difference from ultra2 or no result reported) was seen with one or more of the ion-exchange methods. Following manufacturer instructions for interpretation of chromatograms usually, but not always, prevented reporting of inaccurate results. For 14 variants studied, the Diazyme results were inaccurate; this method does not detect the presence of variants and all results are reported.

Conclusions: In order to insure that accurate HbA1c results are obtained for all patients, it is important to know if a patient has a hemoglobin variant and how that variant can affect their HbA1c results. Laboratories must be cautious about reporting results when the presence of a variant is suspected. Manufacturers may need to clarify and/or tighten their criteria for accepting results and update their software to flag potential variants in an effort to reduce the likelihood of incorrect HbA1c results being reported.

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Evaluation of HbA1c measurement in Trinidad and Tobago

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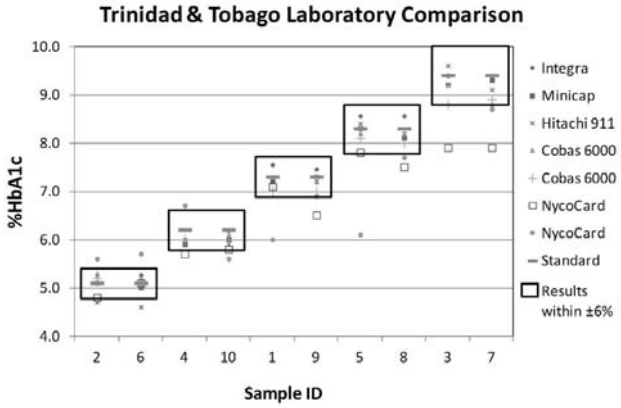
Background: HbA1c is recommended for routine monitoring of long-term glycemic control in patients with diabetes and also for diabetes diagnosis. The prevalence of diabetes in Trinidad and Tobago is over 12%. Although HbA1c testing is offered by public and private sector laboratories, no HbA1c proficiency testing (PT) has previously been performed. Therefore, Johns Hopkins Medicine International and the Diabetes Diagnostic Laboratory (DDL) at the University of Missouri organized a pilot HbA1c Proficiency Testing Study for 7 key laboratories as part of the Trinidad and Tobago Health Sciences Initiative.

Methods: Sets of 10 samples containing blinded duplicates were created from five whole blood pools with HbA1c levels from 5.1 to 9.3% HbA1c and shipped to participating laboratories. To assess within-day imprecision, the pooled estimate of the SDs between the duplicates (Sp) was calculated; 0.229 was the acceptable limit based on the current NGSP HbA1c standardization program monitoring criterion. To assess accuracy, each laboratory's results were compared to an NGSP Secondary Reference Laboratory (SRL9 using Tosoh G8).

Results: One laboratory reported results as IFCC %HbA1c; these were aligned to NGSP using the NGSP/IFCC master equation [NGSP%=0.915(IFCC%)+2.15]. Methods used by the laboratories included Roche Tina quant on Cobas Integra, Cobas 6000 and Hitachi 911, Sebia Minicap, and Axis-Shield NycoCard. All laboratories except the two using the NycoCard showed acceptable imprecision. All results from three laboratories (one each using Sebia Minicap, Roche Cobas 6000, and Roche Cobas Integra) were within 6% of the NGSP SRL assigned values; most results from the two laboratories using the NycoCard were outside 6%.

Conclusion: Because inconsistent HbA1c results can negatively impact patient care, it was recommended that all laboratories report NGSP % HbA1c and those using the NycoCard use a more precise method. Laboratories are instituting changes based on these findings and a future study will re-assess performance.



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Identifying G6PD Deficiency: Defining the Lower Reference Limit and Evaluating Pre-analytical Sources of Error

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Background: Glucose-6-phosphate dehydrogenase (G6PD) protects red blood cells (RBCs) from oxidative damage by regenerating NADPH. G6PD deficiency is the most common X-linked enzymopathy that results in hemolytic anemia triggered by oxidative stress. Diagnosis of G6PD deficiency is facilitated by determining its activity in RBCs and interpreting the result against the lower limit of a reference interval (LLRI). Despite the use of similar methods, the LLRI for G6PD varies considerably between laboratories (range, 4.6-8.8; N=4). The objectives of this study was to 1) validate the LLRI of the G6PD reference interval used by our laboratory (7.0-20.5 U/gHb) and 2) to identify pre-analytic sources of error that may influence the accuracy of G6PD test results.

