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 Lab Medicine 2012, the 47th Annual Meeting of ACLPS, May 31 to June 2, 2012, in Milwaukee, WI. Authors receiving a 2012 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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Identification of NLRP Mutations in Head and Neck Squamous Cell Carcinoma

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Head and neck squamous cell carcinoma (HNSCC) encompasses malignancies that arise in the mucosa of the upper aerodigestive tract. Recent high throughput DNA sequencing revealed HNSCC mutation profiles including genes that control several cancer cell characteristics, including dysregulation of proliferation and death signals, induction of inflammatory responses, and reprogramming of cell metabolism. Emerging evidence suggests an evolutionarily conserved family, the pyrin-containing NLR (nucleotide binding domain, leucine-rich repeats containing) proteins, play fundamental roles in modulating the aforementioned processes, however, their functions in carcinogenesis still remain elusive. This study aims to assess the clinicopathologic and genetic characteristics associated with novel *NLRP* genes mutations identified in HNSCC by whole exome sequence analysis. The mutation landscape of 74 HNSCC patients treated at the University of Pittsburgh Medical Center was revealed using whole exome sequencing as previously reported. The mutation profiles of 62 primary conventional squamous cell carcinomas excluding other histologic variants are analyzed here. Associations were tested by Fisher exact test or Mann-Whitney U test as appropriate. *P* less than .1 was considered to be significant. Mutations in *NLRP* genes encoding pyrin-containing NLR proteins were exclusively identified in advanced stage HNSCC. These mutations were clustered at the leucine rich repeats region of pyrin-NLRs; and affected *NLRP* genes are mostly localized at chromosomes 11p15.4 and 19q13.42-19q13.43. Carcinomas that harbor these mutations demonstrated significantly higher mutation rates ($P = .009$). Clinically, NLRP mutations affected both genders equally, and these mutations were more frequently found in SCC arising in the floor of mouth ($P = .034$). Genome mutation rate is an important parameter for evaluating cancer cell genome instability. The identification of novel NLRP mutations in HNSCC is correlated with increased cancer cell genome mutation rates, and such features could be a potential molecular biomarker of HNSCC genome instability.

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Comparison Between the Ark-Levetiracetam Assay on the Olympus AU400 Analyzer and Liquid Chromatography-Tandem Mass Spectrometry

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The objective of this study is development of a user-defined application protocol for measuring levetiracetam on the Olympus AU400 chemistry analyzer and evaluate assay performance by comparison with a liquid chromatography-tandem mass spectrometry (LC-MS/MS) method. Further, we sought to assess the cost-effectiveness and turnaround time (TAT) improvement associated with bringing this assay in-house vs continuing to send it to a reference laboratory. The Ark-Levetiracetam assay (ARK Diagnostics, CA) is a new FDA-cleared homogenous enzyme immunoassay for quantifying levetiracetam in serum or plasma. The assay was evaluated on an AU400 chemistry analyzer utilizing an open channel user-defined method. The assay was calibrated using a 6-point calibration curve (spanning concentrations of 6-46 µg/mL) with 3 levels of QC. Assay characteristics evaluated included linearity, AMR, inter- and intra-assay precision, recovery, and interference (hemolysis, bilirubin, lipemia). Performance of the assay was further compared to LC-MS/MS (reference laboratory) using specimens from patients undergoing routine TDM at our centers (n = 25). The Ark assay was linear between 6.0 and 46 µg/mL, with $r^2 = 0.99$. Intra-assay precision was 5% at 7.7 µg/mL, 2.9% at 29.7 µg/mL, and 2.6% at 74.3 µg/mL and interassay precision was 7.5% at 7.6 µg/mL, 4.2% at 29.6 µg/mL, and 4.3% at 71.4 µg/mL. Significant interferences from hemolysis, bilirubin, or lipemia were not observed. Recovery using spiked samples with concentrations ranging from 5.0 to 100 µg/mL demonstrated an average

recovery of 99.7%. Results of 25 patient samples with concentrations from 2.0 to 58 μg/mL were compared with results from LC-MS/MS. Regression analysis showed approximately 2% negative bias compared with LC-MS/MS, with $r^2 = 0.99$. The upfront cost associated with the initial validation was very minimal; ie, $500 (technologist time to perform validation at $50/h, reagents provided free of charge by Ark). At a yearly volume of approximately 400 tests per year, the cost of in-house testing in the first year using the Ark-Levetiracetam assay was estimated as $19,200 ($48.50/test), compared with $45,200 for sendout testing ($113.00/test), for a savings of approximately $25,500 per year. Currently, the TAT for the send out test is approximately 30 to 48 hours, which includes specimen shipping, analysis, and reporting. By offering this test in–house, the TAT can be as little as 30 minutes, which is compatible with STAT testing. The Ark assay compares favorably with LC-MS/MS analytically and is well suited for routine TDM use on an AU400 instrument. The advantages of in-house testing include reduction of preanalytical problems associated with specimen shipping, reduced cost of testing and a rapid TAT of 30 minutes. The rapid TAT will permit STAT testing required during emergency evaluation of seizures or overdose situations.

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Web-Based Tracking System for Weekend Resident Hand-offs in a Multisite Clinical Pathology Training Program: A Solution to JCAHO and ACGME Mandates

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We developed a web-based, automated tracking system to facilitate patient hand-offs in a multisite, multirotation, academic clinical pathology training program. Transfer of patient care responsibility from one provider to another over weekends carries risks for introducing error, delay, or suboptimal quality of care. Both ACGME and JCAHO have recently mandated hospitals to enact patient care transfer procedures that incorporate both verbal and written communication between the transferring and the receiving physician. We will discuss the process of implementation, the successes, and remaining areas of improvement for hand-offs after developing a web-based hand-off system for clinical pathology residents. The system is a Health Insurance Portability and Accountability Act–compliant web-based hand-off portal that allows entry of patient information every Friday from 3 hospitals, including a tertiary care academic medical center, a county hospital, and a Veteran's Administration hospital. Residents from the various clinical pathology services enter patient identification and information useful to the weekend on-call resident. The on-call resident is also notified by a phone call (or email if a phone call is not possible) in addition to being able to review the hand-off online. Compliance with online hand-offs is tracked by the percentage of total residents who completed a hand-off at the end of each week. From July 22, 2011, through January 23, 2012, the system has been used to track 67 hand-offs by 10 residents. While clinical pathology residents cover 10 core rotations at 3 hospitals on any given month, only 7 services have had any cases to hand-off in the last 6 months. Over the initial 6 months the compliance rate was 94%. Noncompliance tended to be among the more senior trainees. A midyear survey of the clinical pathology residents revealed support for the system and a desire that it be continued. In conclusion, a web-based tracking system can be used to facilitate transfer of patient care between residents at the various rotations and the weekend on-call resident. The hand-off portal facilitates hand-offs but may not optimize the quality of those hand-offs. Further study into improving quality of hand-offs is needed.

