abstracts of papers

Young Investigator Awards Program Abstracts

The Academy of Clinical Laboratory Physicians and Scientists (ACLPS) established the Paul E. Strandjord Young Investigator Awards Program in 1979 to encourage students and trainees in laboratory medicine to consider academic careers. Each year, a call for abstracts is sent to each member, inviting submission of scientific papers.

All submitted abstracts are peer reviewed by a committee of ACLPS members selected confidentially by the director of the Young Investigator Program, Alexander J. McAdam, MD, PhD. Reviewers are blinded to authors and institutions. Young Investigator Award recipients are granted free registration to the annual meeting, reimbursement for a portion of travel expenses, and the opportunity to present their scientific work before an audience of peers and mentors.

The following abstracts were presented at the 46th Annual Meeting of ACLPS, June 9-11, 2011, in St Louis, MO. Authors receiving a 2011 Young Investigator Award are marked with an asterisk (*).

ACLPS abstracts are published in the *American Journal of Clinical Pathology* (*AJCP*) as received by ACLPS without *AJCP* editorial involvement. Content and typographical errors and inconsistencies in these abstracts are the responsibility of the abstract authors.

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Plasma Procoagulant Phospholipid Activity Compared to Platelet Microparticles: A Testing Challenge

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The procoagulant phospholipid activity in plasma is related to the presence of anionic phospholipid (APL) within microparticle (MP) plasma membranes. We evaluated 2 different functional assays to quantify APL activity: the STA Procoag-PPL (STA) and the Zymuphen MP-Activity (Zym). The STA test is a clot-based assay where platelet-poor patient plasma is mixed 1:1 with phospholipid depleted plasma, calcium, and factor Xa. The clotting time is dependent on the amount of APL present, with a shorter time representing higher amounts of APL. In the Zym test, plasma is added to a microplate well coated with annexin V, which binds phosphatidylserine. Factors Va and Xa and calcium are added to generate thrombin, which is measured using a chromogenic substrate. Thrombin generation is proportional to the APL content.

Twenty-four samples from healthy donors (14 female; 10 male; age, 35 ± 11 years) were double spun at 1,500*g* to produce platelet-poor plasma and tested with both assays. The average APL concentrations (in phosphatidylserine-equivalent activity) with the STA and Zym were 590 nmol/L (± 414) and 27 nmol/L (± 17), respectively. The correlation between the assays was low, r^2 = 0.29. We hypothesized that the calculated higher PS concentration in the STA assay could be due to the contact system activation. This was ruled out by running the samples in the presence of corn trypsin inhibitor, which showed a decrease of only 8% in the APL concentration. To test whether the difference in the concentration was related to differences in MP size, patient samples were filtered using 0.45-µm and 0.22-µm filters. Patients' plasma filtration with 0.45- and 0.22-µm filters showed decreased APL concentrations with the STA assay of 82% (± 6) and 93% (± 1), respectively. In contrast, the Zym assay showed a decrease in the concentrations of 42% (± 10) and 61% with the 0.45 and 0.22 filters, respectively. This finding supports the idea that the Zym assay is measuring the APL content of the smaller microparticles.

To evaluate the relation between APL concentration and platelet MP number, 12 patients' samples were also analyzed by flow cytometry. Microparticles were gated by size (<1 µm) and CD 41-positive expression. The correlation between number of platelet MP by flow cytometry and the calculated APL concentration by the STA was high, r^2 = 0.82. In contrast, the correlation between flow and the APL concentration by Zym was low, r^2 = 0.23.

We conclude that Zym measures the APL present only in smaller microparticles and that the STA assay shows good correlation with platelet MP quantification done by flow cytometry.

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Validation of a Quantitative PCR Assay for Detection of BK Virus in Transplant Patients

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BK virus (BKV) is an established cause of nephropathy in renal transplant recipients. Latent BKV in the epithelial cells of the urinary tract may reactivate in the setting of immunosuppression, leading to BKV nephropathy and possible allograft rejection. Early detection of reactivated BKV would allow for timely clinical intervention and minimize renal damage. BKV DNA can be noninvasively monitored in the blood and urine using PCR.

We have validated a new quantitative PCR assay from Focus Diagnostics for detecting BKV DNA in serum and urine. We optimized the PCR conditions by independently varying primer annealing and extension times and annealing temperature. The linear range of the assay was found to be of 500 to 2.6. × 10^{10} cp/mL.

The limit of detection, the lowest concentration at which 95% of samples were detected, was 688 cp/mL for urine and 660 cp/mL for plasma. The lower limit of quantitation, determined by evaluating the variability of replicates near the lower end of the linear range, was found to be between 1,399 and 696 cp/mL.

To assess accuracy, we compared results for patient specimens to those obtained by an outside reference laboratory. The assay was determined to be 100% sensitive for both plasma and urine specimens, and 80% and 92% specific for plasma and urine specimens, respectively. Quantitatively, the average differences were +7.4% for plasma and +7.1% for urine, with no evident systematic bias. The assay met reproducibility standards of CV less than 30% or variation less than 2,000 copies/mL for both intra-assay and interassay precision. To assess specificity, the assay was performed on samples containing potentially cross-reactive viruses. No positive results were obtained, demonstrating the assay is specific for BKV. We also ran potentially interfering substances commonly found in plasma with low positive specimens. Interference was observed with hemolyzed blood and heparin. Plasma and urine samples for this assay are stable at 4ºC for a minimum of 4 days. Both extracted and preextraction samples were found to be stable for a minimum of 3 freeze-thaw cycles.

The Focus Diagnostics BKV DNA assay was found to be a valid method for detecting BKV in plasma and urine with a high degree of sensitivity and specificity. Review of patient charts demonstrated significantly higher viral loads in urine than plasma, indicating that urine is a sensitive sample for routine posttransplant monitoring. Viral titers determined by the assay correlated with clinical disease progression, with some patients remaining viremic for extended periods of time. Changes in viral titer correlated with acute exacerbations of BK nephropathy and reductions in immune suppression. Monitoring BKV replication using PCR may lead to improved outcomes in immunosuppressed patients.

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Laboratory Medicine Best Practices: Methods for Evidence-Based Patient-Centered Quality Improvement

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The study objectives are to apply and test newly developed systematic review methods designed to evaluate published and unpublished evidence of the effectiveness of preanalytic and postanalytic quality improvement practices. These practices will support the development of new evidence-based recommendations and guidelines in laboratory medicine leading to improved health care quality outcomes consistent with the Institute of Medicine's aims (safe, timely, effective, efficient, equitable, patient-centered).

The Laboratory Medicine Best Practices (LMBP) "A-6" systematic review methods were applied (A-6: Ask, Acquire, Appraise, Analyze, Apply, Assess) by several multidisciplinary teams, expert panels, and the LMBP Workgroup as described in detail in a 2010 technical report (http://www.futurelabmedicine.org).

The pilot test application of the LMBP A-6 methods produced systematic reviews of 7 quality improvement practices in 3 distinct quality improvement topic areas: patient specimen identification (bar-coding systems and point-of-care test bar coding), critical value test result communication (automated notification and call centers), and blood culture contamination (venipuncture, phlebotomy teams, and prepackaged prep kits) used to support evidence-based recommendations. Preliminary systematic review results, including meta-analyses of each practice, support a "best practice" recommendation for 4 of these practices (bar-coding systems, point-of-care test bar coding, venipuncture, phlebotomy teams) based on sufficient evidence of practice effectiveness for improving patient-related outcomes.

The LMBP A-6 systematic review method for evaluating and recommending laboratory quality improvement practices has been demonstrated to be a robust and reliable method for implementing evidence-based quality improvement linked to improved patient outcomes.

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Pediatric Reference Intervals for Serum Copper and Zinc

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Zinc and copper are essential trace elements, which have important roles as cofactors in many metabolic pathways. Multiple aspects of normal human physiology are adversely affected by copper or zinc deficiency. Therefore, it is important to establish reference intervals to guide diagnosis and therapy of deficiencies. Pediatric reference intervals for copper and zinc are often based on adult data or are derived from small groups of young children. The aim of this study was to establish age- and sex-specific reference intervals for copper and zinc with a large healthy pediatric population.

A total of 2,115 clinically healthy children were enrolled in this IRB-approved study: 1,098 boys and 1,017 girls 0.5 to 18 years of age. Serum samples were collected from each child after parental permission had been obtained. The children 6 years and younger had fasted overnight, prior to sample collection. The children 7 years and older did not fast. Serum copper and zinc concentrations were measured using previously validated inductively coupled plasma–mass spectrometry (ICP-MS) methods. Normality of the data was assessed using a Shapiro-Wilk test. As distributions were significantly nonnormal (nongaussian), nonparametric methods were applied. Quantile regression was used to determine whether gender or age significantly affected reference intervals. Additionally, regression trees were calculated to find the best partitions based on age and gender. All calculations were performed using the R software package, version 2.9.2 (The R Foundation for Statistical Computing). There were no significant effects of gender on the copper or zinc intervals. There were also no significant effects of fasting on these data.

For copper, but not zinc, there were significant differences in the reference intervals by age. The resulting 95% reference interval for serum zinc was 62 to 121 µg/dL. The age-dependent 95% reference intervals for serum copper were 75 to 153 µg/dL for those less than 10.3 years, 64 to 132 µg/dL for those 10.3 to 12.5 years, and 57 to 129 µg/dL for those over 12.5 years. Our data provided a sufficient number of results to define reference intervals for serum zinc and copper values in an American clinically healthy pediatric population.

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Rapamycin-Mediated Growth Inhibition in Human Prostate Cancer Cells

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The specific causes of prostate cancer remain unknown. In the United States in 2010, it is estimated that 217,730 men will be diagnosed with and 32,050 men will die of prostate cancer. The objective of this study is to investigate whether rapamycin, a drug known to inhibit mediators of the mammalian target of rapamycin (mTOR) signaling pathway, can affect survival of human prostate cancer (LNCaP) cells.

LNCaP cells, after reaching 50% to 60% confluence, were treated with various concentrations of rapamycin (0, 0.1, 0.5, 1, and 10 µmol/L) for 24, 48, and 72 hours. The cell death and proliferation were determined by electron microscopy and MTS Cell Proliferation Assay (Promega) assay. Protein expression of phospho-mTOR (pmTOR), phospho-70S6 kinase (p70S6K), phospho-Akt1/2/3 (pAkt 1/2/3), and phospho-STAT3 (pSTAT3) was determined utilizing Western blotting and immunofluorescence techniques. Rapamycin levels in the media were determined by a liquid chromatography combined with mass spectrometric method (LC/MS/MS) using asomycin as the internal standard, 24 and 48 hours after incubation. The detection limit of the assay is 1 ng/mL.

At 24 hours posttreatment, the 1- and 10-µmol/L concentrations displayed 16% and 28% growth inhibition. In the 48-hours and 72-hours treatment groups, we observed 25% and 38% growth inhibition even at the lower rapamycin concentration of 0.5 µmol/L. Electron microscopy showed apoptosis and autophagic cell death. Western blotting studies, following 24 hours of rapamycin exposure, showed a decrease of pmTOR at 0.5-, 1-, and 10-µmol/L concentrations. After 48 hours of rapamycin exposure, p70S6K showed a decrease in its expression starting from 0.5 to 10 µmol/L, whereas p Akt 1/2/3 and p-STAT3 appeared to have no change in their expression. Immunofluorescence studies confirmed these results. After 24 and 48 hours of incubation, no rapamycin level was detected in the respective media using LC/MS/MS, indicating that human cancer cells were capable of uptaking rapamycin from the media within 24 hours.

These results not only demonstrate that rapamycin significantly down-regulates mTOR and p70S6 kinase signaling pathways, but also indicate both a concomitant inhibition of growth and also a decrease in cell viability and induction of apoptosis and autophagy in human prostate cancer cells. Therefore, rapamycin may aid in chemotherapy of prostate cancer.

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The Role of Microstructural Polymorphisms in Class I HLA-B Peptide Binding: A Computational Molecular Dynamics Study

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Human leukocyte antigen (HLA) molecules are critical determinants of the innate immune surveillance to distinguish self from non-self. The HLA-peptide complex at the cell surface mediates a successful transplantation outcome and modulates the course of autoimmune disease pathology. This study focuses on the role of structural polymorphisms of class I HLA-B 4402, 4403, and 4405 molecules in antigenic peptide binding. These 3 HLA molecules differ from each other by a single amino acid (A.A. 116 and 156). Inexplicably, the binding affinity of the T-cell receptor to HLA-peptide complex differs by an order of magnitude (HLA-B 4402/4403 vs HLA-B 4405). The current study aims to explain these binding differences using computational molecular dynamics (MD).

Six computational MD simulations were performed on 3 crystal structures, HLA-B 4402, 4403, and 4405. The HLA molecules are complexed with a decapeptide, immunodominant viral epitope, EENLLDFVRF (abbreviated EENL) derived from the Epstein-Barr virus (EBV) nuclear antigen 6. The first 182 amino acids of the HLA molecules corresponding to the α1-α2 binding domains of the HLA molecule were simulated for a total duration of 25 nanoseconds. The simulation contained ~16,500 water molecules with a final system size of ~69,000 atoms. The simulations were carried out at a temperature of 310K. Added Na+ and Cl– ions maintain the system osmolarity at 100 mmol/L.