Methods: A G6PD reference interval was determined empirically and by the Hoffman method. For the empirical approach, whole blood was collected from 120 reference subjects (59 males and 61 females; 19-75 years) and G6PD determined by kinetic spectrophotometry using commercially available reagents (Trinity Biotech USA) at 37°C. For the Hoffman method, 20,736 G6PD test results were extracted from the laboratory information system and linear regression performed over the linear portion of the cumulative frequency distribution. The LLRI and upper limit of the reference interval (ULRI) were calculated as LLRI=2.5(slope)+intercept and ULRI=97.5(slope)+intercept. The effects of pre-analytical sources of error were investigated using samples submitted to the laboratory for G6PD testing and included the method of vortex mixing, incubation time for RBC lysis, and analytical dwell time. The study was approved the University of Utah Institutional Review Board.

Results: G6PD activities in 120 reference subjects ranged from 9.0-14.7 U/gHb (median=11.6). The non-parametric reference interval was 9.9-14.1 U/gHb. G6PD activities in the 20,736 clinical subjects ranged from 0.3-86.2 U/gHb (median=13.2). 6.1% of the samples were less than the current LLRI of 7.0 U/gHb. The Hoffman technique yielded a reference interval of 9.9-16.6 U/gHb. Applying this LLRI to the clinical subjects identified 8.9% as deficient. The method of vortex-mixing was evaluated in 40 samples and did not significantly affect G6PD activity. Compared to the reference technique (single-tube with single-tube vortex mixer) which produced a mean G6PD activity of 13.0 U/gHb, the use of a tube rack with multi-tube vortex mixer or a tube rack with single-tube vortex mixer produced mean activities of 12.9 and 13.2 U/gHb, respectively ($p>0.24$). The incubation time for RBC lysis after vortex-mixing did affect G6PD activity. Compared to the reference time of 5 minutes, a 3-minute incubation had no effect (mean difference 0.2 U/gHb, $p=0.05$) but 15- and 30-minute incubation times produced significantly lower results (mean difference -1.0 and -2.1 U/gHb, respectively; $p<0.0001$). Analytical dwell time (40 minutes) had no significant effect on G6PD activity as determined from 70 aliquots of a whole blood sample that produced a CV of 1.5% at a mean of 15.6 U/gHb ($r=0.12$, $p=0.32$). Conclusions: The G6PD LLRI should be 9.9 U/gHb as determined empirically and by the Hoffman method. The incubation time for RBC lysis is a variable that should be controlled when performing G6PD testing.

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Diurnal Variation of analytes: an underestimated pre-analytical factor in clinical chemistry

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Objectives: Diurnal variation is a well known cause of biological variation. We set out to identify analytes affected by diurnal variation, the amplitude of the within day difference and whether collection instructions specified the impact of collection time on test interpretation.

Design and Methods: We identified analytes reported to undergo diurnal variation through a review of Tietz's Textbook of Clinical Chemistry and through a search of Pub Med to capture recent citations. We checked if the time of collection, and its potential impact on test interpretation, was specified in the posted collection procedures issued by two large commercial labs in Ontario.

Results: Our search identified 21 analytes affected by diurnal variation. As expected, cortisol, the archetypal diurnally changing analyte, was the most cited. Hormones represented the major class of identified analytes, but fasting plasma glucose (FPG), MR-proANP, and hepcidin were other noteworthy inclusions. Diurnal amplitude ranged from a low of 1.1 for FPG (still sufficiently large to degrade test performance in ruling diabetes in or out using the cutpoint of 7.0 mM) to a maximum of 65-fold for melatonin. Of the 14 analytes listed in posted collection instructions, the importance of the time of collection was specified for only 5 (36%).

Conclusions: The confounding effect of diurnal variation on test interpretation is underappreciated given that cautions regarding when analytes should be collected were issued in collection instructions only 36% of the time.

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Can platelet aggregation testing by Multiplate be influenced by one minute tourniquet application?

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Background: The study of the role of platelets in the pathogenesis of ischemic vascular diseases and the monitoring of antiplatelet drugs require reliable platelet-function tests. Presently several techniques are in use to measure platelet aggregation.

Assessment of platelet function by multiple electrode aggregometry (Multiplate® Roche, Germany) is common in laboratory practices. Indeed, sample collection and processing are essential steps for quality assurance, so that the fulfillment of standardized preanalytical conditions are key factors in maintaining patient safety. The collection of diagnostic blood specimens for Multiplate is traditionally performed by medical staff using a tourniquet for evidencing veins. The Clinical and Laboratory Standards Institute H03-A6 document, recommends the use of the tourniquet for localizing suitable veins and the tourniquet time can not in any case be extended over 60 sec. This study was aimed to assess the impact of 60 sec tourniquet application on platelet function evaluated by multiple electrode aggregometry (Multiplate).