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Appropriateness of Utilization of Serum Protein Analysis; SPEP and IFE

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Screening for monoclonal gammopathies is usually done by serum protein electrophoresis (SPEP) and serum free light chain (SFLC) tests. SPEP may be followed by immunofixation (IFE). IFE may be ordered by the ordering physician or be at the discretion of the pathologist. We examined the appropriateness of IFE ordering by physicians and report on our findings, follow-up changes to the ordering process and results of the change. We retrospectively analyzed the data from our laboratory from April 2009 onwards. In April 2009, 3 options for test order were available for the clinicians; SPEP only, SPEP with IFE, and SPEP with reflex IFE. Over the course of the following year, it appeared that SPEP with IFE was ordered excessively. To validate this premise we conducted a retrospective review of the SPEP with IFE orders. From April 2009 through April 2010, we identified 92 orders for SPEP with IFE. Without revealing the results of the IFE, we asked the pathologist, if given the SPEP findings and his review of the clinical history he would have performed an IFE. The pathologist identified 18 cases in which he would have performed an IFE and in 10 of 18 there was a positive finding. It is noteworthy that IFE was noncontributory in all of the remaining 74 cases. Based in this finding and following discussion with medical leadership, ordering option was limited to SPEP with reflex IFE only. We compared the rates of SPEP and IFE performed in the 2 periods, ie, April 2009 to April 2010 and from May 2010 to December 2011. From April 2009 through April 2010, a total of 451 SPEP and 210 IFE were performed with IFE/SPEP ratio of 0.47. From May 2010 through December 2011, a total of 514 SPEP and 112 IFE were performed with IFE/SPEP ratio of 0.21. There was a 25.70% decline in the utilization of SPEPs and 65.32% decline in the utilization of IFEs. Decline in IFEs was statistically significant ($P < .05$). In the time period of April 2009 to April 2010, 37 SFLC tests were ordered. In the May 2010 to December 2011 period, 83 SFLC were ordered. Our experience exhibited that IFE can be excluded from the screening panel without compromising the test sensitivity and thus significantly reducing the laboratory workload. SPEP and serum FLC assay constitute a sensitive screening panel for monoclonal gammopathies. IFE can be performed at the discretion of the pathologist if initial SPEP is suggestive of some abnormality. The change reduced the number of SPEP and IFEs performed thus improving the efficiency of the Laboratory. Even with increase in SFLC tests, the total volume of tests for investigation of monoclonal gammopathy was reduced.

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Reference Interval Confirmation and Stability Analysis of Macro Aspartate Aminotransferase

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We report the case of a 50-year-old healthy, asymptomatic male patient with a chronic fluctuating elevated aspartate aminotransferase (AST) found incidentally on a routine clinic visit. Other liver function tests, imaging studies, and physical examination findings were normal. Since elevated enzyme activities may be due to the presence

of macroenzymes, their identification is important to prevent further unnecessary diagnostic procedures. We investigated the presence of macro-AST by removal via polyethylene glycol (PEG) precipitation and ultrafiltration (UF) methods. Results were compared with previously reported reference intervals (Wyness et al, *Clin Chim Acta*, 2010). Monomeric AST recoveries for PEG precipitation and UF were determined by dividing the activity of the supernatant or ultrafiltrate by the neat activity and converting to a percentage (% monomeric AST). To assess stability, PEG precipitation and UF were repeated daily for 7 days. In addition, we investigated AST concentrations longitudinally over a period of 7 days at 4°C storage, as decreasing concentrations over time at 4°C has been proposed to indicate the presence of a macroenzyme. Both PEG precipitation (13%) and UF (22%) methods revealed % monomeric AST values that were below the previously described reference intervals (PEG: 26%-88%; UF: 50%-89%), indicating the presence of a macroenzyme. Using PEG precipitation, % monomeric AST results were stable through day 3 of testing (<5% change), while UF results actually doubled within the first day. Over time, we found a 63% decrease in AST values by day 5 in the patient sample stored at 4°C, while no change was observed in control patients. Elevated and fluctuating AST values, low monomeric AST recoveries by two independent methods and decreased AST values over time in samples stored at 4°C all indicated the presence of macro-AST in the patient presented here. These findings support previously proposed reference intervals for macro-AST. Due to ease of sample preparation and the apparent stability through day 3, PEG precipitation appeared to be the preferred method for AST macroenzyme detection. The stability data provides a reasonable testing window for the laboratory investigation of questionable AST results. Decreased AST activity with 4°C storage may be attributed to antibody precipitation over time. Therefore, concerns regarding the lack of specificity of PEG precipitation may be addressed by adding 4°C stability to the diagnostic workup.

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Detection of THC in Infant Urine: Truth or Fallacy?