All 3 HLA molecules demonstrate enhanced flexibility of the α1 and α2 domains in the absence of the binding peptide. The α2-1 region shows uniquely increased flexibility in HLA-B 4402/4403 in proximity to the tapasin binding site. The EENL peptide exhibits distinctive fluctuation patterns, with increased root mean squared fluctuation in the P- terminal end of HLA-B 4402/4403, while HLA-B 4405 showed enhanced mobility. In addition, we observe a unique role of "bound, ice-like" water molecules in the determining the stability of the HLA-bound peptide. Normal rotational correlation time of bulk water molecules is ~20 picoseconds. We observed distinct water molecules in the HLA peptide binding sites with a correlation time of 1.2 nanoseconds (~60 times slower than in bulk water). We hypothesize that ice-like water molecules can potentially explain the differences in the peptide binding affinity of HLA-B 4402, 4403, and 4405, underscoring the important role played by water molecules in maintaining the HLA-peptide binding complex stability and, potentially, T-cell recognition.

We demonstrate, for the first time, the role of microstructural polymorphisms and associated bound water molecule dynamics in determining the HLA-peptide binding kinetics using the technique of computational MD.

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Fenretinide Reverses a Subset of Polyunsaturated Fatty Acid Alterations in Cystic Fibrosis Cells

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Cystic fibrosis (CF) is the most common inherited disorder affecting Caucasians in the United States. CF is caused by mutations in the cystic fibrosis transmembrane regulator (CFTR). These mutations cause a multitude of pulmonary, gastrointestinal, and endocrine defects leading to significant morbidity and early mortality. Studies have shown consistent alterations in lipid metabolism in CF patients as well as animal and cell culture models of the disease. The most common of these are alterations in polyunsaturated fatty acids (PUFA) levels, including decreased linoleic acid (LA; 18:2n-6) and docosahexaenoate (DHA; 22:6n-3) and increased arachidonic acid (AA; 20:4n-6). These may contribute to the pathophysiology of CF by altering levels of eicosanoids, oxygenated PUFA metabolites. Recent studies have indicated that fenretinide (FEN), a synthetic retinoid with antitumor properties, may modulate lipid levels, including PUFAs, in CF. However, the mechanism is unknown. We hypothesized that FEN can correct fatty acid alterations by modulating PUFA metabolism.

Experiments were carried out in 16HBEo- human bronchial epithelial cells stably transfected with plasmids expressing either sense (CFTR wild-type) or antisense (CFTR-negative) partial transcripts of CFTR. Cells were treated with varying concentrations of FEN for 24 hours and fatty acids measured by gas chromatography/mass spectroscopy (GC/MS). In FEN-treated cells, LA was increased and AA decreased, indicative of decreased LA to AA metabolism. In contrast, the n-3 pathway showed increased linolenic acid (LNA; 18:3n-3) with higher eicosapentaenoic acid (EPA; 20:5n-3) levels and unchanged DHA. This pattern is suggestive of suppressed LNA to EPA metabolism with increased retroconversion of DHA to EPA. The degree of these changes is greater in sense than antisense cells.

These data indicate that FEN can alter PUFA composition, perhaps by modulating metabolic activity in the n-3 and n-6 pathways, and correct some of the alterations typical of CF by increasing LA and decreasing AA. It may also stimulate retroconversion of DHA to EPA. Future studies will examine the precise mechanism of FEN action on PUFA metabolism and CF pathology, especially the role of FEN in inducing free radicals generating enzymes.

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Sphingosine-1-Phosphate Receptor Modulation of Platelet Function

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Sphingosine-1-phosphate (S1P) is a bioactive lipid which signals via specific G protein–coupled receptors (S1P-$R_{1,2,3,4,}$ and $_5$) found in various combinations on multiple cell lineages, including platelets, lymphocytes, and endothelial cells. The effect of S1P stimulation on platelets is controversial as there are reports that S1P inhibits aggregation, whereas other reports indicate an aggregation promoting response. Of added clinical importance, a drug called fingolimod (FTY720), which binds to S1P-$R_{1,3,4,}$ and $_5$, was recently released as an FDA-approved treatment for multiple sclerosis; however, its effect on platelet aggregation has gone unreported.

We sought to determine the effect of S1P or FTY720 stimulation on platelet function. Our studies consisted of light transmission aggregometry (LTA) of platelet-rich plasma (PRP) after incubation with various doses of S1P/FTY720 with subsequent stimulation of aggregation with either collagen, adenosine diphosphate (ADP), or arachidonic acid (AA).

Neither S1P nor FTY720 induced an aggregation response in PRP. S1P was found to have an inhibitory effect on ADP-induced aggregation at 100 μmol/L. FTY720 (10 nmol/L) on the other hand, displayed an inhibitory effect on collagen-induced aggregation, which could be overridden by high doses of collagen. Additionally, FTY720 appears to be associated with an increase in disaggregation when ADP is the platelet agonist.

In summary, our studies demonstrate that S1P receptor signaling can modulate platelet response to various physiological aggregation agonists. Furthermore, these results are the first to document the effect of fingolimod on platelet activity, which may have important clinical implications for the bleeding risk of patients being treated with the drug. Our future goals are to dissect the contributions of the different S1P receptors on platelet activation response from which we may be able to identify novel biotargets which may result in new antiplatelet therapies.

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Investigation of Interfering Hemoglobin Variants in the Olympus AU400 HbA_{1c} Assay

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In 2009, the ADA added hemoglobin A_{1c} (HbA_{1c}) of 6.5% or more as a diagnostic indicator for diabetes mellitus. The Diabetic Control and Complications Trial showed a strong relationship between increasing HbA_{1c} and increasing risk of vascular complications related to disease progression.

While widely recognized as a useful measure in monitoring the glycemic status of diabetic patients, HbA_{1c} has only recently been recommended for diagnosing diabetes, due in part to difficulties in standardization and susceptibility to interferences by hemoglobin variants by some methods. Using HbA_{1c} as a diagnostic criterion for diabetes is an attractive alternative to fasting plasma glucose or the 2-hour oral glucose challenge because fasting is not required, there is less preanalytical instability of the analyte, and HbA_{1c} is a better index of long-term glycemic exposure. Immunochemical HbA_{1c} assays are attractive because they are fast and automated. However, they rely on antibodies that bind to specified epitopes in the hemoglobin molecule, and so there is the potential for analytical interferences in patients with hemoglobinopathies, such as those with hemoglobin S. Our objective in this study was to validate the Olympus HbA_{1c} assay on patients with wild-type hemoglobin and examine the effects of hemoglobin variants on measurement accuracy.

Intra-assay precision values ranged from 1.29% to 2.09%. Interassay precision values ranged from 4.14% to 5.78%. Correlation with a cation-exchange HPLC method (Variant II) yielded the following relationship Olympus = 0.95 HPLC – 0.26, $r^2 = 0.97$. HbA_{1c} of several well-characterized hemoglobin variants were measured on both platforms and showed varying degrees of interference. The 2 most common variants (heterozygote HbS and HbC) resulted in spurious increases up to 20%. Because HbF more than 10% is known to decrease measured HbA_{1c}, we measured 3 individuals with HbF less than 10% and 2 individuals with more than 10%. With HbF less than 10%, a small decrease was observed (–8%), and with HbF more than 10%, a large decrease of –36% was observed. Heterozygote Hb variants B2 and E showed no significant interference. Hb Hasharon showed a decrease of –8%. Additional variants could not be analyzed by HPLC and instead were compared to the Rapid DCA 2000 immunoassay: compound heterozygotes carrying both S and C alleles showed a spurious increase of 31%, and 2 Hb J-Baltimore variants and 1 Hb G-Philadelphia showed no significant interference.

The Olympus AU400 HbA_{1c} assay performs satisfactorily in patients with wild-type hemoglobins, but clinically significant biases are evident with several hemoglobin variants that are seen in our hospital laboratory.

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Examination of Laboratory Test Repeat Practices

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A long-standing practice in clinical laboratories has been to automatically repeat laboratory values that are above or below a

critical threshold or that fail a "delta check." These practices were established when laboratory instruments were less reliable than today's analyzers. We examined the reproducibility of repeated laboratory values to determine whether this practice is still necessary and whether it may be possible to reduce redundancy and improve efficiency and turnaround time for reporting critical values.

Repeat testing from 10 routine chemistries (AST, BUN, Ca, Cl, CO_2, creatinine, glucose, K, lipase, and Na) performed on a Roche Modular P system were examined between December 14, 2010, and January 15, 2011. Monthly volumes at our institution are ~38,000 for electrolytes, BUN, glucose; ~16,000 for AST; and ~1,400 for lipase. Captured data included date, result times, initial result, verified result, instrument ID, and accession number. Absolute and percent difference between the initial value and the final verified value was calculated. The number of differences between the initial and verified values that exceeded College of American Pathologists/ Clinical Laboratory Improvement Act (CAP/CLIA) allowable error (AE) limits was determined.

The number of repeat tests examined were 254 for AST, 567 for BUN, 619 for Ca, 472 for Cl, 505 for CO_2, 575 for creatinine, 233 for glucose, 451 for K, 116 for lipase, and 495 for Na. The percentages of these repeated tests where the differences between initial and verified results exceeded the AE was 0 for lipase, 1.6% for calcium, 1.7% for creatinine, 3.7% for BUN, 5.4% for K, 3.1% for AST, 6.1% for Cl, 12.0% for glucose, 7.2% for Na, and 7.9% for CO_2. Of these, the vast majority were the result of the initial value being below the analytic measurement range presumably due to "short sampling." For instance, 95% of the repeat BUN results that exceeded the AE were due to results of less than 3 mg/dL from the initial analysis. The percent of repeated values that exceeded the AE after exclusion of these short samples was 0 for lipase, 0.16% for calcium, 0.17% for creatinine, 0.18% for BUN, 1.5% for K, 2.0% for AST, 2.1% for Cl, 2.5% for glucose, 3.4% for Na, and 6.1% for CO_2.

These preliminary findings suggest the need for additional data from multiple tests and instrument platforms and a potential pathway to improving laboratory efficiency that may have a positive impact on turnaround time and patient care.

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Analytical Performance of Platelet Factor-4 (PF4) Assay

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Heparin-induced thrombocytopenia (HIT) is a serious complication associated with heparin therapy and is characterized by the presence of antibodies directed against the heparin/platelet factor-4 (PF4) complex causing platelet activation resulting in a prothrombotic state. Identification of patients at risk for developing a severe thrombotic event is fundamental to improving outcomes. Due to the ease of implementation, enzyme-linked immunosorbent assays (ELISAs) designed to detect antibodies reactive with PF4 are used routinely in large medical centers, often replacing the more labor-intensive functional assays. Notably, these immunoassays are marketed as a purely qualitative assessment of anti-PF4 antibodies. Recent literature suggests that optical density (OD) results can be used to stratify patients for the likelihood of developing HIT. The analytical performance of these assays has not been thoroughly reviewed in the literature.

The objective of this study is to evaluate the analytical performance of the GTI PF4 Enhanced ELISA and assess its utility in the quantitative evaluation of anti-PF4 antibodies. Interassay imprecision studies were performed for three categories of OD reactivity (<0.40, 0.40-1.00, and >1.00) over a 30-day period. Each OD category was comprised of at least 6 patient specimens. Each specimen was analyzed 10 times over the course of the study.

Results of this analysis reveal that the correlation of variability (CV) of this ELISA is on average 27% across the analytical range. Specifically, for results in the negative range (OD < 0.4), the CV is 30%, for results in the "weak" positive range (OD = 0.4-1.0), the CV is 27%, and for results in the "strong" positive range (OD > 1.0), the CV is 25%. Because this assay is designed to qualitatively detect antibodies to PF4, there is no internal normalization for protein concentration. The negative and positive controls provided are designed to be used qualitatively and have CVs similar to what was found with our patient specimens. Interestingly, clinical laboratories routinely report PF4 results as an OD.

While there are data to support an increased risk for thrombosis with increased OD measurement, it is important for physicians to understand the limitations of the assay when making critical clinical decisions.

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Interference of HbA_{1c} Measurements by Rare Hemoglobin Variants

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HbA_{1c} is the most important marker for long-term assessment of the glycemic state in patients with diabetes mellitus and has been endorsed by an international expert committee for the diagnosis of diabetes. Many studies have shown that Hb variants can interfere with HbA_{1c} measurements. The objective of this study is to compare results of HbA_{1c} measurements obtained with 16 rare hemoglobin variants using 6 different methods.

Whole blood samples were collected in EDTA tubes for HbA_{1c} analysis. Hb variants were identified by inspection of chromatograms obtained with a Bio-Rad Variant analyzer using the beta-thalassemia program. HbA_{1c} analysis was performed using 6 different methods (Bio-Rad D-10, Diazyme enzymatic, Roche Tina-Quant, Tosoh G7 Variant Mode, Tosoh G8 Variant Mode, and VII Turbo 2.0). The Trinity Biotech ultra^2 boronate affinity HPLC method was used as the comparison method. Results were analyzed by a regression model to assess the effects of method, sample, phenotype, and the interaction of phenotype and analyzer.

We identified 26 samples containing 16 rare variants (4 α,12 β). Forty-seven nonvariant samples were also included as controls. The interaction of phenotype and method was statistically significant ($P <$ .0001). Ten β variants (Andrew-Minneapolis, Austin, Camperdown, Deer Lodge, Hope, N-Baltimore, Rambam, Riyadh, Sherwood Forest) were associated with clinically (>15.0% relative bias) and statistically ($P <$ .005) significant differences on the G7, G8, VII Turbo and D-10. However, chromatograms from these HPLC methods generally showed abnormalities indicating the presence of an Hb variant. The Diazyme method was only affected by 1 β variant (Austin). The Roche method was only affected by 1 β variant (Deer Lodge) as well. The presence of Hb variants cannot be detected by the Roche immunoassay or the Diazyme enzymatic methods. No methods were affected by the α variants tested.