Methods: Ten volunteers, after 12-hours fasting, were maintained seated during 15 minutes to eliminate possible interferences from both lipemia and blood distribution due to different posture. After this time frame, 6mL of blood were collected by venipuncture with a 20G straight needle directly into two 3.0mL Hirudin Blood Tube for Multiplate® analysis (proprietary vacuum tube 06670105001, Roche Diagnostics GmbH, Penzberg, Germany) from two different procedures: Procedure I (no stasis) - a radial vein was localized on right forearm by a subcutaneous tissue transilluminator device without tourniquet, to prevent interference from venous stasis. Procedure II (stasis) - a radial vein was localized on the left forearm by tourniquet application during 60 sec prior to venipuncture. To eliminate any potential interference due to either the contact phase or the tissue factor all 1st tubes from each volunteer for both procedures were discarded. All diagnostic blood specimens were mixed gently and carefully by five times inversion, as recommended by the manufacturer. All samples were processed for assessment of platelet function (ADP-test®, ASPI-test®, COL-test®, RISTO-test® and TRAP-test®) by multiple electrode aggregometry (<15 min after collection) on the same Multiplate (Roche Diagnostics GmbH, Penzberg, Germany). In the Multiplate Test Cell, activated platelets adhere to and aggregate on the sensor wires. This leads to an increased resistance between the sensor wires, which in continuously recorded and expressed via the area under the curve in arbitrary units. The significance of differences between samples was assessed by paired Student's t-test after checking for normality by the D'Agostino-Pearson omnibus test. The level of statistical significance was set at $p<0.05$.

Results: All platelet functions tested showed lower values from samples collected with tourniquet than without tourniquet. Significant differences ($p<0.05$) were observed for RISTO-test (-16%), ADP-test (-10%) and TRAP-test (-8%) determination in samples collected after 60 sec tourniquet application.

Conclusion: The significant variations after 60 sec tourniquet application suggest that platelets could undergo a sort of pre-activation by venous stasis, thus decreasing the sensitivity to subsequent activation by agonists. Furthermore, this effect can compromise clinical results interpretation and jeopardize patient safety. In conclusion tourniquet application should be avoided during phlebotomy for Multiplate analyses.

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Impacts of sample volume and stopper on the stability of ethanol in lithium heparin plasma

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Background: It is recommended that ethanol samples should be analyzed immediately upon opening the tube, but there is no evidence about how long the ethanol is stable in real specimen tubes without stoppers. The objective of this study is to evaluate the stability of ethanol with different sample volumes in unstoppered and stoppered tubes at room temperature (RT).

Methods: Heparin plasma samples with ethanol ≥ 10 mg/dL were selected for this study and stored at -20°C until analysis. Ethanol was analyzed on UniCel DxC800 Systems by an enzymatic assay. To determine the impact of stopper on ethanol at RT, 30 plasma samples with a volume of 100 µL stored in 5 mL plain tubes at RT with and without stoppers and ethanol was measured after 1, 2 and 3 hours of baseline measurement. To determine the influence of sample volume on ethanol stability, 30 heparin separator tubes each with a total volume (including cells, gel, and plasma) of 3, 4 and 5 mL with plasma volume of 0.5, 1 and 2 mL respectively were stored at RT without stoppers and ethanol was measured after 1, 2 and 3 hours of baseline measurement. The allowable total error (ATE) for ethanol was 25%.

Results: The average ethanol concentrations in samples with different volumes with and without stoppers and recoveries were shown in table1.

Conclusions: Ethanol concentrations are within ATE for samples stored for at least 3 hr without stopper at RT with a minimum of 0.5 mL plasma in original heparin plasma separator tubes and for samples stored in plain tubes with stoppers at RT for at least 3 hr with a minimum volume of 100 µL, whereas ethanol in this low volume of sample in plain tubes is unstable for more than 2 hr at RT without a stopper.