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This study sought to investigate the observation that infant urine samples which test positive for cannabinoids by screening methods do not test positive using confirmatory methods. The rate of unconfirmed positive tetrahydrocannabinol (THC) immunoassay results was compared between infant and adult samples. Nine consecutive months of laboratory test results were searched retrospectively. The screening assay used (Syva EMIT II Plus, Siemens Healthcare Diagnostics) has a cutoff of 20 ng/mL 11-nor-Δ^9-carboxy THC (9-COOH-THC), which is also the target analyte for the liquid chromatography tandem mass spectrometry (LC-MS/MS) confirmatory assay (5 ng/mL cutoff). Of urine samples with positive screen results, samples from infants (≤2 years old) were less likely to confirm positive by LC-MS/MS, compared with adult samples. The rate of false-positive screen results was significantly higher in infants (18/38; 47%) than noninfants (24/3,024; 0.08%) (χ^2 = 601, P < .0001). A similar discrepancy was observed in data from an independent hospital laboratory using a different immunoassay platform (THC2, Roche Diagnostics, 50 ng/mL 9-COOH-THC cutoff), where 75% of screen-positive infant samples failed to confirm (6/8) while only 3.7% of positive adult results were not confirmed (3/81) (P < .0001, Fisher exact test). We hypothesized that infant urine contains an alternate THC metabolite accounting for this discrepancy, specifically 11-hydroxy-tetrahydrocannabinol (11-OH-THC). This metabolite is known to be present in meconium samples and cross-reacts in THC immunoassays (47% cross-reactivity in the EMIT II assay, 13% in the Roche assay). To test this hypothesis, urine samples from twenty infants which were negative for 9-COOH-THC by LC-MS/MS were analyzed using the EMIT II immunoassay. Half screened positive. The same samples were then analyzed using an LC-MS/MS method designed to quantify the 11-OH-THC metabolite (detection limit ~7 ng/mL). 11-OH-THC was not detected in any of the samples. Thus, while the cannabinoid immunoassay false-positive rate was higher in infants as compared to adults, 9-COOH-THC and the 11-OH-THC metabolites were not detectable in infant urine. Screen results may reflect the predominance of other cannabinoid compounds in infant urine or may indicate true false-positive results. Until the precise compound responsible for positive results in infants is identified, cautious interpretation of positive screen results is necessary.

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Development of a Multiplex, Real-Time PCR Assay for the Detection of *B pertussis* and *B parapertussis* From Different Specimen Types Using the Smart Cycler

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Pertussis is an infectious respiratory disease caused by the fastidious bacterium *Bordetella pertussis*. Pertussis afflicts unvaccinated, previously vaccinated children, and adults in whom immunity has waned. Infants are at particular risk for severe disease and complications. *Bordetella parapertussis* may cause a similar illness, however the symptoms are less severe and of shorter duration. Pertussis is a highly contagious disease and early diagnosis is essential. Studies have shown that PCR is 2 to 4 times more likely than culture to detect *Bordetella pertussis*. To date there are no FDA–licensed PCR test kits and no standardized protocols, reagents or reporting formats. We developed a multiplex, real-time PCR assay using the Cepheid ASR beads with primers and probes that targeted the multicopy insertion gene sequences *IS481* and *IS1001* of *Bordetella pertussis* and *parapertussis*, respectively. These specific ASRs were used in conjunction with Cepheid Smartmix. These beads contained premixed, predispensed blend of reagents for performing PCR amplifications. Included in the ASR is a competitive internal control to evaluate the performance of the PCR reaction. After DNA extraction, amplification and detection were done on the Smart Cycler System, which performs integrated amplification and detection automatically in a single step. The presence of amplified product is confirmed when the fluorescent signal exceeds a user-defined threshold. Specificity of the assay was confirmed using multiple distinct bacterial strains. Sensitivity of the assay and extraction efficiency were evaluated on DNA isolated from pure bacterial cultures and on spiked respiratory specimens. We also spiked different swab types and transport media to evaluate for interfering substances. To assess accuracy, we studied different patient specimen types received from outside laboratories that used similar or different methods to detect *Bordetella pertussis* and *Bordetella parapertussis*. Comparison of results revealed very good concordance. Our assay was found to be a valid method for the simultaneous detection of *Bordetella pertussis* and *Bordetella parapertussis* in multiple specimen types. The possibility of testing

different specimen types in various transport media reduces the need of collecting multiple samples from each patient for other assays.

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Use of Multiplexed LC-MS/MS Opioid Confirmation Assay With Built-in Quality Control to Investigate Interfering Oxycodone Metabolites

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When chronic pain patients are suspected of being noncompliant, their therapy can be withdrawn. As a result, sensitive and specific confirmatory testing is important in identifying diversion and adherence in this population. Many labs are using multiplexed LC-MS/MS confirmatory methods to measure opioids. We found that several oxycodone metabolites interfere with codeine in our assay, and are likely to cause a problem in other assays. We designed a stringent built-in quality control process in our LC-MS/MS method to simultaneously measure 14 opioids and 6 opioid glucuronides. Samples were processed by centrifugation and diluted in equal volume with deuterated internal standard. For 2 of the glucuronides, internal standards were not available to critically evaluate for matrix effects in each sample. Instead, we designed a novel approach to achieve rigorous quality control: we evaluated the recovery of each analyte in each negative sample. Ten µL were injected for analysis by LC-MS/MS (Waters Xevo TQ). Separation of all compounds was complete in 9 minutes using an Acquity UPLC HSS T3 column (2.1 x 50 mm, 1.8 µm). The assay was linear between 10 and 1,000 ng/mL. Intra-assay imprecision (500 ng/mL) ranged from 1.0% to 8.4% CV. Interassay imprecision (500 ng/mL) ranged from 2.9 to 6.0% (n = 20). Recovery was determined by spiking 5 patient specimens of varying composition with opioid and opioid glucuronide standards (100 ng/mL). Recoveries ranged from 82% to 107% (median 98.9%). Morphine-positive patients were misclassified 10% of the time when not monitoring for the glucuronides. Oxymorphone-positive patients had a misclassification rate of 29%. The misclassification rate of hydromorphone- and codeine-positive patients was 24% and 40%, respectively. Buprenorphine was not detected in any of 168 consecutive patients, but 2% of patients were positive for norbuprenorphine glucuronide. In addition, we discovered interference in the codeine channel that was consistent with oxycodone metabolites not routinely measured (ie, not oxymorphone or noroxycodone). We tentatively identified these interferents as N-oxide metabolites of oxycodone using orthogonal mass spectrometric methods. We established a method with built-in recovery experiments to give added confidence to results that could cause a change in patients' therapy.