All of the HbA_{1c} methods tested showed some degree of interference with the variant hemoglobins tested. Ion-exchange methods were the most affected, but in many cases, the presence of the variant could be detected such that an incorrect HbA_{1c} result would not be reported. Laboratories should be aware of their method's limitations with respect to these interferences.

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Determination of Cocaine Metabolites and the Adulterant Levamisole in Urine Using UPLC-MS/MS

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Levamisole, an antihelminthic agent, is increasingly being discovered as an adulterant of street cocaine. The US Drug Enforcement Agency reported that, in July 2009, levamisole was found in as much as 69% of seized cocaine. The previous human uses of levamisole were as an immunomodulator in treatment of rheumatoid arthritis and as an adjuvant treatment with fluorouracil for colorectal cancer. Recent reports have linked levamisole found in street cocaine to agranulocytosis and retiform purpura in cocaine users.

We have developed a UPLC-MS/MS method for the determination of the major urinary cocaine metabolites: benzoylecgonine and ecgonine methyl ester, cocaethylene, and the adulterant levamisole and its urinary metabolite 4(OH) levamisole. We performed these assays on a Waters ACQUITY UPLC with an ACQUITY TQ Detector equipped with a Waters ACQUITY UPLC BEH C18 column using an ammonium acetate/formic acid mobile phase. Urine was extracted using methanol after the addition of benzoylecgonine-D3, ecgonine methyl ester-D3, cocaethylene-D3, and aminorex ((*RS*)-5-phenyl-4,5-dihydro-1,3-oxazol-2-amine) as internal standards. MS/MS operates with positive electrospray ionization (ESI).

Both within-run (4.7%-17.1%) and between-run (3.7%-7.3%) imprecision was acceptable using drug-free urine spiked with known low, medium, and high concentrations of the analytes. The limit of detection ranged from less than 1 ng/mL to 6.3 ng/mL. We tested 69 urine samples sent for toxicology screening at Grady Memorial Hospital: 51 samples tested positive for cocaine, and 18 tested negative by immunoassay and LC-MS/MS. There was 100% concordance between the immunoassay and LC-MS/MS results. 25 patients tested positive for opiates as well. Levamisole and 4(OH) levamisole were detected in 70.6% of the cocaine-positive urine samples, while the metabolite cocaethylene (indicator of concomitant alcohol consumption) was detected in 31.4% of cocaine-positive urine samples. Levamisole was not detected in opiate-positive urines in the absence of cocaine.

This method was successfully used to detect cocaine metabolites and its adulterant levamisole in urine and investigate the incidence of agranulocytosis in the setting of levamisole-tainted cocaine.

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Utility of Screening Bone Marrow Specimens for Monotypic Plasma Cells by Flow Cytometry

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The choice of which antibodies to use in flow cytometry panels is not standardized and typically depends on an assessment of relative value vs cost. Prior to June 2008, a tube to detect monotypic plasma cells (PCs) was run only if there was a history of PC dyscrasia, standard flow cytometric findings that may be suggestive of PCs, or if increased PCs were identified on the Wright-stained smears made on all cases. The goal of this study was to assess any benefit from running our PC tube on all bone marrow aspirate specimens, which detects CD38, CD45, CD56, cytoplasmic κ, and cytoplasmic λ and has a sensitivity of 0.01%.

Bone marrow cases submitted for flow cytometry phenotyping between June 2008 and November 2010 were retrospectively reviewed to identify those with 2% or less monotypic PCs. This value was chosen because 2% PCs would not have triggered running our PC tube. We then excluded all cases with a previous history of plasma cell dyscrasia (due to either a previous flow study or provided clinical history), cases with a concomitant monotypic B-cell population, and cases representing diagnostic samples for acute leukemia. Wright-stained aspirate smear slides were also reviewed for atypical, clustered, or increased PCs.

A total of 1.94% (157/8,097) of all bone marrow cases analyzed during the study period contained small monotypic PC populations that would have been missed by our laboratory prior to June 2008. An additional 0.22% (18/8,097) of cases contained small populations of PCs that were suspicious for being monotypic, with κ/λ ratios at the upper or lower limit of normal.

Including antibodies for the sensitive detection of plasma cells (PCs) in bone marrow flow cytometry screening panels is necessary to identify at least 2% of cases with small monotypic PC populations that would otherwise be missed by review of clinical history, initial histograms, or aspirate smears. Furthermore, screening all bone marrow samples for PCs can improve up-front laboratory workflow by eliminating the need to add PC tubes throughout the day and can also eliminate PC testing on poorer viability specimens initiated by delayed requests. Our approach of assessing multiple PC markers during the initial screen has the additional advantage of not needing to later run additional tubes to assess PC clonality or CD56-positive subpopulations. Screening for PCs in all marrow specimens appears to have several benefits that outweigh the minimal additional costs of the up-front testing.

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Expansion of a Second-Tier Newborn Screening Panel for Congenital Adrenal Hyperplasia to Include 21-Deoxycortisol and 11-Deoxycortisol Measurements From Dried Blood Spots

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Congenital adrenal hyperplasia (CAH) is a genetic disorder of steroidogenesis resulting in the inability to synthesize cortisol. Approximately 90% of CAH cases are a result of 21-hydroxylase deficiency; ~ 7% are a result of 11-hydroxylase deficiency. In both cases, cortisol will be decreased, while 17-hydroxyprogesterone (17OHP) and androstenedione will be increased. Measurement of 21- and 11- deoxycortisol facilitates the differential diagnosis of 21α-hydroxylase and 11β-hydroxylase deficiency. Our goal was to expand our current second-tier newborn screening panel for CAH, which measures cortisol, 17OHP, and androstenedione, to also

include the quantification of 11- and 21-deoxycortisol using ultra performance liquid chromatography–tandem mass spectrometry (UPLC-MS/MS).

All 5 steroids were extracted from dried blood spots (DBS) using 20:80 water/acetonitrile containing the internal standards and reconstituted in 10% acetone in water. Ten microliters of the sample was separated using a 2.1 × 50-mm, 1.7-µm, bridged ethyl hybrid C_{18} reverse phase column at 40°C. Using a flow rate of 0.6 mL/min and a gradient that progressed from 40% to 70% methanol, separation was completed in 4 minutes. A Waters Xevo MS/MS was used in positive ion mode with selective reaction monitoring for detection and quantitation. As internal standards, deuterated analogs of each steroid were added to the samples, allowing for accurate quantification.

The upper limit of linearity was 222 ng/mL serum for all 5 analytes, and the recovery was 100% to 105%. The lower limit of quantitation was 3.5 ng/mL serum. Intra-assay and interassay precision were determined using calibrators in DBS at 8 concentrations from 0 to 222 ng/mL serum. For the intra-assay precision, 3 to 5 replicates of each concentration of the 5 steroids were analyzed, with average CVs ranging from 7.6% to 11.6%. Interassay precision calculated over 3 days was 7.0% to 12.6%. The method was further validated by the quantification of the 5 steroids in CAH dried blood spot calibrators from the Centers for Disease Control.

We have successfully expanded our second-tier newborn screen for CAH to include 21-deoxycortisol and 11-deoxycortisol. The addition of these analytes will further improve the sensitivity and the specificity of neonatal screening for CAH.

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Suspected Patient Adulteration of Urine by Ex Vivo Addition of Oxycodone- and Buprenorphine-Containing Formulations

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Routine monitoring of opioids in urine is an established means for clinicians to determine patient compliance and to deter drug diversion. Evidence of patient compliance with chronic opioid therapy can be established by expected patterns of parent drug as well as drug metabolites (eg, glucoronidated, hydroxylated drug) in random urine samples. One consequence of noncompliance includes denial of future opioid therapy. To maintain access to opioid therapy, a patient may adulterate his or her own urine specimen to mimic compliance.

The purpose of this study was to evaluate urine samples positive for oxycodone or buprenorphine and suspicious for adulteration by ex vivo addition of, for example, crushed tablets. To initiate this study, urine samples were selected which contained more than 5,000 ng/mL parent drug (free) and little to no corresponding metabolite(s). Oxycodone and metabolite oxymorphone, as well as buprenorphine and metabolites (buprenorphine glucuronide, norbuprenorphine, norbuprenorphine glucuronide) were detected and quantified by LC-MS/MS. Specimens were demonstrated to be valid urine based on routine specimen validity testing (pH, creatinine, specific gravity, and oxidant testing). Next, the samples were analyzed for unmetabolized companion drugs acetaminophen, by HPLC-UV, and naloxone, by LC-MS/MS. Oxycodone is often formulated with acetaminophen (eg, Percocet), and buprenorphine is often combined with naloxone (eg, Suboxone). Acetaminophen is extensively metabolized, and only 3% to 7% of the parent molecule is passed in the urine. Naloxone, when taken orally, is metabolized in the gut, not absorbed extensively, and is not expected in the urine.

Sixteen suspicious urine specimens were analyzed. Five urine specimens with more than 5,000 ng/mL oxycodone and less than 10 ng/mL oxymorphone contained acetaminophen ranging from 68 to 510 µg/mL of urine. The acetaminophen/oxycodone ratios clustered around 17:1 to 23:1 (4 samples) with 1 sample being 86:1, which approximates the tablet formulation ratios (30:1-260:1) and suggests ex vivo origin. Eleven urine specimens with more than 5,000 ng/mL buprenorphine (5,821-49,619 ng/mL) and less than 40 ng/mL metabolite (sum of the 3) contained naloxone (1,984-11,305 ng/mL). The average buprenorphine/naloxone ratio was 3.4:1, approximating the ratio of Suboxone (4:1) and suggesting ex vivo origin.

Together, these data suggest that some patients may be adulterating urine to mimic compliance with prescribed opioid therapy. The possibility of intentional adulteration could be detected by quantitative analysis of parent drug, metabolites, and common companion drugs.

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Can the Quantitative Serum hCG Test Replace the Qualitative Detection of hCG in Serum?

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The qualitative detection of chorionic gonadotropin (CG) in serum using rapid, point-of-care tests is used for pregnancy diagnosis. Laboratories may also perform quantitative CG tests which, compared to qualitative tests, may have greater analytical sensitivity but longer analytical times. The objectives of this study were to compare the turnaround times (TAT) and the analytical sensitivities for qualitative and quantitative serum CG tests and to assess physician perceptions of the clinical performance of these tests.

The TATs for 920 qualitative and 1,186 quantitative CG tests performed at the University of Utah Hospital laboratory over 6 consecutive months were determined. Two TAT definitions were used: the total TAT (TTAT) was the time from test order to result reporting, and the lab TAT (LTAT) was the time from sample receipt to result reporting. Samples with a TAT more than 24 hours or less than 5 minutes, add-on tests, and samples from women younger than 18 years were excluded. 739 samples had enough volume to perform both qualitative (detection limit, 25 IU/L) and quantitative CG tests. A Web-based survey regarding perceptions and expectations of CG tests was sent to 1,058 University physicians.

The mean ± SD LTAT for 910 qualitative CG tests was 43 ± 48 minutes and was significantly ($P < .0001$) shorter than the 58 ± 41 minutes for 1,109 quantitative tests. The mean ± SD TTAT for 904 qualitative CG tests was 203 ± 231 minutes and was not significantly different ($P = .20$) from the 133 ± 147 minutes for 1,115 quantitative tests. Of 739 samples, 50 had a CG concentration more than 5 IU/L (considered positive). Compared to quantitative tests, qualitative tests were 80% sensitive, 100% specific, and had positive and negative predictive values of 100% and 98.5%, respectively. CG concentrations ranged from 6 to 31 IU/L in the 10 false-negative samples. 183 (17%) of survey respondents provided sufficient data for analysis. When pregnancy status needed to be known with greatest certainty, 59% and 25% preferred to use quantitative and qualitative CG tests, respectively ($P < .0001$); 88% and 80% were willing to wait up to 45 minutes for results from quantitative and qualitative tests, respectively ($P = .05$).

Qualitative serum CG tests can be performed faster than quantitative tests, but the TTATs are similar for both. Qualitative tests may fail to detect low CG concentrations. Physicians prefer quantitative serum CG tests when pregnancy status is to be known with high certainty and are willing to wait up to 45 minutes for results from either test. Quantitative tests can be substituted for qualitative tests and still fulfill physician expectations.

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Comparison of Gender-Specific Clinical Laboratory Reference Ranges in Transgender Patients

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Clinical practice guidelines for the treatment of transgender persons were recently published and call for routine laboratory monitoring of patients on cross-sex hormone therapy. However, many of the recommended tests have gender-specific reference ranges, leaving clinicians with the dilemma of having to decide what levels are "normal" for each individual patient.

The goal of this study is to identify consistent changes in analyte levels with hormone therapy, which could indicate whether changes from pretherapeutic levels should be considered indicative of potential adverse effects or may simply be a part of the desired physiological changes induced by the therapy.

IRB approval was obtained through Emory University. Twenty-two self-identified male-to-female transgender patients on hormone therapy were enrolled, and laboratory data were abstracted from the medical records. Preliminary analyte reference ranges generated from data from these patients was compared to normal range data from 25 male and 25 female nontransgender subjects.