Table1: Stability of ethanol with various volumes at room temperature

		Base Line	1h	2h	3h
100 µL of P* (NS)	Mean ± SD (mg/dL)	143 ± 95	126 ± 84	107 ± 72	91 ± 60
	R (%)	100	88	76	66
100 µL of P* (ST)	Mean ± SD (mg/dL)	143 ± 95	144 ± 95	144 ± 95	144 ± 96
	R (%)	100	101	101	101
3 mL TV (0.5 mL P*) (NS)	Mean ± SD (mg/dL)	142 ± 94	141 ± 94	137 ± 92	131 ± 87
	R (%)	100	99	96	92
4 mL TV (1 mL P*) (NS)	Mean ± SD (mg/dL)	153 ± 100	150 ± 98	147 ± 95	143 ± 94
	R (%)	100	98	95	94
5 mL TV (2 mL P*) (NS)	Mean ± SD (mg/dL)	142 ± 73	141 ± 72	138 ± 71	136 ± 71
	R (%)	100	99	97	96

TV: Total Volume, P*: Plasma, R: Recovery, ST: Stopper, NS: No-Stopper

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Evaluation of a method to detect prozone/hook effect/antigen excess phenomenon for Free Light Chain quantification using a simple pooling protocol

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Background: Antigen excess is an important issue that can lead to underestimation of patient's results and misdiagnosis. With patient samples which can range from less than 1mg/L to over 100 000mg/L, serum free light chain (FLC) measurement is especially prone to this interference. Some institutions choose not to implement on site testing for FLC, due to the lack of an instrument platform which can automatically detect antigen excess and in which concerns over workflow interruptions and personnel costs inhibit implementation of an antigetn excess protocol.

Methods: We propose a methodology based on sample pooling for a fast and cost effective method to detect of antigen excess phenomenon for serum free light chain quantification. Our strategy was evaluated on the Immage (Beckman) and on the BNII (Siemens) nephelometers using the Freelite® assay (The Binding Site).

Results: First, we evaluated the sensitivity of our strategy using a pool of 15 mixed samples spiked with different concentrations of kappa or lambda free light chain. Secondly, patients with antigen excess ranging from 1192 to 8500 mg/L of kappa free light chain were efficiently detected using our strategy. Finally, a preliminary evaluation of the size of the pools was conducted using pools ranging from 15 to 42 different samples. Based on the precision of the method, our preliminary data suggest that the number of samples in a pool can reach up to 43 different patients to detect an antigen excess of 1192 mg/L.

Conclusion: We described and validated a strategy based on sample pooling for detection of antigen excess on free light chain measurement. This approach could be suitable for any laboratory measuring serum free light chain that does not currently have an instrument platform for detection of antigen excess.

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Performance Evaluation of Rapid Test Strips for the Identification of EDTA-Containing Specimens

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Background: Most laboratory assays have specimen acceptability requirements. Proper identification of ethylenediaminetetraacetic acid (EDTA) containing specimens is frequently necessary when investigating mislabeled specimens, suspected primary tube mixtures, and aliquots. The purpose of this study was to evaluate the performance of commercially available EDTA test strips using biological specimens.

Methods: Studies included human sera and plasma specimens, bovine calf serum, and phosphate-buffered saline. Specimens were applied to test strips (Quantofix® EDTA; Macherey-Nagel) using a pipet ("drop mode"). These test strips are embedded with bismuth nitrate, xylenol-orange, and citric acid to detect chelating agents such as EDTA. Test strips were blotted to remove excess fluid; visual evaluation was made at 15 seconds. Reactions were scored on a scale from red (no EDTA), orange (low EDTA), to yellow (EDTA). A NODE+Chroma color detector (Variable, Inc) on a custom 3D-printed test strip channel was also used to capture quantitative RGB values

on a Bluetooth-linked iPhone 5, thus permitting additional statistical analysis and graphical display. Potentially reactive chelating agents tested included alpha lipoic acid (ALA), deferoxamine (DEF), 2,3-dimercapto-1-propanesulfonic acid (DMPS), dimercaptosuccinic acid (DMSA), K_2EDTA, Na_2EDTA, ethylene glycol tetraacetic acid (EGTA), and penicillamine (PEN). Results were compared to K^+, Ca^{2+}, Mg^{2+}, and serum indices (Roche cobas 8000), as well as a sodium tetraphenylborate method of K^+ detection. Finally, supernatant bismuth concentrations were evaluated using inductively coupled plasma mass spectrometry (ICP-MS).