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Zinc: A Possible Interference in Drug Testing

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Techniques to alter drug screening results can be easily researched over the internet. It was recently reported (*JAT.* 2011;35:333-340) that ingestion of zinc sulfate ($ZnSO_4$) can interfere with ELISA-based detection of cocaine, methamphetamine and cannabinoids (THC) in urine. False negative results could be due to (1) direct interference of Zn with immunoassays, (2) undefined components excreted as a consequence of $ZnSO_4$ ingestion that directly interfere with immunoassays or (3) altered excretion of drug analytes. The objective of this study was to evaluate the possibility of Zn interference with EMIT-based immunoassays (Syva EMIT II Plus reagents, Siemens Healthcare Diagnostics): acetaminophen, barbiturates, benzodiazepines, cocaine, marijuana, methadone, opiates, phencyclidine, and propoxyphene. Drug-free patient urine, deidentified and characterized using validated clinical assays for Zn content, was spiked with increasing concentrations of $ZnSO_4$ (Sigma), and analyzed by EMIT. Significant spectral interferences were observed for amphetamine, cocaine metabolite, MDMA, opiates and THC assays, when challenged with Zn > 2,500,000 µg/dL (reference interval for urine, Zn: 15-120 µg/dL). Concurrently, we obtained residual patient urine samples for which drug testing was requested, that produced spectral interference by EMIT, and measured Zn concentration by a previously validated ICP/MS method. We found 16 (48%) of 33 samples reported with spectral interference by the EMIT assay had elevated Zn concentrations, ranging from 123 to 1,440 µg/dL. These data suggest that the donors of these urine specimens may have intentionally ingested Zn to interfere with drug testing. The Zn concentrations observed in this sample set were approximately 1,000-fold lower than that determined to interfere with EMIT testing in spiking studies, suggesting that direct interference of Zn with immunoassays is an unlikely mechanism for interference based on $ZnSO_4$ ingestion. In conclusion, direct addition of at least 2.5 g Zn to 100 mL of urine may interfere with EMIT-based drug screens, but it is unclear if and how interference due to ingestion of Zn may occur.

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Using Plasma or Recombinant Factor VIIa (rFVIIa) for Placing Intracranial Pressure Monitors (ICPM) in Patients With Acute Fulminant Hepatic Failure (FHF): A Decision Analysis Approach

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The severe coagulopathy in pre–liver transplant patients with acute FHF, who are at risk of increased intracranial pressure, poses a challenge for successful ICPM placement, which is part of their optimal management. Traditionally, plasma is transfused preprocedure to correct the coagulopathy transiently and, thereby, reduce hemorrhagic risk. Several small studies suggested that rFVIIa could be used as an alternative. Donor plasma and rFVIIa both have risks and benefits; thus, to determine the best treatment option, we created a decision analytic framework to model the use of these products for ICPM placement in patients with FHF. Three outcomes were modeled using TreeAge 9.2: cost, successful ICPM placement, and time-to-ICPM placement. A 70-kg patient entering the model with a platelet count $\geq 100\times10^9$/L and a fibrinogen level ≥100 mg/dL could receive either 1 round of plasma (6 units) alone or 4 units of plasma and 40 µg/kg rFVIIa. ICPM is placed without coagulation testing after rFVIIa administration, whereas coagulation testing is performed when plasma alone is given. If the internationalized normal ratio (INR) is calculated to be less than 1.6 after a dose of plasma alone, ICPM is placed. Otherwise, another round of plasma is given. If the INR does not correct after 2 rounds of plasma, then rFVIIa is administered. The risks of transfusion reaction as well as the thrombotic risk of rFVIIa were taken into account. Model parameters, such as probability of INR correction and risk of complications, were

estimated based on the literature, theoretical calculations, and institutional experience. The model was run for patients with INRs of 2, 3, 4, 5, 6, and >6 with concomitant adjustments to estimated model parameters. Our models predict lower cost and higher probability of successful ICPM placement using plasma, regardless of initial INR. When only time-to-ICPM is considered, plasma is superior only if INR ≤2. Finally, considering a ratio of outcome (ICPM placement) to time-to-outcome, initial use of rFVIIa results in successful placement of ICPM in the shortest amount of time when INR ≥3. In conclusion, we developed a decision analytic model to estimate the cost, outcome, and time-to-outcome of ICPM placement in coagulopathic, pre–liver transplant patients with acute FHF. An algorithm using rFVIIa up front achieves the best ratio of outcome to time-to-ICPM placement in patients with INR ≥3. Future modeling will include actual patient data to refine our estimated parameters.

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Technical and Clinical Validation of a Next-Generation Sequencing Diagnostic Assay for Fanconi Anemia

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Diagnostic testing for Fanconi anemia (FA), a rare heterogeneous genetic disorder, is currently performed through a multiple-step process including chromosomal breakage studies, complementation group testing, and mutation analysis of 1 of 15 known FA genes. This multistep process leads to considerable costs, long turnaround times, and in some cases, inconsistent results. Next-generation sequencing (NGS) provides highly accurate sequencing for large amounts of genomic data in a timely fashion. Our group developed and validated an NGS panel for clinical use in the diagnosis of FA. Utilizing the Agilent SureSelect platform, we designed a preliminary panel that included genes associated with FA (*FANCA*, *FANCB*, *FANCC*, *FANCD1 [BRCA2]*, *FANCD2*, *FANCE*, *FANCF*, *FANCG*, *FANCI*, *FANCL*, *FANCM*, *FANCN [PALB2]*, and *BRIP1*). Using data from the initial validation, sequence capture was optimized and baits were designed for 2 additional FA-associated genes (*RAD51C* and *SLX4*) that will be included in our final panel. The 13 genes included in our preliminary panel comprised 265 exons. With 7 million reads, the average coverage for each exon was 295×. Minimum coverage of 30× at every position was achieved in 252/265 (95%) of the exons analyzed. The 13 exons that did not achieve 30× coverage belonged to the following genes: *FANCA* (7 of 43 exons), *FANCE* (3 of 10 exons), *FANCD2* (1 of 42 exons), *FANCG* (1 of 14 exons), and *PALB2* (1 of 13 exons). The high coverage level (≥30× coverage) across a majority of the exons allows a high confidence in detection of point mutations and small indel mutations. The 13 exons with <30× coverage will be sequenced using PCR followed by next-generation sequencing. The FA panel will be further validated using 10 samples with known mutations. We anticipate the use of NGS will enhance the diagnosis of FA by timely identification of the full spectrum of mutations and improved ability to perform genotype-phenotype correlations.