Preliminary ranges for hemoglobin and hematocrit resembled the normal female reference ranges and were significantly different from the normal male ranges ($P < .0001$ and $P < .005$, respectively). All other analytes, including creatinine, sodium, potassium, albumin, blood urea nitrogen (BUN), alkaline phosphatase (ALP), alanine transferase (ALT), aspartate transferase (AST), and cholesterol, had notable overlap between the normal male and female ranges.

Use of correct gender-specific reference ranges plays an important role in the interpretation of laboratory results. Preliminary data suggest that there may be a difference in analyte reference ranges for transgender patients receiving hormone therapy compared to nontransgender patients. More extensive studies are needed to generate reliable statistical data confirming these differences.

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***KRAS* and *BRAF* Mutation Detection With Multiplex Amplification and Pyrosequencing**

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Targeted therapies against receptor tyrosine kinase signaling cascades play a key role in personalized medicine. For metastatic colorectal carcinomas (mCRCs), anti–epidermal growth factor receptor (EGFR) humanized monoclonal antibodies have been shown to improve patients' outcome. However, these therapies are reportedly effective in only 10% to 20% of such patients. Mutations in the *KRAS* gene (most frequently codons 12/13) can explain approximately 30% to 40% of nonresponsive cases. In addition, mutations in the *BRAF* gene, located directly downstream of *KRAS* in the EGFR-RAS-RAF signaling pathway, and less frequent mutations in codon 61 of *KRAS* have also been shown to confer resistance to anti-EGFR therapy. It is thus desirable to test for these mutations, ideally as a multiplex assay.

We designed a multiplex PCR protocol to amplify segments containing *KRAS* codons 12/13, *KRAS* codon 61, and *BRAF* codon 600. The PCR products are electrophoretically separated to confirm amplicon size. Pyrosequencing is performed on the same multiplex amplification reaction. A *KRAS* mutant cell line (HCT116), a wild-type control (placental DNA), and specimens of papillary thyroid carcinoma (*BRAF* mutant) were used to test this approach.

The PCR results showed adequate multiplex amplification of the *KRAS* codon 61, *KRAS* codons 12/13, and *BRAF* codon 600 amplicons. Pyrosequencing demonstrated expected sequencing patterns; mutated (GGC>GAC; G13D) sequences for *KRAS* in the HCT116 cell line, mutant *BRAF* V600E in 2 of 3 PTCs, and wild-type sequences in the control DNAs. However, there was increased background noise compared to non–multiplex PCR reactions.

We have demonstrated that the clinically significant codons of *KRAS* and *BRAF* in CRCs can be amplified simultaneously and then tested sequentially on the pyrosequencing platform. Optimization of the assay to reduce background noise and validation with a large number of samples will be required for clinical implementation of this assay.

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Detection of Internal Tandem Duplications (ITD) in the *FLT3* Gene by Different Electrophoretic Methods

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The internal tandem duplication in the juxtamembrane domain of the *FLT3* gene is one of the most frequent genetic alterations in acute myeloid leukemia (AML), associated with poor prognosis. A complex evaluation of the analytical properties of the 3 most frequently used detection methods—PCR followed by agarose (AGE), polyacrylamide (PAGE), or capillary electrophoresis (CE)—was performed on 95 DNA samples obtained from 73 AML patients.

We could verify the presence of a mutant allele in 20 samples of 18 patients, independently of the detection method used. AGE and PAGE could detect the presence of about 1% to 2% mutant allele, while the sensitivity of CE was 0.28%. However, acceptable reproducibility (interassay CV% <25%) of the mutant allele rate determination was only achievable above 1.5% mutant/total allele rate. The reproducibility of the ITD size determination by CE was much better (interassay CV% <11%), but the ITD size calculated by PeakScanner or GeneScan analysis showed 7% lower values compared to values obtained by DNA sequencing. The presence of multiple ITD was overestimated by PAGE and AGE due to the formation of heteroduplexes in these methods.

Based on these data, we suggest the use of CE combined by PeakScanner analysis in the diagnostics and the follow-up of AML patients.

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A Retrospective Analysis of Measured Versus Estimated Free Phenytoin Concentrations

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Measurement of free (unbound) concentrations of phenytoin in plasma has been recommended in a variety of clinical situations, such as in patients with low albumin concentrations. However, measurement of free phenytoin concentrations is more time-consuming and analytically more challenging than determining total phenytoin concentrations, leading some clinical laboratories to estimate the free phenytoin concentration or an "adjusted" total phenytoin concentration by a formula that corrects for albumin concentration. The present study analyzed data from inpatients and outpatients over 14 years at an academic medical center.

Following IRB approval, the electronic medical record was searched for patients that had total and free phenytoin plasma concentrations determined on the same plasma sample and also an albumin concentration within 7 days of the phenytoin measurements. This yielded 1,753 data points from 756 patients.

The results showed that the Sheiner-Tozer equation for calculating an estimated free phenytoin more frequently underestimates than overestimates the measured free phenytoin. Three-by-three contingency tables that divided concentrations into below therapeutic range, within therapeutic range, and above therapeutic range showed that estimated and measured free phenytoin concentrations were concordant 69.6% of the time. In contrast, measured total and free phenytoin were concordant by a similar analysis only 46.6% of the time. Discrepancies between total and free phenytoin were more common at plasma albumin concentrations less than 3 g/dL, but there was considerable scatter in the data across all albumin concentrations. Patient age, gender, or patient location (eg, inpatient, outpatient, emergency room) were not significant variables in the predictive value of estimated free phenytoin. In addition, using albumin concentrations determined up to 7 days prior to measuring phenytoin concentrations did not limit the predictiveness of estimate free phenytoin.

The results suggest that a measured free phenytoin should be obtained where possible to guide phenytoin dosing. If this is not feasible, then an estimated free phenytoin can supplement a total phenytoin concentration.

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A Retrospective Analysis of Laboratory Tests to Diagnose Ingestion of Toxic Glycols and Alcohols

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Patients who ingest ethylene glycol and nonethanol "toxic" alcohols (methanol, isopropanol) may present with nonspecific signs and symptoms (eg, altered mental status) and be difficult to diagnose clinically. Definitive measurement of glycols, methanol, and isopropanol is by gas chromatography (GC); however, few hospitals have clinical laboratories that perform this assay on site with fast turnaround time. Consequently, laboratory tests, including electrolyte panels (yielding anion gap), plasma osmolality (yielding osmolal gap), and arterial blood gas measurements, are commonly used to help diagnose or rule out toxic alcohol ingestion.

The present study analyzed laboratory and clinical data over 14 years at an academic medical center in an IRB-approved study. In this time period, 22,994 patients had results from a screening panel that determined plasma ethanol and estimation of osmolal gap (corrected for plasma ethanol concentration, if present). 321 patients had GC performed for alcohols and glycols. Of these, 57 were from patients that had detectable ethylene glycol, propylene glycol, methanol, and/or isopropanol; the remaining 264 were negative for all alcohols and glycols.

There were 7 patients who had osmolal gap less than 14 who, nonetheless, had toxic alcohols or glycols by GC. Six of these patients were positive for ethylene glycol and had histories of consuming small amounts of antifreeze. One patient was a toddler who consumed a small quantity of windshield fluid. All patients with known toxic alcohol ingestions had an anion gap or osmolal gap or both. Other than toxic alcohols or glycols, the most common suspected causes for an elevated osmolal gap (>14) were ethanol consumption (mostly patients in withdrawal with low plasma ethanol concentrations), renal failure, diabetic ketoacidosis, activated charcoal, mannitol, and multiorgan failure. Only 9 patients with osmolal gap more than 50 and no patients with osmolal gap more than 100 were found to be negative for glycols and toxic alcohols.

This study demonstrates the utility of osmolal and anion gaps in the evaluation of toxic alcohol ingestion.

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Determination of Whole Blood Everolimus Concentrations Using Architect Sirolimus Immunoassay and Mathematical Equations: Comparison With Everolimus Values Obtained by Liquid Chromatography–Tandem Mass Spectrometry

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The FDA in 2010 approved everolimus, which requires therapeutic drug monitoring in whole blood. Currently, there is no FDA-approved immunoassay for everolimus, and only chromatography–tandem mass spectrometric (LC/MS/MS) methods are available. Many hospital laboratories do not have this instrument.

We hypothesized that by taking advantage of the structural similarity between sirolimus and everolimus, it is possible to rapidly determine the everolimus concentration from the apparent sirolimus concentration obtained by the Architect sirolimus immunoassay and mathematical equations. These equations are derived by curve-fitting methods based on observed apparent sirolimus concentrations and true everolimus concentrations as determined by LC/MS/MS. We analyzed 8 everolimus standards prepared in whole blood (concentration range, 1-30 ng/mL) using both the Architect sirolimus immunoassay and the LC/MS/MS everolimus assay. In order to determine the validity of our approach, we also analyzed 25 specimens from patients receiving everolimus using both the immunoassay and LC/MS/MS.

Everolimus standards exhibited 74% to 100% cross-reactivities with the sirolimus immunoassay. We attempted various methods of curve-fitting (linear, polynomial, exponential, logarithmic, and power) to obtain the best correlation between apparent sirolimus

concentrations and true everolimus concentrations. For this purpose, we plotted apparent sirolimus concentrations on the x-axis and true everolimus concentration on the y-axis and observed best curve fitting using a polynomial equation: $y = -0.0042x^2 + 1.3985x - 0.4322$ ($r = 0.99$). Next best fitting was observed with a linear equation: $y = 1.2944x - 0.0689$ ($r = 0.97$). Using these equations, the everolimus concentration (y) can be easily calculated from the apparent sirolimus concentration (x). We observed good correlation between calculated everolimus values and true everolimus values; although there was an average 9% positive bias in calculated everolimus values using a polynomial equation ($y = 1.09x - 0.38$; $r = 0.98$) and a 12% positive bias using a linear equation ($y = 1.12x - 0.47$; $r = 0.97$). Out of 25 specimens analyzed, only 1 specimen showed positive bias over 20% (23.2% using a polynomial equation, 25.0% using a linear equation).

We conclude that everolimus concentrations can be calculated using mathematical equations and apparent sirolimus concentrations, which correlate reasonably well with true everolimus values as determined by the LC/MS/MS method.

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Lupus Anticoagulant (LAC) Prolongation of Activated Partial Thromboplastin Time in 1:1 Mix Is Affected by Incubation Time and LAC Strength, Which Complicates Factor VIII inhibitor Screening

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Our study analyzes the effects of length of incubation time and strength of lupus anticoagulant (LAC) on clotting times and prolongation of aPTT 1:1 mix assays with incubation. We also looked at the effect of a longer incubation time (2 hours vs 1 hour) on screening for factor VIII (F8) inhibitors by aPTT 1:1 mix with incubation.

We performed a retrospective analysis of aPTT 1:1 mix assays with incubation encompassing periods during which incubation was performed for either 1 hour (9 months) or for 2 hours (7 months) at the University of Washington Medical Center. Patients that had contemporaneous 1:1 mix and LAC assays were included in the study. A clotting time prolongation comparing immediate mix to incubated mix of either 6-7s (borderline) or 8s or more was used to screen for possible F8 inhibitors; eligible specimens were then retested by a F8 inhibitor Bethesda assay. Patients that screened positive for both incubation times were analyzed for true- and false-positives for F8 inhibitor. A second analysis compared the strength of the LAC (weak, moderate, or strong) with prolongation of 1:1 mix clotting times, stratified by 1-hour and 2-hour incubations.

With the 2-hour incubation, 12 out of 83 patients screened positive (14.5%) for prolongation of clotting times 6s or more, of which none were found positive for F8 inhibitor, and 8 were confirmed as LACs. With the 1-hour incubation, 4 of 166 patients screened positive (2.4%), of which 1 was found positive for F8 inhibitor and 1 was a strong LAC. When prolongation times were compared to strength of LAC, there was an increase of average prolongation (seconds) with LAC strength (nonreactive, 1 ± 0.4; weak, 2 ± 3; moderate, 3 ± 4; strong, 5 ± 5). When stratified for incubation time, the prolongation was increased with the longer incubation (1 hour: nonreactive, 1 ± 0.4; weak, 2 ± 1; moderate, 2 ± 2; strong, 4 ± 3; 2 hour: nonreactive, 1 ± 0.4; weak, 2 ± 6; moderate, 7 ± 6; strong, 9 ± 8). Our study suggests that prolongation of clotting times for aPTT 1:1 mixes from patients with LACs are proportional to both length of incubation time and strength of the LAC. Also, we observed that using the longer incubation time (2 hours) to screen for possible F8 inhibitors increases the frequency of false-positives, most of which are due to LACs.

When using aPTT 1:1 mixes to screen for F8 inhibitors, it is important to consider the effect of a possible LAC on the interpretation of the aPTT prolongation and to be aware that this effect is influenced by both assay incubation time and strength of the lupus anticoagulant.

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Alleviation of IgM Monoclonal Protein Interference in Nephelometric Assays by Pretreatment With Reducing Agent in a Chaotropic Salt Solution

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The presence of monoclonal IgM proteins can cause substantial false elevation of nephelometric-assayed analyte concentrations, including CRP, IgG, and IgA, in a subset of patients. The mechanism of this interference has not been completely elucidated. Here, we report that the addition of reducing agent in a chaotropic salt solution can alleviate artifactual elevation of nephelometric determinations of IgG and IgA concentrations.