Results: Test strips detected EDTA in specimens taken from the following primary tube types: royal blue (Na_2EDTA), tan (K_2EDTA), lavender (K_2EDTA), and pink top (K_2EDTA). Test strips did not falsely detect EDTA in serum tubes including gold (serum separator), red (serum), and orange top (thrombin / serum separator), although three discordant red top serum results - due to pour off and/or aliquot errors - were identified using EDTA test strips in preliminary experiments. Test strips did not falsely detect EDTA in most other plasma specimens, including light green (lithium heparin / plasma separator), green (sodium or lithium heparin), and light blue top (sodium citrate), although one type of gray top tube (potassium oxalate with sodium fluoride) produced an orange "low EDTA" reaction. EDTA test strip reactivity was observed with a variety of chelating agents (order of reactivity: DMSA≈EGTA>K_2EDTA=Na_2EDTA>DMPS>PEN; ALA and DEF were non-reactive at 10 mM), although in general reactivity was only observed at concentrations higher than might be expected during chelation therapy. No pH effect was observed between pH 2-12. Lipemia did not interfere with test strip performance. Marked hemolysis caused an orange appearance to otherwise yellow reactions from EDTA-containing specimens, while visible icterus caused a slightly brown appearance to normally red reactions from non-EDTA specimens. ICP-MS demonstrated that bismuth levels were higher in specimens which had test strips dipped into the solution, arguing that a "dip mode" method may interfere with certain clinical assays.

Conclusions: EDTA test strips reliably detected the presence of EDTA in clinical specimens. Indeterminate or "low EDTA" orange results may require further investigation. "Dip mode" can produce analytical complications for assays which measure (or are interfered by) test strip constituent reagents.

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Lack of tube mixing can be validated as regards ISO-15189 standard - preliminary validation

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Background: Gentle and careful mixing by inverting 5 times is standardized as good laboratory practice by manufacturers and endorsed by Clinical Laboratory Standard Institute. Correct mixing after blood collection is claimed to be important. Nowadays laboratory quality managers have evidenced that phlebotomists do not mix vacuum tubes as recommended by manufacturers. As regards ISO-15189 international standard, all necessary improvements and potential sources of nonconformities, either technical or concerning the quality management system, shall be identified and all laboratory process shall be validated. The aim of this study was to evaluate whether it is really necessary to mix both K2EDTA- and lithium heparin-vacuum tubes immediately after blood collection.

Methods: Blood collection: Samples from 100 volunteers were drawn in three 3.0mL vacuum tubes containing 5.9mg K2EDTA and in three 3.5mL vacuum tubes with 52.5USP U of lithium heparin and gel separator. To eliminate any potential interference due to either contact phase or tissue factor, ~2mL of blood were preliminarily collected in a discard tube without additive. Blood collection was accurately standardized, including the use of needles and vacuum tubes of the same lot.

Processing: All vacuum tubes (one from each kind of additive) were processed using 3 different methods. Method 1: Gold Standard (M1): All specimens were mixed gently and carefully by inverting 5 times as recommended; Method 2: Rest time (M2): All specimens remained 5 min in upright position, followed by gentle careful mixing by inverting 5 times; Method 3: No mix (M3): All specimens were left in upright position without mixing afterwards. The influence of the primary tube mixing procedure was evaluated for routine hematology- and clinical chemistry-testing by paired t-test.

Laboratory testing: All samples were processed for routine hematological testing immediately after collection (<15min) on the same Sysmex® XE-2100D, Automated Hematology Analyzer (Sysmex Corporation®, Kobe, Japan). Routine clinical chemistry was performed on the same Cobas® 6000 c501 module (Roche Diagnostics GmbH, Penzberg, Germany). The parameters tested included erythrocytes, hemoglobin, hematocrit, mean corpuscular volume, RBC distribution width, reticulocytes, white blood cells count and differential, including neutrophils, lymphocytes, monocytes, eosinophils and basophils, platelet count and mean platelet volume, glucose, total cholesterol, high-density lipoprotein cholesterol, triglycerides, total protein, albumin, C-reactive protein, urea, creatinine, uric acid, alkaline phosphatase, amylase, pancreatic amylase, aspartate aminotransferase, alanine aminotransferase, g-glutamyl transferase, lactate dehydrogenase, creatine kinase, total bilirubin, direct bilirubin, phosphorus, calcium, magnesium, iron, sodium, potassium, chloride, lipase and hemolysis index.

Results: No fibrin filaments or microclots were observed in any samples. Significant differences ($P<0.01$), were found only for: a) erythrocytes (0.5%) and haematocrit (1.1%) when M1 was compared with M2; b) alanine aminotransferase (-3.0) when M1 was compared with M3; c) erythrocytes (-0.9%) and haematocrit (-0.8%) when M2 was compared with M3.