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Creating Software to Generate an Antibiogram Based on Patient Demographics Using Structured Query Language (SQL)

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The antibiogram is an important clinical tool that is generated by the microbiology laboratory summarizing the susceptibility data for important infectious agents, tailored specifically to the needs of the institution. At the University of Iowa Hospitals and Clinics, expansion of the pediatric inpatient and outpatient services into a new pediatric hospital has led to the request for an antibiogram specific for the pediatric population. Our current laboratory information system (LIS) has built-in tools for generating antibiograms but lacks the flexibility to generate an antibiogram tailored to a specific subpopulation within the hospital. The goal of this study is to produce software that can retrieve relevant clinical data from the LIS (microbial culture and susceptibility data, patient age, hospital location, inpatient/outpatient status, specimen site, and cystic fibrosis status) and present it in a clinically useful fashion. To bypass the limited data-exporting ability of our LIS software, susceptibility data is retrieved via "screen scraping." The software stores this data in a parallel database that can be queried with SQL code to provide susceptibility data for population subsets of interest. Results can be graphed over time to illustrate trends in susceptibility. The resulting data provide up-to-date information to guide clinical decisions in the hospital. In this communication I describe a user-friendly tool to enable pathologists in the microbiology lab to create custom antibiograms tailored to specific hospital populations. Inflexible LIS software can be supplemented with laboratory-produced software to support clinicians with relevant, population-tailored antibiotic susceptibility data.

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Prevalence of Howell-Jolly–like Inclusions in HIV-Positive Patients and Their Correlation With CD4 Counts and HIV RNA Viral Load: A Potential Biomarker for Good Prognosis

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Previously published reports have described the rare occurrence of detached nuclear fragments resembling Howell-Jolly bodies (referred to as Howell-Jolly body–like inclusions) in the peripheral smears of patients with HIV infection, organ transplant recipients, and patients on chemotherapy or receiving immunosuppressive drugs. These inclusions have mostly been identified in neutrophils but at this point any potential clinical significance of this phenomenon is unknown and pathologists tend to disregard their presence. Therefore, we attempted to find any clinical significance of such inclusions. In the present study we examined the peripheral smears of HIV positive patients for the presence of Howell-Jolly–like inclusions in neutrophils and also investigated the temporally corresponding CD4 counts and HIV RNA viral loads to evaluate the state of their disease. CD4 counts were determined by flow cytometry and HIV RNA viral load by the Roche COBAS AmpliPrep instrument and the Roche COBAS TaqMan HIV-1 real-time PCR system, respectively. Slides were all prepared using the Wright-Giemsa stain. A total of 65 peripheral smears from HIV patients were reviewed and only 5 contained Howell-Jolly–like inclusions, yielding a prevalence of 7.7% in this study which is consistent with literature references.

CD4 counts within 1 month as well as viral loads were available in every case. Of the 5 cases with Howell-Jolly–like inclusions, complete set of laboratory data were reviewed. In this subset, 4 had CD4 levels >200 cells/μL (highest 966 cells/μL) and viral loads <850 copies/mL (total range from "undetectable" to 849 copies/mL). The fifth one had a CD4 count of 60 cells/μL and viral load of 8,690 copies/mL. Among the cases where Howell-Jolly–like inclusions were absent (60 cases), most had viral loads > 10,000 copies/mL including nine with viral load >1,000,000 copies/mL and CD4 count < 40 cells/μL. The lowest CD4 count among the patients lacking Howell-Jolly–like inclusions was 3 cells/μL and highest viral load was 1,400,000 copies/mL. Based on these findings we conclude that the presence of Howell-Jolly–like inclusions may be viewed as a biomarker, indicative of low risk for disease progression and/or good response to highly active antiretroviral therapy (HAART) as evidenced by none-detected to relatively low viral counts and relatively favorable CD4 counts compared with patients where Howell-Jolly–like inclusions were absent. The exact association between these inclusions and HIV infection as well as disease progression remains unknown but very intriguing.

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Criteria for Cost-Effective Flow Cytometric Analysis on Cerebrospinal Fluid Specimens

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Flow cytometry (FC) is a commonly ordered adjunct study to routine analysis of cerebrospinal fluid (CSF) at our institution. To help optimize resources, labor, and time without forfeiting clinically important data, we sought to determine evidence-based indications and criteria under which future FC requests on CSF samples would be considered. Results of 309 CSF FC specimens received from January 1, 2011, to December 19, 2011, were reviewed and compared with clinical and other parameters, including history of hematologic malignancy; manual total nucleated cell (TNC) count (normal range, 0-5/μL); and results of any concurrent cytology. One hundred eighty-six samples (60%) were from patients with a history of hematologic malignancy. A clonal process was identified or suspected in 19/186 cases (10%) by FC, with TNC counts ranging from 0 to 1,050/μL (median, 3/μL). Eighteen such cases had concurrent cytology, 8 of which (44%) were positive or suspicious for malignancy. Of 186 samples, 120 (65%) showed polyclonal B cells (0% to 3%) and normal T cells (mean, 17%), and 48 (26%) were insufficient for FC analysis. Of the 123 samples (40%) from patients without a history of hematologic malignancy, 2 were positive or suspicious for a clonal process by FC, leading to a diagnosis of a hematologic malignancy; both cases had elevated TNC counts (39/μL and 302/μL) and abnormal cells by cytology. In 4 separate samples from 3 patients, cytology demonstrated malignant cells while FC did not (3 samples with large cell lymphoma [TNC counts of 4/μL, 21/μL and 358/μL] and 1 sample with metastatic carcinoma [TNC count of 128/μL]). The remainder of the evaluable samples (104/123; 85%) showed a wider range of polyclonal B cells (0%-52%) and a higher mean T-cell percentage (34%) than seen in samples from patients with a history of hematologic malignancy, and fewer samples (13/123; 11%) were insufficient for FC analysis. Based on these results, we propose the following criteria for FC assessment on CSF specimens at our institution: history of hematologic malignancy or, in the absence of such a history, TNC count ≥30/uL. If these criteria were applied to the current cohort, 80 (26%) of 309 samples would have been deferred for testing. In addition, our findings suggest that cytological analysis may be preferred over FC in CSF samples in which a diagnosis of large cell lymphoma or carcinoma is suspected.