A 59-year-old female presented with a high serum viscosity and 1-year history of achiness, fatigue, and bleeding. Nephelometric analysis at 37°C on the Siemens BNII instrument revealed significantly elevated concentrations of CRP and IgG, IgA, and IgM immunoglobulins. Immunofixation electrophoresis (IFE) resulted in nonmigrating protein aggregates. A 1:9 solution of 10% B-mercaptoethanol and dilution fluid containing chaotropic salts (Fludil, Sebia) were added to patient serum sample. This pretreatment resulted in the resolution of cross-linked immunoglobulins into a distinct IgM κ monoclonal protein band by IFE. Treatment with Fludil alone did not resolve the cross-linked monoclonal protein. A diagnosis of Waldenström macroglobulinemia was confirmed by bone marrow pathology.

Patient specimens were treated with reducing agent solution as described above and measured IgG, IgA and IgM concentrations compared in parallel to untreated patient samples. A ~50% drop in IgG and IgA concentrations was observed in the presence of reducing agent, while IgM concentrations were unchanged. Treated and untreated control specimens with IgM, IgG, and IgA monoclonal proteins from patients that did not exhibit cross-linking by IFE were also examined. The control specimens exhibited no significant change in assayed immunoglobulin concentrations upon treatment.

These results are consistent with dissociation of higher order IgM complexes by reducing agent and chaotropic salts, leading to alleviation of nonspecific light scattering by IgM aggregates in the IgA and IgG nephelometric reactions. The preservation of measured immunoglobulin in control specimens indicates that the assay was not disrupted by the mere presence of reducing agent and chaotropic salts. While these agents should be utilized with caution as they may be deleterious to analytical instrumentation, this study indicates that similar pretreatment protocols could be used to identify or alleviate nephelometric interference in the presence of cross-linked monoclonal proteins.

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ADAMTS13 Distinguishes Thrombotic Thrombocytopenic Purpura (TTP) From Other Forms of Thrombotic Microangiopathy (TMA), and Rituxan Is an Effective Adjunct Treatment in Refractory TTP

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ADAMTS13 is a metalloproteinase that cleaves ultra-large vWF multimers to generate normal-sized multimers present in normal circulation. Its severe deficiency (<10%) leads to persistence of ultra-large vWF multimers that are responsible for formation of platelet-vWF-rich microthrombi in microcirculation. Severe ADAMTS13 deficiency due to genetic mutations is diagnostic of congenital TTP; however, severe deficiency due to autoantibodies is still not universally accepted as diagnostic of acquired TTP.

We aim to show that ADAMTS13 deficiency can be used to diagnose acquired TTP and distinguish it from TMA.

We performed a retrospective analysis of consecutive patients (January 2006 to October 2010) with TMA that had ADAMTS13 activity performed. Demographics, presenting clinical and laboratory features, responses to plasma exchange (PLEX) and Rituxan, discharge diagnoses, and other underlying clinical conditions were recorded. Statistical analysis was performed using an unpaired *t* test to compare means and the Fisher exact method for contingency tables.

We divided our cases based on severe ADAMTS13 deficiency (<10%) as TTP (26/50 patients) and nonsevere deficiency (>10%) as TMA (24/50 patients). TMA included quinine-induced HUS (1), malignant hypertension (1), HIV (5), drugs (3), preeclampsia (1), sepsis (3), MCTD/SLE (2), HELLP (1), AML (1), parvovirus B19/unspecified TMA (1), ITP (2), PCKD/HIT (1), malignancy (2). Statistically significant differences between TTP and TMA included lower presenting platelet count in TTP (mean of 26×10^9/L vs 65; $P = .004$). The TTP group also had a more significant improvement in platelet count (87% vs 25%; $P = .005$) and LD (83% vs 25%; $P = .002$). TMA patients either did not respond to PLEX or improved without PLEX. The mortality was higher in the TTP vs TMA group (17% vs 4.2%, respectively). Rituxan was a useful adjunct in TTP patients with high inhibitor titers and slow responders.

ADAMTS13 activity less than 10% was diagnostic of TTP vs higher levels (>20%) were found in patients with TMA. Symptoms such as altered mental status, renal failure, and fever were not statistically different and did not separate TTP from TMA. TTP patients presented with significantly lower platelet counts than the TMA. Additionally, the TTP group had a better response to PLEX as compared to TMA.

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Validation of Calculated Free and Bioavailable Testosterone Using the Vermeulen Formula

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Testosterone (T) binds nonspecifically to albumin and specifically to sex hormone binding globulin (SHBG). Free and albumin-bound T are referred to as bioavailable T (BioT). BioT rather than direct measurement of free T (FT) is believed to be more accurate in the estimation of hormonal status. Biological changes affecting SHBG and albumin have a direct effect on BioT, particularly in individuals with nephrotic syndrome, males with low total T (TT), and females with evidence of hyperandrogenism. The Vermeulen formula allows calculation of FT and BioT using TT, SHGB, albumin concentrations, and known binding constants (*J Clin Endocrinol Metab.* 1999;84:3666-3672).

To validate the use of the Vermeulen formula in our clinical practice, we compared calculated FT and BioT to equilibrium dialysis and differential precipitation coupled to liquid chromatography–mass spectrometry (LC-MS/MS), respectively. FT directly measured by radioimmunoassay (RIA) was also compared to equilibrium dialysis. Consecutive serum specimens (11 females, 19 males; age range, 20-77 years) were used after institutional review board approval. TT and SHBG were measured using a paramagnetic particle, chemiluminescent immunoassay on the Beckman DXI analyzer. FT and BioT concentration were calculated using the Vermeulen formula or measured either by competitive analog-tracer radioimmunoassay (RIA, Siemens Coat-A-Count) or equilibrium dialysis and differential precipitation techniques coupled to LC-MS/MS. For the purpose of the correlations, albumin concentration was assumed to equal 4.3 g/dL. Data were analyzed using Microsoft Excel (Microsoft) and EP Evaluator (Data Innovations).

Calculated FT and BioT demonstrated excellent correlation with equilibrium dialysis and differential precipitation. Linear regression for TT was TT (Beckman) = 1.0335 (LC-MS/MS) – 17.624. FT and BioT correlations were FT (calculated) = 0.5098 (equilibrium dialysis) + 0.5683, and BioT (calculated) = 0.9113 (differential precipitation) + 23.112, respectively. For FT measured directly, the regression was FT (RIA) = 0.6791 (equilibrium dialysis) + 0.3753. The correlation coefficients ranged from 0.92 to 0.94. Calculated FT was approximately 50% lower than the concentration measured by equilibrium dialysis. This difference is reflected in the method-specific reference ranges.

Calculated FT and BioT indices provide an accurate, convenient, and reliable alternative to direct analysis while avoiding limitations of the direct assay.

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A Multi-Marker Approach to Differentiate Sepsis From SIRS

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Sepsis is a major cause of mortality in the critically ill and is challenging to differentiate from systemic inflammatory response syndrome (SIRS). SIRS is identified by the presence of 2 or more of the following: abnormal body temperature and white count, tachypnea, and/or tachycardia. Sepsis is SIRS in the presence of infection. Rapid identification and treatment of septic patients with antibiotics and volume resuscitation significantly reduces morbidity and mortality; SIRS patients do not require antimicrobial therapy. A laboratory test that could rule-in sepsis among ICU patients with SIRS may help facilitate fast and appropriate treatment decisions. Individual biomarkers are unable to reliably predict sepsis. A panel of biomarkers reflecting the complex pathophysiology of sepsis may best predict disease.

Our objective was to identify a panel of biomarkers that detects sepsis in ICU patients with SIRS.

We included 169 leftover plasma samples collected from ICU patients on the first day they had SIRS (identified through an automated electronic medical record scan). Of these, 67 had sepsis

and 102 had noninfectious SIRS. Concentrations of 5 markers, CRP, LBP, IL-6, IL-10, and TNFα, were determined by immunoassay on the Siemens Immulite 1000. ROC analysis for each biomarker was used to generate the area under the curve and select optimal cutoffs. The ability to predict sepsis at optimal cutoffs was assessed for single and combinations of biomarkers.

Areas under the curve for the individual biomarkers were 0.76, 0.72, 0.78, 0.73, and 0.74 for CRP, TNFα, IL-6, IL-10, and LBP, respectively ($P < .0001$ for all). Optimal cutoffs to identify sepsis were CRP, 87 mg/dL (75% sensitivity, 72% specificity); TNFα, 22 pg/mL (52% sensitivity, 82% specificity); IL-6, 58 pg/mL (76% sensitivity, 74% specificity); IL-10, 12.7 pg/mL (60% sensitivity, 84% specificity); and LBP, 13.8 μg/mL (84% sensitivity, 63% specificity). Several biomarker panels showed excellent diagnostic strength to rule-in sepsis: positive likelihood ratios were 33.5 for all 5 markers, as well as TNFα, IL-6, IL-10, and LBP, and 32.0 for the combination of CRP, TNFα, IL-6, and IL-10 (all with positive predictive values of 96%). Patients positive for all 5 markers within 5 days of meeting SIRS criteria were more likely to die (odds ratio = 3.2), while those negative for all markers had an excellent prognosis (odds ratio = 0.04).

A combination of biomarkers, CRP, TNFα, IL-6, IL-10, and LBP, shows robust diagnostic strength to rule-in sepsis and predict mortality in critically ill patients with SIRS. These markers should be prospectively evaluated in ICU patients.

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Analytical and Clinical Validation of the Immulite 1000 hCG Assay for Quantitative Analysis in Urine

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Currently, no automated immunoassay is routinely used to quantitatively measure human chorionic gonadotropin (hCG) in urine for the diagnosis and monitoring of pregnancy. Distinct hCG variants are present in urine of women at different times during pregnancy. Ideally, an hCG assay would detect all known variants of hCG found in the urine during pregnancy, including total hCG dimer (hCG), free β subunit (hCGβ), and β core fragment (hCGβcf). The Siemens Immulite hCG assay detects all major hCG variants in serum. However, to date, no one has investigated its ability to quantitatively measure hCG and its variants in urine. Our objective was to validate the Immulite hCG assay for quantitative determination of total immunoreactive hCG in urine for routine clinical detection and monitoring of pregnancy.

Validation experiments were performed by adding known concentrations of hCG from pregnancy urine and/or purified 1st WHO reference reagent for hCG, hCGβ, and hCGβcf into hCG-free urine from males 18 to 40 years old. Precision, linearity, recovery, lower limit of quantitation, and the hook effect were assessed. Reference intervals were established by nonparametric methods from hCG measured in the urine samples collected from 120 reference subjects in each of 3 cohorts: females younger than 55 years, females 55 years or older, and males 20 to 70 years old.

The Immulite hCG assay was precise for measuring hCG from pregnancy urine with intra-assay and interassay imprecision for all concentrations measured less than 20% CV. The assay was linear over a dynamic range of 2 to 5,000 mIU/mL with pregnant urine, hCG, and hCGβ. The assay was nonlinear for hCGβcf with 2 separate linearities from 2 to 200 and 100 to 5,000 mIU/mL. The lower limit of quantitation for pregnancy urine was 2 mIU/mL. No hook effect was observed at concentrations up to 1,200,000 pmol/L, much greater than physiologically expected, for hCG (previous study), hCGβ, or hCGβcf. The reference intervals were 0 to 1.5 mIU/mL for males, 0 to 2.1 mIU/mL for females younger than 55 years, and 0 to 12.2 mIU/mL for females 55 years or older.

The Immulite 1000 hCG assay accurately quantitates hCG in urine throughout pregnancy. Caution should be used when serially monitoring pregnancies in which hCGβcf is the major urine isoform as measurements are nonlinear throughout the dynamic range of the assay.

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Thrombotic Microangiopathy (TMA) in Patients With Antiphospholipid Syndrome Is an Important Cause of Acute Renal Graft Failure: A Case Report

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Antiphospholipid syndrome (APS) is a clinical disorder characterized by a tendency to recurrent arterial and venous thrombosis. APS can be primary or secondary to a number of conditions, including systemic lupus erythematosus (SLE), malignancy, drugs, DIC etc.

We present a case of a 48-year-old parous female with a history of SLE, end-stage renal disease maintained on peritoneal dialysis, and under consideration for renal transplantation. Her HLA phenotype using the reverse sequence specific oligonucleotide probe (rSSOP) method was as follows: HLA-A*03, 29; B*07, 15 (Bw6); C*03, 15; DRB1*04, 13; DRB3*03, B4*01; DQB1*03, 06. She had a class I panel reactive antibody (PRA) of 72% and a class II PRA of 0%. She received an unrelated living donor kidney transplant from her sister-in-law whose HLA profile was as follows: HLA-A*01, 03; B*07, 15 (Bw6); C*03, 07; DRB1*04, 11; DRB3*02, B4*01; DQB1*03. No donor-specific antibodies (DSA) were detected at the time of transplant, and T-cell and B-cell flow cytometric crossmatches were negative.

Four days posttransplant, the patient developed fever, rising creatinine, and dropping platelet counts. A Doppler ultrasound showed no arterial or venous flow. The patient underwent an emergency nephrectomy of the kidney graft. Histology showed widespread microvascular, arterial thrombi, consistent with thrombotic microangiopathy (TMA), and acute tubular injury with focal cortical infarction. There was no pathological evidence of antibody-mediated rejection (AMR), C4d staining was negative, and DSA were not detected.