Conclusion: This preliminary evaluation has shown that K2EDTA- and lithium heparin- tube mixing after collection with evacuated system appears unnecessary. Moreover, this outcome indicates that not mixing vacuum tubes should not viewed as a non conformity for quality system and in conclusion the lack of tube mixing can be validated as regards ISO-15189 standard.

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Comparability of urine total protein assays in patients with monoclonal proteinuria

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Background: Urine contains various proteins including albumin, Tamm Horsfall protein, and low molecular weight proteins. Several different reagents and instrument platforms are used for urinary total protein measurement, with no established gold standard. All methods appear to measure albumin consistently, yet detection of non-albumin proteins appears more variable. This study compared the performance of five different reagent kits using urine from patients with and without kidney disease and also with known monoclonal M-spikes.

Methods: Urine samples submitted for random urinalysis (RUA) and monoclonal protein electrophoresis testing were analyzed using four pyrogallol red urine protein assays (Pointe Scientific Microprotein Reagent Set, Canton MI; Quantimetrix QuanTtest Red, Redondo Beach CA; Wako Diagnostics Autokit Micro TP, Richmond VA; and Siemens Healthcare Diagnostics Total Protein_2 (Urine), Tarrytown NY), and one benzethonium chloride assay (Roche Diagnostics Total Protein Gen. 2, Indianapolis IN) on the Roche cobas 6000 c501. Pyrogallol red assays were all performed using identical instrument settings, while the benzethonium chloride assay was performed per manufacturer's instruction. The Wako pyrogallol red assay served as the reference assay for the analysis. Samples were electrophoresed and stained on a SPIFE 3000 system (Helena Laboratories, Beaumont TX). M-spike quantitation was performed using a Helena Laboratories Quick Scan 2000. The Quick Scan 2000 scans and calculates the relative percentage of each electrophoretically separated protein fraction based on its staining intensity. The original urinary concentration of the M protein band is obtained by multiplying its relative percentage on the gel by the urinary total protein concentration.

Results: Among RUA samples with varying levels of proteinuria (1- 2632 mg/dL; mean=130 mg/dL; median=52 mg/dL), Passing and Bablok regression analysis revealed good correlation between all methods and the reference (Wako) assay (Pointe: y=1.15x+1.90, n=100; Quantimetrix: y=0.97x+0.39, n=325; Siemens: y=0.99x+5.04, n=25; Roche: y=1.01x+4.31, n=100). In the urine samples with a known M-spike but total protein content >25% albumin, linear correlations with the Wako assay were still reasonably good (Pointe: y=1.0273x+7.8232 R^2=0.9985, n=18; Quantimetrix: y=0.9549x-3.6582 R^2=0.9974, n=22; Siemens: y=1.0114+4.1414 R^2=1, n=4; Roche: y=0.9496x+11.007 R^2=0.9946, n=18). Urine samples with M-spike concentrations <100 mg/24 hours also compared reasonably well, regardless of the albumin percentage (Pointe: y=1.0731x+3.0911 R^2=0.9564, n=44; Quantimetrix: y=0.9101x+0.4658 R^2=0.9781, n=54; Siemens: y=0.9059x+6.181 R^2=0.9843, n=10; Roche: y=1.0416x+3.0244 R^2=0.884, n=44). However, among samples with M Spikes ≥100 mg/24 hours and albumin ≤25%, comparisons between assays were quite variable (Pointe: y=1.1156x+10.509 R^2=0.8207, n=52; Quantimetrix: y=0.2386x+18.907 R^2=0.6697, n=66; Siemens: y=0.9536x+15.617 R^2=0.9735, n=14; Roche: y=1.2409x+15.676 R^2=0.7977, n=52).

Conclusions: All urine protein assays in this study compared well for testing random urinalysis samples obtained from a population of patients with and without kidney disease in whom albumin is usually a major protein species. However, these total protein assays performed quite variably when testing urine samples that contained large quantities of monoclonal protein, particularly those with <25% albumin and

a calculated M-spike greater than 100 mg/24 hours. In particular, urine total protein assays can vastly underestimate protein excretion in certain patients with large M-spikes.