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A Retrospective Analysis of Transient Hepatitis B Surface Antigen Positivity in Recently Vaccinated Patients

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Hepatitis B vaccination has been shown to produce temporarily detectable levels of hepatitis B surface antigen (HBsAg) in patients. However, the time course and duration of this effect is unclear. In cases of recent vaccination, a positive HBsAg test can lead to unnecessary confirmatory laboratory testing, with added expense for the patient as well as the stress of a false positive result. Unrecognized false positives can lead to additional downstream effects such as reporting to public health authorities and use of isolated dialysis equipment for renal failure patients. The present study aims to clarify the nature and duration of transient postvaccination HBsAg positivity. The study was performed at the University of Iowa Hospitals and Clinics. Following IRB approval, the electronic medical record was searched for all HBsAg tests performed within a 17-month period. This yielded 11,719 tests on 9,930 patients. By institutional policy, a reactive result for HBsAg by electrochemiluminescence immunoassay (Roche Diagnostics cobas HBSAg assay) is defined by a cutoff index (COI) of > 20. A weakly reactive ("grayzone") result is defined by COI ≥ 1 to ≤ 20. All reactive and grayzone results are automatically retested, with persistent grayzone results sent to a reference laboratory for confirmatory testing. Thirty-three HBsAg tests were performed on patients within 14 days of a dose of hepatitis B vaccine. Of these 33 tests, 10 were resulted as grayzone; the remaining 23 were reported as nonreactive (COI < 1). All 10 patients with grayzone results received Engerix-B (GlaxoSmithKline) vaccine at a dose of either 20 μg (1 patient) or 40 mcg (9 patients). Further examination showed the COI for the grayzone results reached an average peak of 1.583 at 2 to 3 days following vaccine administration before decaying to an average of 0.578 by day 14. After 14 days, the COI was indistinguishable from the average COI of patients nonreactive for HBsAg who had no prior vaccination. No reactive or grayzone results were reported in patients who were tested for HBsAg between 15 and 180 days following a vaccination dose (168 patients) or for 2 patients who received a dose of a combined hepatitis A/hepatitis B vaccine (Twinrix, GlaxoSmithKline) within 14 days of HBsAg testing. The results of this study confirm that transient HBsAg positivity can occur in patients who have recent hepatitis B vaccination. The results also suggest that this positivity is unlikely to persist beyond 14 days postvaccination. False-positive HBsAg results due to recent vaccination may become more common as marketed assays continue to increase sensitivity for low levels of surface antigen relative to earlier generation assays.

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Reducing Laboratory Costs With Education-Based Gate-Keeping

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The need for reducing unnecessary test costs is a critical issue in laboratory medicine. Many physicians, particularly residents, are unaware of the costs associated with specific laboratory tests and/or the reimbursement models employed in the hospital setting. It has been shown that educating ordering physicians about the cost and relative clinical value of laboratory-based testing can result in substantial cost savings. One area of growing concern is referred testing for molecular studies. These tests are extremely expensive and are often not reimbursed through the current DRG system. They can also have limited value to the inpatient stay because of the associated long turn-around time. At Northwestern Memorial Hospital, all referred laboratory tests costing >$500 require approval by the laboratory medical director or designee. When notified, the director or designee contacts the ordering physician to discuss the clinical utility of the test and provide education on the cost of the test and expected turnaround time. We evaluated ordering practices for paraneoplastic panels, a referred test costing > $1,750 and having a turnaround time of 10-21 days. Over the 90-day study period, 15 paraneoplastic panels were ordered on inpatients at Northwestern Memorial Hospital. In each instance the ordering physician was contacted. In many of the cases, a consulting physician was also contacted. Of the 15 paraneoplastic panels ordered, 6 were approved and 9 were canceled, resulting in a cost of $10,022.50 and a savings of $15,817.50. Of the 6 that were approved, none were positive, and all patients had been discharged from the hospital prior to the results being reported. Gate-keeping of an expensive referred laboratory test combined with physician education resulted in a significant cost savings for the hospital. In many of the cases, ordering physicians were unaware of the cost, prolonged turnaround time or hospital reimbursement policies for send-out tests. Education resulted in decreased laboratory expenditures without limiting patient access to the tests when they provided clinical value. As the number of expensive molecular tests increases, improving physician education on the process of hospital costs, billing and reimbursement, starting at the resident level, may be a useful future direction to further affect these areas.

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Determining the Effects of the UGT1A1*28 and UGT2B7*2 Polymorphisms on the Production of Ethyl Glucuronide In Vivo

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Although ethanol is primarily metabolized via oxidation in the liver by alcohol dehydrogenase and aldehyde dehydrogenase, a small percentage is conjugated by uridine diphosphate-glucuronosyl transferase (UGT) and subsequently excreted. The product of this conjugation, ethyl glucuronide, has been widely utilized as a marker for monitoring ethanol abuse and can be detected in urine for up to 5 days. There is substantial controversy over testing for ethyl glucuronide because a number of studies have demonstrated that incidental exposure to products containing small amounts of ethanol can lead to false positives, depending on the cutoff utilized. In addition, the principal enzymes that catalyze the formation of ethyl glucuronide from ethanol, UGT1A1 and UGT2B7, are highly polymorphic and mutations in these genes such as the UGT1A1*28 and UGT2B7*2 have been demonstrated to affect enzyme activity in vitro. Although these polymorphisms are common in humans, it is unclear whether their presence significantly alters ethanol metabolism in vivo. Determine the effects of the UGT1A1*28 and UGT2B7*2 polymorphisms on the formation of ethyl glucuronide in vivo. A dilute and shoot method for measuring ethyl glucuronide was developed utilizing a Thermo Exactive Orbitrap TLX-1 mass spectrometer in negative mode. Genotyping was performed on a BD Max thermocycler for UGT1A1 and an ABI 7500 thermocycler for UGT2B7. Ethanol consumption studies were performed at the clinical research center at San Francisco General Hospital. Subjects consumed a calorie-controlled meal and subsequently ingested 0.5 g/kg ethanol over a period of 20 minutes. We conducted controlled ethanol consumption studies on 26 genotyped individuals that fell into 1 of 4 categories: wild-type, UGT1A1*28 homozygous, UGT2B7*2 homozygous, UGT1A1*28/UGT2B7*2 double-homozygous. Ethyl glucuronide levels at 7 time points over 24 hours were measured and normalized to creatinine. The area under the resulting curve was subsequently utilized as a surrogate for total ethyl glucuronide production. The average amount of ethyl glucuronide produced from individuals harboring UGT1A1*28 or UGT2B7*2 alleles was not significantly different from wild-type individuals. From these data, we conclude that the common polymorphisms found in UGT1A1 and UGT2B7 do not significantly affect the production of ethyl glucuronide in vivo. Consequently, testing for ethyl glucuronide as a marker of ethanol ingestion remains a robust test and genotyping for UGT1A1 and 2B7 does not need to be considered.