On the fourth postoperative day, testing for lupus anticoagulants showed elevated anti–β_2-glycoprotein antibody titers (IgG, 40.7 U/mL and IgA, 35.5 U/mL). It is possible that the patient's TMA could be attributed to the undiagnosed lupus anticoagulants. Seven months posttransplantation, antibody testing indicated the presence of de novo HLA-A1 DSA and increased PRA. We speculate that there may be an additional component of AMR in this case. On subsequent follow-up, the patient is back on the transplant waiting list with an even higher PRA (93%). High PRA and the presence of lupus anticoagulants make the decision for retransplantation particularly challenging.

TMA is an infrequent but important cause of acute graft failure. In patients with a history of APS with a high PRA, aggressive medical management with plasmapheresis, immunosuppression, and anticoagulation may be necessary prior to and after transplantation.

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Effect of Skin Color on Transcutaneous Bilirubin Measurements: BiliChek Versus Drager Method

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Bilirubin measurements are required for all newborns for the prevention of kernicterus. Our hospital has been utilizing a noninvasive point-of-care (POC) transcutaneous bilirubin (TcB) meter, the BiliChek system by Philips, and found the system provided reliable results while reducing the need for serum bilirubin testing in our core laboratory. The essentially instantaneous results facilitate our 24-hour discharge protocol for all well newborns. Recently, we initiated an evaluation of a more cost-effective Drager Jaundice Meter (Model JM-103), which eliminates the extra expense of disposable shields.

Comparison studies were performed to establish the accuracy and precision of the new Drager meter. Initially, 73 newborns were tested for TcB using the BiliChek and Drager devices as well as serum bilirubin testing in the core laboratory on our Beckman Coulter DxC800 chemistry analyzers. All sternum TcB measurements were performed by our trained nursing personnel following manufacturers' recommendations.

On comparison of the Drager method with our BiliChek method, we observed the following: TcB-Drager = 0.90 x TcB-BiliChek + 0.40; R^2 = 0.67. After observation of a significant positive bias on the Drager meter in 3 darkly pigmented newborns, we initiated an additional 47-patient TcB comparison study focused on the effect of skin color. In all studies, both the BiliChek and Drager TcB methods showed reasonable correlation with serum bilirubin levels: TcB-BiliChek = 0.99 x DxC800 + 1.35; R^2 = 0.78; TcB-Drager = 1.03 x DxC800 + 0.56; R^2 = 0.73). While the initial study showed significant bias of the Drager meter on 3 dark-skinned newborns (8.4, 9.3, 9.2 mg/dL serum vs 13.3, 14.5, 14.1 mg/dL Drager, respectively), our follow-up skin color studies failed to confirm this observed bias. Indeed, accounting for skin color, the Drager had the following results when compared to serum bilirubin: light-skin sternum, TcB-Drager = 1.02 x DxC800 – 0.67; R^2 = 0.76; dark-skin sternum, TcB-Drager = 1.13 x DxC800 – 0.50; R^2 = 0.86. The same study was performed on the BiliChek meter with results as follows: light-skin sternum, TcB-BiliChek = 0.92 x DxC800 + 0.91; R^2 = 0.73; dark-skin sternum, TcB-BiliChek = 1.10 x DxC800 + 0.54; R^2 = 0.85.

We concluded that the Drager meter compared reasonably well with both the BiliChek and the DxC800 methods regardless of skin color and can be safely used in our clinical setting.

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Classification Error Plots: A New Method for Determining the Effect of Bias and Imprecision on the Probability of Correct Classification for Cardiovascular Disease Risk

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Laboratory results are often used to classify patients into categories used for risk assessment. An important example is the National Cholesterol Education Program ATP III (NCEP ATP III) guidelines for cardiovascular disease (CVD) risk based on LDL-C. Method performance required to accurately categorize patients is, however, often poorly defined. For LDL-C, the NCEP ATP III guidelines recommend a total allowable error (TEa) of less than 12%. TEa is often defined as bias plus 2 times imprecision (CV).

The aim of this study was to evaluate the effect of bias and imprecision on the classification of patients. This was done by simulating the effect of bias and imprecision on a data set of 1,000 results with distribution of results similar to that of men, aged 60 to 64 years, surveyed in the National Health and Nutrition Examination Survey (NHANES) study. Each result was categorized into cardiovascular risk category as defined by NCEP ATP III criteria before simulating the effect of all possible combinations of bias (–15% to 15%) and imprecision (ranging from 0% to 14%) on classification. The probability of a patient being correctly classified after introduction of bias and imprecision was then calculated for each result. The mean probability of correct classification was calculated for each CVD risk category, and an overall probability of correct classification (P_{CC}) was calculated by summing the mean probability of correct classification in each risk category multiplied by the percentage of population falling into that risk category. The effect of various bias and imprecision combinations on P_{CC} is then demonstrable on a 3-dimensional classification error surface plot.

Some points on this plot, all corresponding to a TEa = 12%, are P_{CC} = 0.80 when bias = 0% and CV = 6%; P_{CC} = 0.82 when bias = –2% and CV = 5%; P_{CC} = 0.81 when bias = 2% and CV = 5%; P_{CC} = 0.81 when bias = –4% and CV = 4%; P_{CC} = 0.79 when bias = 4% and CV = 4%; P_{CC} = 0.75 when bias = –6% and CV = 3%; and P_{CC} = 0.73 when bias = 6% and CV = 3%. P_{CC} is dependent on the underlying distribution of results, the number and size of classification categories, and analytical performance and does not closely correspond with TEa. We also describe a mathematical equation for calculation of P_{CC} at any given bias and imprecision.

Use of classification error plots instead of TEa provides better guidance for evaluating method performance because it is a direct assessment of the probability of correct classification. It can also be a useful tool for deciding optimum cut points in the development of national guidelines for the interpretation of clinical laboratory test results.

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Establishment of Reference Intervals and Clinical Decision Limits for Detection of MacroAST

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Macro–aspartate aminotransferase (macroAST) complexes are benign macroenzymes that exhibit reduced clearance from blood and result in increased serum AST activity. Confirmation of macroAST is important to reduce unnecessary and invasive diagnostic procedures. Several methods have been proposed to detect macroAST, including polyethylene glycol (PEG) precipitation. However, reference intervals and cutoffs for confirming the presence of macroAST are needed.

The purposes of this study are to (1) establish reference intervals for AST in PEG-precipitated samples from normal and liver disease samples and (2) validate a cutoff for the detection of macroAST.

Residual samples (IRB No. 10-007098) with the following characteristics were obtained: (1) AST, alanine aminotransferase (ALT), and alkaline phosphatase (ALP) activity within reference intervals (normal, n = 120); (2) AST, ALT, and ALP results exceeding upper reference limits (elevated liver enzymes, n = 120); (3) AST more than 100 U/L, AST/ALT more than 2.0, and ALT and ALP activity within reference intervals (possible macroAST, n = 38); and (4) clinically suspected macroAST based on physician order (suspected macroAST, n = 14). Samples were treated with PEG6000 (12.5% final concentration) followed by centrifugation (1,500*g*, 5 minutes). Protein G columns (Pierce) were used to confirm the presence of IgG-AST complexes. AST was measured on a Roche Cobas c501, and % PEG-precipitated AST was calculated. Reference intervals were determined by nonparametric analysis. Assay performance characteristics were determined using PEG-treated samples.

Precision, recovery, and analytical measurable range were comparable to assay characteristics observed in non–PEG-treated samples. Reference intervals for % PEG-precipitated AST in normal and elevated liver enzyme samples were 0% to 51% (90% CI upper limit, 45%-71%) and 5% to 51% (90% CIs, 3%-7%, 47%-64%), respectively. The mean % PEG-precipitated AST in possible macroAST samples was 26% ± 14% (range, 0%-57%). Suspected macroAST samples yielded an average 94% ± 5% PEG-precipitated AST (range, 84%-99%). In 7 of 8 suspected macroAST samples, protein G columns bound 69% ± 17% of AST activity (range, 43%-86%). Protein G columns bound 25% ± 8% of AST activity (range, 14%-41%) in 10 control samples.

Reference intervals for % PEG-precipitated AST were comparable between normal and elevated liver enzyme samples. PEG precipitation can be used to identify macroAST, and a clinical decision limit of more than 80% PEG-precipitated AST activity is sensitive and specific for detecting macroAST.

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Myofibroblastoma of the Breast: An Ambiguous Tumor With Myofibroblastic and Smooth Muscle Differentiation

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Myofibroblastoma (MFB) of the breast is a rare, benign mesenchymal neoplasm commonly affecting older adults with a reported male predilection. It is typically a small, well-circumscribed lesion with a fascicular growth of bland spindle cells separated by hyalinized collagen bands. The lesional cells have immunophenotypic characteristics of myofibroblasts, as evident by CD34 and smooth muscle actin (SMA) expression, although a clear-cut definition for classifying a cell as a "myofibroblast" has not been generally adopted. In fact, smooth muscle differentiation of MFB by ultrastructural analysis has also been previously suggested.

Here, we report 2 cases of MFB. The first case was a 1.0-cm nodule incidentally found in a 72-year-old female with invasive mammary carcinoma treated by lumpectomy. The second case occurred in a 53-year-old male who presented with a 2.2-cm breast mass. Both tumors were grossly nodular and well-demarcated with a tan-white cut surface. Histologically, both lesions showed fascicles of bland, uniform spindle cells dissecting broad bands of hyalinized collagen. The neoplastic cells were diffusely immunoreactive with CD34 and SMA as well as h-caldesmon. Given that the latter is exclusively expressed in smooth muscle and myoepithelial cells but is virtually absent in myofibroblastic lesions, we further explored the histogenesis of MFB at the ultrastructural level in the second case. Features characteristic for smooth muscle differentiation, such as bundles of myofilaments with fusiform dense bodies, were not identified in the lesional tissue by transmission electron microscopy analysis.

Thus, MFB represents an ambiguous tumor with both myofibroblastic and smooth muscle differentiation and is likely derived from CD34+ progenitor cells of mammary stroma capable of multidirectional differentiation, including fibroblasts and smooth muscle. Further immunophenotypic and ultrastructural analysis with a larger number of cases to better characterize this tumor is needed.

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Early Detection of Relapse in Multiple Myeloma

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With novel drugs, most newly diagnosed MM cases are responsive to treatment, but almost all MM patients who respond to initial treatment will relapse and require additional therapy. Traditionally, immunotyping a patient's immunoglobulin is accomplished by immunofixation electrophoresis (IFE), but the M protein associated with some patient's disease presents as a broadly restricted band. In such cases, a recurrence of small concentrations of monoclonal protein cannot be easily distinguished from the polyclonal background. Our objective was to determine if the newly developed Hevylite (HL) assay can be used to monitor for relapse. This nephelometric assay utilizes antibodies specific for the intersection of the heavy and light chain of each immunoglobulin and allows separate quantitation of each immunoglobulin heavy chain and light chain combination.

Reference ranges for each of the immunoglobulins were established (n = 147, age 22-90): IgGκ, 407 to 1,130 mg/dL; IgGλ, 188 to 583 mg/dL; IgAκ, 55 to 292 mg/dL; IgAλ, 39 to 249 mg/dL; IgMκ, 16 to 182 mg/dL; and IgMλ, 10 to 92 mg/dL. Reference ranges for HL pairs were also established: Gκ/Gλ, 1.2 to 3.6; Aκ/Aλ, 0.7 to 2.2; and Mκ/Mλ, 0.9 to 3.7. An IgAκ myeloma case in which a yearlong monitoring of remission revealed consistently normal serum and urine PEL and IFE, normal FLC ratios, but a steadily increasing IgA concentration was used in our study. Elevations in immunoglobulin concentration, however, are not indicative of clonal increases and are insufficient to diagnose recurrence.

To give insight to the clonality of the IgA, the Hevylite test was performed on current as well as selected stored frozen serum samples. Results of this analysis revealed the Aκ/Aλ ratios never normalized in this patient. In addition, during the period of increasing IgA levels, Aκ/Aλ ratios continued to steadily rise, supporting the existence of an abnormal clone despite the apparently normal IFE. The ratio of the IgAκ and the IgAλ concentrations indicated there was a preferential synthesis of IgAκ. Importantly, even when the IgA level transiently decreased after chemotherapy was increased, the HL ratio revealed the clonal expansion of the IgAκ continued to progress. The patient's recent SPEP and IFE and progression of boney disease indicate relapse of disease.

The HL test can be used to distinguish a clonal expansion of immunoglobulin from a polyclonal background, specifically in the case of patients that present with a broadly restricted band on electrophoresis. In addition, the HL test may have previously heralded MM

disease relapse in this case by the steadily increasing ratio of HL and could be a useful tool in remission monitoring.

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Sphingolipid Profiles in Cystic Fibrosis Are Characterized by Increased Atypical Sphingolipids, Sphinganine-1-Phosphate and Decreased Sphingosine-1-Phosphate

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Cystic fibrosis (CF), a chronic lung disease, is caused by mutations in CFTR, a chloride channel. We reported that defective CFTR increases serine-palmitoyl transferase (SPT)-dependent de-novo synthesis of sphingosine (SO) and sphinganine (SA), precursors of the immune-active sphingolipids sphinganine-1-phosphate (Sa1P) and sphingosine-1-phosphate (S1P). 1-Deoxysphinganine (DSA) is generated by SPT using alanine as a substrate rather than serine. We measured serum sphingolipid profiles from CF patients and controls and evaluated their relationship to LDL, HDL, triglyceride (TG), and total cholesterol (TC) profiles that are known to be altered in CF.