A-277

Elevated Cerebrospinal Fluid Total Protein caused by Povidone-Iodine (Betadine) Interference

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Background: The measurement of cerebrospinal fluid (CSF) total protein (TP) is useful in the diagnosis of meningitis and the detection of other inflammatory diseases. The laboratory was contacted by a clinician concerned with a clinically discrepant elevated CSF TP. The specimen received for chemistry testing was the first tube of the CSF collection. The CSF TP was 417 mg/dL (reference interval 15- 45 mg/dL) and CSF glucose was 87.2 mg/dL (reference interval 40-70 mg/dL). CSF cell counts were: red blood cells 6/mm3 and white cell count 1/ mm3. Another specimen from the patient (tube 2) taken at the same time and submitted to hematology was analyzed for CSF TP. The CSF TP result of tube 2 was 20 mg/dL and was confirmed on repeat analysis.

Objectives: To investigate falsely high CSF TP results, suspected to be caused by preparation of collection site by povidone-iodine (Betadine) solution.

Design and Methods: We performed interference studies to determine the effect of Betadine, iodine only and povidone only on the CSF TP concentration. A CSF diluent was prepared by pooling clear CSF specimens to which reagent grade water was added to give a final ratio of 90%:10% v/v CSF to water. An initial sample of the CSF diluent and either the povidone-iodine or iodine only or povidone only solution was prepared to give a final ratio of 90%:10%v/v CSF to solution. This initial sample was then serially diluted with the prepared CSF pool to give different final concentrations of povidone-iodine/iodine/ povidone. CSF TP was then measured for these prepared samples using the laboratory routine chemistry analyzer, the Siemens Dimension Vista (Siemens Healthcare Diagnostics, Tarrytown, USA). This assay involves the reaction of protein in the sample with the pyrogallol red (PGR) sodium molybdate complex to form a bluish-purple colored complex, which absorbs at 600 nm. Further CSF TP measurements were made on the Siemens Dimension Xpand using the PGR method. In addition to this CSF samples were analysed for TP at a reference laboratory using the modified biuret method. To investigate if the positive interference was a result of the iodine in the Betadine solution and its direct absorbance at the same wavelength as the PGR- protein complex, spectrophotometric absorbance studies were also performed.

Results: The experimental data of the interference confirmed a positive interference for the PGR assay when Betadine containing povidone or povidone alone were added to a CSF sample. The Betadine solution did not interfere the modified biuret method for TP. The false positive TP interference was clinically significant (> 10% change) even at Betadine concentrations (as low as 0.0025%) where the CSF sample appeared clear on visual inspection. Spectrophotometric studies of the Betadine solution and patient's sample showed no absorbance at 600nm.

Conclusions: Low levels of povidone-iodine contamination of CSF specimens can lead to clinically significant positive interference for TP results. Alternate iodine solutions not containing povidone should be used for preparation of sites for CSF collection.

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Extending the Time Restriction on Transit Time for Lactate Measurement

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Background: Plasma Lactate is useful for assessing tissue perfusion in critically ill patients. When using sodium fluoride/potassium oxalate (FlOx) as a preservative, test reagent manufacturers require that blood specimens be processed within 15 minutes from collection. This transit time limit, however, cannot always be met, particularly when the laboratory is distant from patient care, as the case in our institution. 3% of lactate samples arrive late into the laboratory and are thus rejected. In an attempt to reduce the number of rejected lactate specimens, we examined the effect on lactate values following extended transit time to 30 minutes.

Methods: Lactate samples were collected in triplicate from 50 patients (with prior physician orders for lactate) and from 50 normal volunteers. One sample set was kept at room temperature (RT) and processed at 15 minutes from collection according to manufacturer's instruction. Of the remaining two sample sets, one was kept at RT and the other on ice (4°C) for 30 minutes before lactate analysis. Lactate was measured using Roche Cobas® LACT2. Accuracy was determined with lactate values obtained at 30 minutes (both RT and 4°C) compared to the standard 15 minutes. Intra-assay precision was obtained using 10 aliquots from the same samples spanning the analytical measuring range. Inter-assay precision studies were performed on 20 patients.

Results: Lactate values ranged from 0.6 to 25.4, and from 0.7 to 2.1 mmol/L in patients and volunteers, respectively. There was good correlation between RT lactate values measured at 15 and 30 minutes (r= 0.9993, bias 0.04). There was good correlation (r=0.9992, bias -0.06), between RT lactate values measured at 15 minutes and those at 30 minutes (4°C). There was good correlation (r=0.9993, bias -0.01) between RT and 4°C lactate values measured at 30 minutes. Intra-assay imprecision for RT samples processed at 15 minutes ranged from 1.6%, 1.0%, 1.1%, and 0.9% at lactate levels of 1.3, 5.5, 9.0 and 12.4 mmol/L respectively. For RT samples processed at 30 minutes, imprecision ranged from 0%, 1.2%, 1.0%, and 0.8% compared with 4°C samples, 0%, 1.3%, 0.9%, and 1.1%, at lactate levels of 1.3, 5.5, 9.0 and 12.4 mmol/L respectively. Inter-assay imprecision for RT samples processed at 15 minutes ranged from 0.0-6.7%; RT samples process at 30-minutes ranged from 0.0-13.1% compared with 0.0-10.2% for samples kept at 4°C. All precision studies met our acceptance criteria of <14%.