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Intracellular Neuron-Specific Enolase: Intra- and Interindividual Variability and Effects of Hemolysis

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Quantitation of neuron-specific enolase (NSE) in serum may be performed in patients post–cardiac arrest to assess for hypoxic-ischemic brain injuries or utilized as a neuroendocrine tumor marker. The accuracy of NSE concentrations is affected by even slight hemolysis, largely because NSE is found within the red blood cells (RBCs). The current practice of rejecting specimens with free hemoglobin (Hb) >20 mg/dL and NSE concentrations >15 ng/mL has resulted in a rejection rate of 14%, due primarily to hemolysis. The purpose of this study was to reduce the hemolysis rejection rate by deriving and validating a correction factor accounting for the concentration of NSE contained within the RBCs of each individual. This also required the differentiation of the NSE concentration contribution due to hemolysis, from that which is clinically relevant and found in vivo. Paired EDTA whole blood and SST serum specimens were collected from normal donors (n = 10) on 3 nonconsecutive days. RBCs isolated from normal donors were lysed daily and the intracellular NSE concentrations were measured on the Kryptor Compact (Thermo Fisher Scientific) immunoassay platform. A spectrophotometric method utilizing the NanoDrop (Thermo Fisher Scientific) for determining free Hb in the paired serum samples was developed and determined to be highly accurate and sensitive, as required for implementing a correction factor based on the degree of hemolysis. In addition, various concentrations of hemolysate from a single normal donor were spiked into 5 serum pools with known NSE concentrations. Results indicate that the intraindividual variability of intracellular NSE from day-to-day is insignificant (<10% CV). However, the interindividual variability resulted in almost a 2-fold difference in the intracellular NSE concentrations (1.57-2.85 pg NSE/mg Hb and $P < .001$), indicating the need for an individualized approach to providing corrected NSE concentrations in the event of sample hemolysis. There was a direct linear analytical suppression of NSE concentrations as a function of free Hb (slope = –0.0844 ± 0.009). Furthermore, for a single normal donor, the increase in NSE as a

function of hemolysate spiked into each serum pool was also shown to be linear (y = 0.144x + 1.31 and r^2 = 1.00). Collectively, these experiments investigated the orthogonal effects of hemolysis on the measurement of NSE. A correction equation based on the degree of hemolysis, including both the analytical interference of free Hb and the individually determined RBC contribution to NSE concentration was determined: $NSE_{corr} = NSE_{meas} – (Hb_{serum})(NSE_{RBCs/Hb}) + 0.0844(Hb_{serum})+1.1$. Applying an individualized correction equation will greatly reduce the number of rejected samples due to hemolysis and maintains increased accuracy when compared to other correction methods previously reported in the literature.

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Strength of Donor-Specific Antibodies Detected by Luminex Single Antigen Bead Assay Correlates With C4d Positivity in Kidney Transplant Biopsies and Guides Clinical Management to Prevent Allograft Loss

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Although solid-phase single antigen bead (SAB) assay by Luminex has increased the sensitivity and specificity of detecting donor-specific anti-HLA antibodies (DSA), it is regarded as a semi-quantitative method for antibody strength determination. Antibody-mediated allograft rejection (AMR) is directly associated with the number and strength of DSAs. We discuss 2 cases to show how DSA strength monitoring by SAB provides important clinical management information that correlates with C4d deposition in the allograft and with histopathologic diagnosis of AMR. Two kidney transplant patients were sequentially tested for HLA class I and II DSA by SAB. DSA strength was represented by normalized mean fluorescence intensity (MFI). Serum creatinine and histological examination of protocol allograft kidney biopsies with C4d immunostain were performed. Case 1: 53-yr-old Hispanic female with pretransplant 94% class I PRA and 57% class II PRA. She had DR4 and DR53 DSAs pretransplant. She received plasmapheresis pretransplant to achieve negative cross matches. Fifteen days posttransplant, she had strong DR4 and DR53 DSAs that correlated with elevated serum creatinine, features of AMR and diffuse C4d positivity on biopsy. Her DSAs responded well to repeated plasmapheresis. Case 2: 39-yr-old AA male pretransplant 0% class I and II PRA and negative cross matches. Nine months posttransplant. serum creatinine started to increase and de novo strong DQ7, DQA1*03:03, and DQA I*04:01 DSAs were detected. Biopsy showed features of AMR and diffuse C4d positivity. His DSA responded to splenectomy. Luminex SAB gives useful information regarding strength of DSA that correlates with biopsy C4d deposition and diagnosis of AMR. Routine posttransplant testing for presence and strength of DSA is recommended to aid clinical management and prevent allograft loss. Close monitoring of DSA strength by SAB is highly recommended in patients who exhibit low-level DSA pretransplant as they may develop early AMR. Routine posttransplant monitoring for DSA is a useful way to detect de novo DSA. Although MFI values are not standardized, DSA MFI values are an important index to assess risk factors, to evaluate treatment response, and to guide treatment strategy for AMR.