We collected serum from 90 CF patients (37% female; mean age, 9 ± 2 years) and 40 age-matched controls (50% female; mean age, 8 ± 2 years). CF diagnosis was confirmed by genotype and/or abnormal sweat test. Serum sphingolipid profiles (SO, SA, DSA, Sa1P, and S1P) were determined by HPLC-MS/MS. HDL, LDL, TG, and TC were determined by enzymatic methods. Statistical significance was determined using the Student *t* tests, "Proc Corr" and "Proc Genmod," on SAS 9.2 with a compound symmetry correlation matrix.

We analyzed 158 CF and 40 control samples. Univariate analysis showed that DSA was markedly elevated in CF serum (0.9778 ± 0.7 µmol/L) compared to controls (0.197 ± 0.21 µmol/L; $P < .0001$). S1P was significantly decreased (CF, 0.587 ± 0.19 µmol/L; controls, 0.67 ± 0.21 µmol/L; $P = .02$). Sa1P was significantly increased (CF, 0.152 ± 0.008 µmol/L; controls, 0.13 ± 0.0.008 µmol/L; $P = .023$). SO and SA were not different between groups but, by multivariable analysis, they were correlated inversely with DSA. For every unit increase in SA or SO, DSA decreased by 1.1 µmol/L ($P = .0006$) and 0.16 µmol/L ($P = .0036$), respectively. We confirmed lower LDL cholesterol (58.9 ± 26 mg/dL in CF; 78 ± 23 mg/dL in controls; $P < .0001$) and TC (124 ± 37 mg/dL in CF; 140 ± 34.5 mg/dL in controls; $P = .035$). HDL and TG were similar between groups. DSA was independent of TC and LDL measurements. Sa1P and S1P positively correlated with HDL and TC but not LDL. All lipid parameters were independent of genotype.

CF serum is characterized by markedly increased DSA, an atypical sphingolipid of currently unknown function, increased Sa1P, a neutrophil chemoattractant, and decreased S1P, a regulator of lymphocyte chemotaxis. Our data causally link mutations in CFTR to an altered profile of sphingolipids that modulate immune cell migration.

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Pediatric Reference Intervals for Free Thyroxine and Free Triiodothyronine Using Equilibrium Dialysis-LC-MS/MS

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Thyroid hormone concentrations in the blood are known to change during growth and development. To accurately diagnose thyroid disease in pediatric patients, an adequate number of subjects are needed to establish pediatric reference intervals (RIs). The objective of this study was to establish pediatric RIs for free thyroxine (FT4) and free triiodothyronine (FT3) using equilibrium dialysis–high-performance liquid chromatography–tandem mass spectrometry (ED-LC-MS/MS).

Healthy children ages 6 months (m) through 6 years (y) undergoing elective surgeries from Primary Children's Medical Center and healthy children ages 7 through 17 y were enrolled. All samples were tested for thyroglobulin and thyroid peroxidase autoantibodies and thyrotropin to eliminate any patients with possible underlying thyroid disease from inclusion in FT4 and FT3 RIs. RIs were established nonparametrically using statistical analysis software EP Evaluator Release 8. Results were partitioned by age and gender. If no statistically significant differences were observed between adjacent groups, they were combined.

RIs obtained for FT4 were as follows: boys and girls ages 6 m to 6 y (n = 840), 1.4 to 2.7 ng/dL; ages 7 to 17 y (n = 1,373), 1.1 to 2.0 ng/dL. RIs obtained for FT3 were as follows: girls ages 6 m to 6 y (n = 401), 3.8 to 8.5 pg/mL; ages 7 to 12 y (n = 378), 3.6 to 6.5 pg/mL; ages 13 to 17 y (n = 305), 2.9 to 5.6 pg/mL; and boys ages 6 m to 6 y (n = 438), 3.7 to 7.7 pg/mL; ages 7 to 12 y (n = 381), 3.7 to 6.4 pg/mL; and ages 13 to 17 y (n = 310), 3.4 to 6.1 pg/mL. While there is some overlap with adult values, we do see differences from previously established adult RIs of 1.1 to 2.4 ng/dL for FT4 and 3.2 to 6.6 pg/mL for FT3.

Results show that age- and gender-specific RIs are necessary for FT3 but only partitioning by age is necessary for FT4. These new RIs should be clinically useful for diagnosis of thyroid disease in pediatric patients using ED-LC-MS/MS.

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Hemoglobin Variant Characterization by Pepsin-Digestion Mass Spectrometry

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Mass spectrometric characterization of unknown hemoglobin variants has traditionally relied on intact mass measurements combined with sequencing of mass-shifted tryptic fragments. However, trypsin digestion may result in incomplete coverage due to the presence of very short and very long fragments unsuitable for sequencing. Furthermore, tryptic fragments can be too hydrophobic or hydrophilic for efficient HPLC, and others do not ionize well. An alternative protease is pepsin, and, in this study, we characterize an unknown hemoglobin variant through mass spectrometric analysis of the intact and pepsin-digested protein.

The study included a wild-type control, a sickle-trait control, and a patient with inconclusive hemoglobin identification by HPLC and capillary electrophoresis. Blood was hemolyzed, centrifuged, and infused into an LTQ Orbitrap for mass measurement of intact hemoglobin chains. This was followed by a 30-minute in-line pepsin digest of the hemolysate then LC-MS/MS. Data were processed by MS-Alignment, an algorithm that discovers all peptide modifications

in an unrestricted fashion. Those modifications that could represent single nucleotide mutations explaining the intact mass shifts were manually validated.

Mass determination of intact hemoglobin chains revealed no mass shifts in the wild-type but heterozygous β chain shifts in the sickle-trait control and test sample: –30 kDa and –10 kDa, respectively. MS-Alignment analysis of the peptic fragments produced 100% coverage of the α and β chains and a small number of possible mutations explaining the intact mass shifts. Manual validation resulted in a single mutation in both samples: E6V in the sickle-trait control and P5S in the test, otherwise known as Hb Tyne.

In this study we characterize hemoglobin variants by intact mass determination followed by pepsin digest analysis and unrestricted discovery of peptide modifications. Pepsin digest tends to be avoided in mass spectrometry as it is nonspecific, resulting in complex mixtures of overlapping peptide sequences. This is problematic for low-abundance proteins as high peptide diversity reduces copy number. However, hemoglobin is of high abundance, and the enhanced coverage by overlapping sequences clearly reinforces the validation of mutated peptides. Also, in-line digestion allows a simplified workflow with direct connection to the mass spectrometer. This method shows potential for the characterization of hemoglobin variants that remain undetermined after traditional phenotypic analysis in the clinical laboratory.

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Implementation of a Custom Bi-directional Interface for a LC/MS/MS Vitamin D Assay

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An increased awareness of the prevalence of 25-hydroxycholecalciferol (vitamin D) deficiency and associated morbidities, along with the potential for toxicity due to supplementation, has resulted in a large demand for tests of vitamin D. The clinical laboratory at the University of Washington Medical Center performs more than 40,000 vitamin D assays per year using an organic liquid-liquid extraction method coupled to a reverse-phase liquid chromatography and is analyzed using isotope dilution–tandem mass spectrometry. Each assay involves the determination of 2 analytes (D_2 and D_3), a summed total vitamin D level, and multiple quality assurance parameters, resulting in over 120,000 results per year previously requiring manual calculation and entry into our laboratory information system (Sunquest FlexiLab).

To reduce technologist effort and potential for errors associated with manual data entry, we developed a semiautomated bidirectional interface between the Waters instrument (Waters Micromass Quattro Micro APT [MS] with Waters Acquity, or Waters Alliance HT 2795 [LC]) and our LIS in which the following steps are performed: (1) a custom function written in Cache MUMPS is used to create a load list readable by the Waters instrument; (2) the results of the vitamin D assay are exported as an XML file and uploaded to the server LIS where it is converted by a Python script into a format compatible with the Sunquest flat-file interface; (3) the LIS receives the results via a Sunquest flat-file interface; and (4) results auto-file subject to QA calculations.

Implementation of this custom-built interface solution emphasizing open-source software tools resulted in a decreased median interval between first and last result entry into the LIS (12 vs 59 minutes), reduced technologist time required for data entry (median daily result time reduced by ~37 minutes; $P < .001$), decreased turnaround time variability (F test, $P < .001$), and a trend towards a reduction of data entry errors (average of 0.5 vs 2 corrected laboratory reports per month).

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Evaluation of Increased Incidence of Hyperkalemia From an Off-Site Satellite Clinic

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An off-site satellite clinic (Huron) of the University of Chicago Medical Center requested an investigation by the clinical chemistry laboratory (CCL) into several cases of possible falsely elevated potassium (K+) values in their patients. Bloods for K+ and chemistry profiles are routinely collected in mint-green heparinized plasma separator tubes (PST), centrifuged, and transported by courier from the Huron clinic to CCL within several hours. Bloods from on-site phlebotomy areas are similarly collected but sent uncentrifuged to CCL via a pneumatic tube system within several minutes after collection. Investigations included performing extensive quality control (QC) and quality assurance (QA) reviews, a reference range study, and visiting the satellite clinic to assess and test preanalytic handling of specimens.

QC reviews showed that the analytical systems were running within targeted control ranges with no systematic bias observed. However, a QA review of K+ distribution of all patient samples from the Huron clinic during a typical month indicated that 17.7% of patients (n = 170) had K+ levels greater than the upper reference limit (URL) of 4.7 mmol/L, as compared to only 3.2% of samples (n = 1,371) from an on-site outpatient "control" group. An in-house K+ reference range study (n = 51 apparently healthy volunteers) confirmed our current reference ranges: plasma, 3.5 to 4.7 mmol/L; serum, 3.8 to 5.0 mmol/L. K+ values on average are expected to be ~0.3 mmol/L higher when derived from serum separator tubes (SST) due to release of K+ from platelets in the process of clotting. We next collected paired plasma (PST) and serum samples (SST) from 104 Huron clinic patients to assess the effects of specimen handling and transportation. Paired *t* tests showed no significant K+ difference between SST vs PST (mean difference = 0.03 mmol/L; $P = .3110$; n = 97) and a mean K+ difference of 0.13 mmol/L ($P < .0001$; range, 0.09-0.18; n = 85) from the centrifuged PSTs vs aliquots taken out immediately from PSTs postcentrifugation and transported together by courier to CCL. In addition, we took several in-house PST samples that had just been assayed for K+ for a simulated drive, and, on average, these samples showed an elevation of 0.20 mmol/L K+ after the journey.

We conclude that centrifuged bloods collected in PST and undergoing courier transport can result in falsely elevated K+ values leading to an increase in the incidence of hyperkalemia (especially for patients with K+ near the URL). We have changed to collecting bloods for chemistry profiles in SST for our off-site clinics, and preliminary follow-up results indicate a decrease in the incidence of hyperkalemia in this same population.

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Liquid Chromatography–Mass Spectrometry Measurement of Neosaxitoxin, a Promising New Local Anesthetic

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Local anesthetics block neurotransmission of pain by reversibly blocking the voltage-gated sodium channel. However, the short duration of action of current local anesthetics limits their analgesic effectiveness. A long-lasting local anesthetic would dramatically improve pain management in surgical patients.

Neosaxitoxin, a paralytic shellfish toxin, has shown promising results with great potential for clinical applications as the next-generation, long-acting local anesthetic. The goals of this study were to develop and optimize the measurement of neosaxitoxin in biological samples using liquid chromatography (LC) coupled with electrospray ionization mass spectrometry (ESI-MS). This method will be used in the phase 1 clinical trial of neosaxitoxin.

Neosaxitoxin, concentrated by solid phase extraction (SPE), was subjected to LC separation from other compounds and ESI-MS analysis. Preparative procedures to concentrate and extract neosaxitoxin from sera were investigated using the Oasis mixed-mode sorbents (reverse phase retention and ion exchange) SPE system. Four different SPE sorbents were explored under 2 protocols, and the neosaxitoxin standard recovery rates were assessed.

Weak cation exchange SPE was shown to be the optimal choice for sample pretreatment. Three types of LC columns were examined under several elution procedures. We found that hydrophilic interaction chromatography under isocratic elution provided the best separation of neosaxitoxin within 5 minutes. Neosaxitoxin was detected by ESI-MS in multiple reaction monitoring mode based on 2 transitions at (m/z = 316.5 – 298) and (m/z = 316.5 – 240). The former transition was used for quantification and the latter transition for internal validation to increase detection specificity. Gonyautoxins 5 (GTX-5) was spiked into each sample at the beginning of the sample preparation and used as internal standard for final quantification.

This method can detect neosaxitoxin in the linear range between 5 and 200 ng/mL. Analytical sensitivity was 5 ng/mL (16 nmol/L), and functional sensitivity was 7.5 ng/mL (24 nmol/L). The functional sensitivity was defined as the lowest concentration that could be detected at a CV no more than 20%. The optimization of this method in such samples as plasma and urine is under investigation. This assay at specified sensitivity and the short LC analytical duration will provide very important information for neosaxitoxin level monitoring to avoid side effects and toxicity. Therefore, it plays an essential role in the success of the phase 1 clinical trial as well as the subsequent clinical trials of neosaxitoxin.