Conclusion: The performance of the assay for samples kept for 30 minutes either at RT or 4°C was not significantly different from those kept at RT and processed at processed at 15 minutes. Our institution changed the lactate processing protocol to 30 minutes, leading to a reduction of lactate order rejections from 3% to less than 1%.

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Measurement of Serum Total Calcium Using the 5-nitro-5'-methyl-BAPTA Method in the Presence of Four Gadolinium-based Contrast Agents

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Background: Some gadolinium-based contrast agents administered to patients undergoing MRI procedures are known to interfere with the widely-used o-cresolphthalein complexone (o-CPC) dye method used to measure serum total calcium. Patient samples with serum calcium <7.0 mg/dL using this method were routinely re-tested in the clinical laboratory with an Arsenazo III method to avoid reporting falsely decreased calcium results due to gadolinium interference. Roche Diagnostics recently introduced a calcium reagent formulation which utilizes a different ion-selective indicator, 5-nitro-5'-methyl-BAPTA (NM-BAPTA).

Objectives: (1) Verify the manufacturer's claim that gadolinium-containing MRI contrast media at therapeutic concentrations does not interfere with serum total calcium measurement utilizing the NM-BAPTA method. (2) Evaluate laboratory efficiencies gained by implementing a serum calcium method that is not affected by gadolinium-based contrast agents.

Methods: Gadolinium-based contrast agents commonly used at Mayo Clinic were added to five residual patient serum pools with varying concentrations of calcium (6.5-10.3 mg/dL). Gadodiamide, gadobenate dimeglumine, gadoxetate disodium and gadofoveset trisodium were each added to serum pools yielding final concentrations of 0.1, 0.25, 0.5, 1.0, and 2.0 mmol/L contrast agent. Total serum calcium was measured utilizing the o-CPC and NM-BAPTA methods on a Roche Cobas c501 and c701 (Roche Diagnostics) and the Vitros 350 Arsenazo III method (Ortho Clinical Diagnostics). Results obtained were compared to control serum pools with de-ionized water added to account for dilution. Absolute differences between total calcium concentrations in samples with and without contrast agent were calculated. The number of patient samples with calcium <7.0 mg/dL, cost of maintaining a secondary method and turn-around time were assessed over a one year time period.

Results: Addition of gadodiamide at final concentrations of 0.1, 0.25, 0.5, 1.0 and 2.0 mmol/L lowered serum calcium results by an average (mg/dL) of 0.3 (range 0.1-0.5), 0.5 (range 0.2-0.8), 0.8 (range 0.4-1.3), 1.4 (range 0.9-1.9) and 2.4 (range 1.7-3.0) mg/dL, respectively, using the o-CPC method. There were no differences between calcium results in control and gadodiamide-containing samples using the Arsenazo III or NM-BAPTA method (average absolute difference 0.0 mg/dL, range 0.0-0.3 mg/dL). In samples containing gadobenate dimeglumine, gadoxetate disodium and gadofoveset trisodium, serum calcium results differed by <0.2 mg/dL between contrast-added and control samples (average absolute difference 0.0 mg/dL, range 0.0-0.2 mg/dL) at all concentrations of contrast agent using the o-CPC, NM-BAPTA, and Arsenazo III methods. During 2013, 732 patient samples were re-tested using the Arsenazo III method costing >$1,200 in additional reagent, calibrators and quality

control. Turn-around time for reporting of calcium results was 25-35 minutes longer for specimens requiring re-testing.

Conclusions: The Roche Diagnostics NM-BAPTA method for measuring serum total calcium does not show clinically significant interference in the presence of four gadolinium-based contrast agents. By implementing a calcium method that is free from gadolinium interference, the laboratory improved quality and reduced the risk of reporting falsely decreased serum calcium results due to gadolinium interference. Efficienies gained included eliminating a secondary Arsenazo III calcium method, reducing patient re-testing, and eliminating the reporting delays and costs associated with re-testing.