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Laboratory Cost Savings Associated With Pathology Resident Review of Physician Orders

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In the current era of tightening laboratory budgets, superfluous test orders are problematic. ViraCor-IBT Laboratories (Lee's Summit, MO) performs the majority of our clinical microbiology laboratory's send-out quantitative molecular virology testing. Charges to the clinical microbiology laboratory had been increasing steadily each year. In 2007, ViraCor charges approximated $386,000 for inpatients. In an effort to reduce send-out costs associated with potentially unnecessary testing, we proactively involved the pathology residents in prospective reviews of certain inpatient orders received by our laboratory.

A team of microbiologists and clinicians from infectious diseases and transplantation/immunology set guidelines for appropriate testing for over 20 common and uncommon viruses from a wide variety of specimen types and patient populations. Based on these guidelines, orders on inpatients for ViraCor testing which were listed as potentially inappropriate were previewed by the laboratory technologists who passed them on to the pathology resident on service. The resident either approved or cancelled the order, based on discussions with the clinicians. Guidelines were established in 2009, and the number and type of ViraCor orders received and cancelled by the lab were recorded monthly from November 2009 to December 2010.

After the institution of pathology resident screening, 26% of all tests ordered in 2010 were cancelled, leading to a cost savings of approximately $82,500. The percentages of cancelled tests by virus varied month to month by an average of 20%, depending on which resident was rotating. However, the total number of tests cancelled decreased over the 14-month study period. The highest percentages of cancelled tests were herpes simplex virus from specimens other than cerebrospinal fluid (74%) and human herpes virus 8 from any source (89%). The highest numbers of cancelled tests were adenovirus and BK virus. The most common reason for cancellation was multiple orders over a short time course for a single patient.

Cost savings were substantial, albeit dependent on month. As the ordering guidelines became more known, the numbers of tests cancelled decreased over time. The influence of variables, such as ordering trends, hospital census, personnel, and resident aptitude, was considerable. In particular, approval thresholds and patient outcomes are deserving of further study. The findings support the use of prospective order review processes to manage the burden of unnecessary inpatient testing.

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Non-targeted Analysis of Synthetic Cannabinoids in Two Intoxication Cases Involving Herbal Incense Products

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The recreational use of herbal incense has recently gained popularity in a number of states. A majority of these products have been reported to be laced with synthetic cannabinoids (SCs), a group of compounds that mimic the effects of the active component of marijuana, THC. SCs are not detected by THC screens, and setting up a targeted screening for these compounds is challenging because herbal incense manufacturers often change the lacing agents they use. There are more than 400 different SCs that can be synthesized and used. This study tested the suitability of nontargeted analysis using liquid chromatography–time-of-flight mass spectrometry (LC-TOF/MS) to detect and identify SCs in patient and drug samples involved in herbal incense intoxication.

In 2 herbal incense intoxication cases, serum and urine samples along with the herbal incense products used were analyzed using Agilent LC 1200-TOF/MS 6230. The chromatograms obtained were analyzed by nontargeted screening using Agilent MassHunter Qualitative Analysis. Formula matches to specific SCs identified were later confirmed when reference standards for the compounds became available.

Two patients presented to the ED with nausea, vomiting, tachycardia, and mentally altered status on separate occasions. Both patients claimed using herbal incense products immediately before presenting to the ED. Nontargeted LC-TOF/MS screening of the products they used revealed formula matches to a number of SCs (Spike Maxx: JWH-007, JWH-073, JWH-398; Spike 99: JWH-018, JWH-015, JWH-210, JWH-122). All of these compounds were confirmed as soon as reference standards were available. JWH-007 is an interesting finding as it has never been reported yet in herbal incense products. One of the patient's serum had a formula match to JWH-018, which was likewise confirmed later. A targeted SCs panel consisting of 33 compounds and metabolites is currently being validated to complement nontargeted screening.

Targeted screening for designer drugs like SCs can easily miss new compounds introduced in the recreational drugs market. Nontargeted screening through LC-TOF/MS is a good adjunct to targeted screens. It allows discovery of new drugs of abuse in a timely manner that can be added to targeted screens. The use of this approach was successfully illustrated for JWH-007 in 2 herbal incense intoxication cases.

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Determining the Cross-reactivities of Designer Drugs to Amphetamine Immunoassays

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Amphetamine-type stimulants comprise of amphetamine, methamphetamine, MDMA (3,4-methylenedioxymethamphetamine), as well as an increasing number of "designer" drugs. According to the United Nations Office on Drugs and Crime 2010 drug report, the number of people abusing amphetamine-type stimulants will soon surpass the total number of those abusing cocaine and opiates combined. Six major categories of amphetamine-type designer drugs exist, including piperazines, phenylethylamines, β-keto amphetamines, substituted amphetamines, 2,5 dimethoxy amphetamines, and the pyrrolidinophenones. Drugs from the β-keto and pyrrolidinophenones categories are legally sold in the United States under the name "bath salts," and abuse of these has resulted in a large number of toxic exposures and hospitalizations. Although these drugs mimic the effects of amphetamine and are related structurally, it is unclear whether existing immunoassays will detect these compounds.

Our objective was to document the cross-reactivities of designer drugs to 3 leading amphetamine immunoassays: the CEDIA amphetamine/Ecstasy immunoassay, the EMIT II Plus Amphetamines Assay, and the Axsym Amphetamine/Methamphetamine II. Thirty-three amphetamine-type stimulants were spiked into drug-free urine at 100,000, 20,000, and 5,000 ng/mL. Samples were analyzed on 3 separate platforms to determine whether the presence of these drugs would cause a positive result. The CEDIA amphetamine/Ecstasy immunoassay was run on a Siemens Advia Centaur analyzer, while the EMIT II Plus Amphetamines Assay and the Axsym Amphetamine/Methamphetamine II were analyzed on a Beckman Olympus AU 680.

We tested drugs from 5 of the 6 major categories and documented their respective cross-reactivities to the amphetamine immunoassays. At 100,000 ng/mL, over half of the drugs cross-reacted with the CEDIA (21/33) and EMIT II (18/33) immunoassays. Less cross-reactivity was seen with the AxSym II, where only 12 of the 33 drugs caused a positive result. Interestingly, each of the 3 immunoassays cross-reacted with a different subset of designer drugs and displayed unique affinities for the 5 different drug categories.

These results demonstrate that although some designer drugs of the amphetamine type can cross-react with our existing immunoassays, it is clear that many of them will not be detected. If amphetamine abuse is suspected in patients but their urine toxicology is negative, additional testing utilizing methods such as mass spectrometry may be required for drug identification.

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Hypoxia Enhances Doxorubicin-Induced Death in Liver Cells Containing Hepatitis B Virus x Protein

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Hepatitis viral B x protein (HBx) is involved in hepatocarcinogenesis by affecting proliferation and growth. HBx can be proapoptotic or antiapoptotic. Hypoxia influences not only the HCC growth, but also the cell sensitivity to treatments. It is unknown whether and how acute or transient hypoxia would affect the response of liver cells with HBx to chemotherapeutic agents such as doxorubicin. We aimed to test how liver cells carrying different HBx mutants responded to doxorubicin in hypoxia and normoxia.

We established 4 Chang liver cell lines with the full-length HBx (HBx), the first 50 amino acids of N-terminal HBx (HBx/50), the last 104 amino acids of C-terminal HBx (HBx/51), and empty vector (CL), respectively. Cells were cultured in normoxia or hypoxia and treated with doxorubicin.

Results showed that all 4 cell lines responded to doxorubicin. However, doxorubicin was most effective in decreasing the proliferation and enhancing apoptosis in HBx/51 cells. Compared with other cells, HBx/50 cells were most resistant to the treatment. Compared with cells in normoxia, cells in hypoxia were more susceptible to doxorubicin. Hypoxia facilitated the Bid cleavage, especially in HBx/51 cells. The Bid cleavage was positively associated with the phosphorylated p38 MAPK but not Akt. p38 MAPK inhibitor significantly reduced the tBid level and recovered the ability of cells to proliferate.

Collectively, N-terminal HBx and C-terminal HBx function differentially in their ability to regulate cell proliferation and growth, with the former being promotive but the latter being inhibitory. The regulation of HBx on cell proliferation and death involves Bid cleavage and p38 MAPK activation. Acute hypoxia in combination of doxorubicin can significantly facilitate death in liver cells, even the cells with apoptosis-resistant mutant HBx. The finding may provide a novel option to treat HBx-induced resistant liver cancer.

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Implementation of a Clinical Microbiology Diagnostic Management Team

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Utilization management is the cornerstone of a managed care system. An effective laboratory medicine utilization management program can provide optimal patient care while reducing the total cost of medical care for the individual and society. This concept

has resulted in the implementation of a clinical microbiology diagnostic management team (CMDMT) to address consultative and interpretive needs for the laboratory diagnosis of infectious diseases.

The role of the CMDMT includes (1) preanalytical consultation from clinical services; (2) internal pathology consultation from surgical pathology, hematopathology, cytology, and autopsy services; and (3) postanalytic interpretation of sentinel results. Preanalytical consultation involves recommendations for microbiologic studies based on clinical factors, differential diagnosis, and specimen accessibility. Internal consultation involves organism identification by microscopy, guidance on appropriate microbiologic testing, and liaison with the requesting service area (eg, surgical pathology laboratory) to ensure proper specimen handling for microbiologic analysis. Sentinel result monitoring is a system in which all results in the clinical microbiology laboratory are monitored for "significant or unexpected findings," which trigger further investigation and interpretation or intervention as necessary. This surveillance generates a list of patients for whom an electronic health record review is conducted. A determination is made as to whether the result requires proactive discussion with the clinician that could offer improved clinical outcome through follow-up testing or patient referral to a particular specialty service. Examples of CMDMT consultations with brief case descriptions and outcomes shall be presented to demonstrate the effectiveness of a CMDMT.

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Diagnostic and Clinical Utility of Age-Based Reflex Testing for Immunofixation

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We perform reflexive serum immunofixation (SIFE) for all patients over 65 years because the incidence of monoclonal gammopathies increases with age, and a small but significant subset progress to neoplastic disease. To assess the diagnostic and clinical utility of this practice, we examined 5 years of reflexive SIFE results.

We gathered 93,073 SPEP and SIFE results from July 2005 to May 2010 from pathology data records. Patients were filtered based on age older than 65 years, no previous SIFE result, an initial order of SPEP alone, and SPEP and SIFE performed on the same sample.

In the past 5 years, 2,200 patients have been reflexively tested for SIFE based on age. The racial breakdown was 61.1% white, 26.9% black, 9.5% other, and 2.5% unknown. Of the 2,200 patients, 606 (27.6%) had a clonal gammopathy. This includes 16.8% monoclonal (370 patients), 2.0% biclonal (44 patients), 1.9% oligoclonal (42 patients), and 2.7% Bence Jones proteinemia (59 patients). We repeated this analysis excluding patients with a visible spike on SPEP. This decreased the total of reflexively tested patients from 2,200 to 1,908. Of these, 333 patients (17.5%) had an abnormal SIFE (8.8% monoclonal, 167 patients; 0.3% biclonal, 5 patients; 1.2% oligoclonal, 24 patients; 2.1% Bence Jones 40 patients). Chart review and discharge ICD-9 codes for 150 of the 606 newly diagnosed patients showed that 14.9% are given a diagnosis of MGUS, 1.3% had preexisting hematologic malignancy, and 83.8% of these diagnoses were not noted in the chart or discharge diagnoses. Based on the chart review, no patients in our cohort appear to have progressed to neoplastic disease.

Reflexive testing of SIFE based on age alone is a sensitive method of detecting monoclonal gammopathies. The number of new diagnosis (27.6%) is greater than what would be expected based on age- and race-specific incidence rates for M spikes alone (7.7%). Patients with MGUS progress to multiple myeloma at a 1% annual rate; thus, detection of MGUS with closer follow-up can increase early diagnosis of MM. However, the clinical utility of this improved diagnostic rate is limited by apparent poor result retrieval. We are working to address this.

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Two Cases of Invasive Disease in Adults Caused by *Haemophilus influenzae* Type f With Identification by 16S rRNA Sequencing and Multiplex PCR-Based Capsular Typing

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Prior to the introduction of the *Haemophilus influenzae* type b vaccine, invasive infections due to non–type b *H influenzae* were rarely described; however, over the last years, this pattern has been changing, particularly in the adult population. Here, we report 2 cases of invasive *H influenzae* type f infections in adults, a case of sporadic mycotic aneurysm of the abdominal aorta, and a case of pneumonia with associated bacteremia.

The organisms were identified to species using routine biochemical tests (X and V factors) and API NH identification strips; both isolates had identical results on the API strip with a biotype I. In addition, 16S rRNA gene sequencing was used for taxonomic classification, and both isolates were identical to the type strain of *H influenza* type f, which is about 2% different from *H influenzae* type b. Multiplex PCR-based capsular typing and routine serotyping confirmed the capsular type. To our knowledge, this is the first report of mycotic aneurysm due to *H influenzae* type f and one of the first reports of *H influenzae* type f pneumonia with bacteremia in the older veteran patient population.

These 2 cases underscore the clinical relevance and pathogenic capability of *H influenzae* type f in the adult population after the near elimination of invasive *H influenzae* type b disease. Furthermore, they raise questions about the necessity of routine capsular typing of *H influenzae* in the clinical laboratory using accurate and reliable techniques, such as 16S rRNA gene sequencing or multiplex PCR-based capsular typing, to assess the need of developing a type f vaccine.