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FLT3 Mutations in Myeloproliferative Neoplasms: The Beaumont Experience

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The goal of our study is to evaluate the mutational status of the *FLT3* gene in patients diagnosed with a myeloproliferative neoplasm (MPN) or myelodysplastic/myeloproliferative neoplasm (MDS/MPN), in correlation with JAK2 mutational status. The files of the William Beaumont Health System Molecular Pathology Laboratory were searched for all MPN and MPN/MDS cases that were analyzed for *JAK2* gene mutations from February 2006 to December 2011. BCR-ABL–positive cases of chronic myeloid leukemia were excluded. FLT3 mutation analysis was performed on DNA extracted from bone marrow or peripheral blood specimens from 152 patients using PCR amplification and analysis of amplicons by gel electrophoresis for internal tandem duplication (ITD) mutations; and by restriction endonuclease digestion fragment analysis for the D835 point mutation. FLT3 mutation analysis was performed on 67 cases of JAK2-negative MPN, 23 cases of JAK2-negative MPN/MDS, and 62 cases of JAK2-positive MPN; 1 FLT3 mutation (ITD) was identified in the JAK2-negative MPN group (1.5%) and none in the JAK2-negative MPN/MDS and JAK2-positive groups. Our results confirm the absence of both types of FLT3 mutations in JAK2-positive specimens, as previously reported, and positivity in 1.5% of JAK2-negative MPNs.

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Adapting Random Mutation Capture for Quantification of Mutation Frequency in Preleukemic PML-Rarα Expressing Myeloid Progenitors

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It is generally believed that sequential acquisition of multiple mutations underlies tumorigenesis in most cancers. Many studies in murine models of leukemia indirectly support this hypothesis based on the long latency of tumor growth despite constitutive expression of oncogenic fusion proteins. Prominent among these are studies showing that mice which express a fusion between the proteins PML and the Retinoic Acid Receptor α (PML-Rarα)—expressed in >95% of human acute promyelocytic leukemia (APL)—develop a mimic of APL over the course of 6 to 9 months. Strikingly, PML-Rarα has been further shown to interfere with DNA repair machinery, suggesting a causal link between the initiating translocation and subsequent accumulation of cancer-promoting mutations. Nevertheless, changes in mutation frequency or type have not been well documented in PML-Rarα expressing cells. In order to begin to characterize the effect of PML-Rarα expression on mutational burden, we modified the method of random mutation capture (RMC) pioneered by Wright and Campbell et al (*NAR*, 2011), which provides a measure of the frequency and type (ie, transitions, transversions) of single base pair mutations. Here we show that RMC clearly detects single base pair mutants in DNA isolated from murine bone marrow. This work represents the first use of RMC in leukocytes and opens the door to characterization of the mutational spectrum in preleukemic myeloid and lymphoid cells.

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Discrepant Serum and Urine B-hCG Results Due to Production of β-hCG by a Cribriform-Morular Variant of Thyroid Papillary Tumor

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Paraneoplastic syndromes may result in benign elevations in serum hormone levels; others may lead to life-threatening conditions such as hypercalcemia, or unnecessary clinical investigations and therapies. Such disorders are rare in the context of papillary thyroid carcinoma. We report a case of a 31-year-old female patient with Gardner syndrome and recurrent cribriform-morular variant of papillary thyroid carcinoma. The patient presented with an elevated serum hCG of 130 mIU/mL using the Roche Elecsys hCG+β test, while a qualitative urine hCG was negative using the Quidel QuickVue+ one-step hCG combo test. Because there was a concern that the patient may be pregnant versus a potentially false-positive serum hCG, further clinical and laboratory investigations were carried out. Pregnancy was ruled out through extensive clinical workup with exams and ultrasounds. The serum hCG was validated as a true value due to the following: (a) heterophile antibodies (HA) interference was unlikely since HA blocking studies did not appreciably affect the patient's hCG value; (b) dilution of this patient serum sample showed expected recoveries; and (c) the same serum assayed by various commercial hCG assays showed positive values ranging from 50 to 346 mIU/mL. Thus we postulated that the origin of the circulatory hCG was paraneoplastic production by the tumor of thyroid origin. Immunohistochemical (IHC) studies on formalin-fixed, paraffin-embedded tissue samples from a recent resection specimen from the recurrent tumor showed focal positive staining of tumor cell nodules using hCG antibodies. To verify that the IHC staining is specific for hCG, an absorption control was carried out by presaturating the IHC antibody reagent with exogenous hCG before addition to the tissue specimen. Following saturation, the tumor sample showed negative staining, indicative of a true positive result. The apparent discrepancy between urine and the various serum assays is thought to be due to differential epitope specificity in the reagent antibodies employed as well as the existence of different hCG variants in blood versus urine. We conclude that paraneoplastic secretion of β-hCG, this being the first to be reported in a thyroid carcinoma, is uncommon, but an important diagnostic consideration in a female patient of childbearing age with a neoplasm. Misdiagnosis can occur when discrepancies in hCG results are not resolved in a systematic manner involving both clinical and further laboratory confirmations.

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Exon 28 Sequencing in Von Willebrand Disease Evaluation: A Retrospective Review of Testing Performed at a Hemostasis Reference Laboratory

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Type 2 von Willebrand disease (VWD) is characterized by structural defects that impair von Willebrand factor function. The majority of cases of types 2A, 2B, and 2M von Willebrand disease are attributable to heterozygous mutations localized within exon 28 of the VWF gene. We undertook a retrospective review of all *VWF* exon 28 sequence analyses performed in our laboratory in order to assess the degree of genetic diversity underlying types 2A, 2B, and 2M VWD, and to assess the accuracy of clinical diagnosis. Data from 765 consecutive patients evaluated between March 2000 and August 2011 were reviewed. Mutation analysis entailed *VWF* gene-specific PCR amplification of genomic DNA, followed by bidirectional sequence analysis using dye terminator chemistry. Results were entered into our lab database and compared to data available in the ISTH-SSC on-line VWF database (http://www.vwf.group.shef.ac.uk) and with clinical phenotype data which was supplied by the referring physician and laboratory data generated in our laboratory. Of the 765 patients evaluated, 213 (27.84%) patients had a single mutation and 50 (6.54%) patients had >1 mutation; 5, 10, and 5 different mutations previously associated with types 2A, 2B, and 2M VWD, respectively, were identified. A total of 52 previously unreported candidate mutations were identified. Inconsistency between clinical phenotypic diagnosis and genotype data was observed in 9 of 183 (4.92%) patients where sufficient data (including VWF:Ag, VWF:RCO, VWF:multimers, platelet count, platelet aggregation, and clinical history) were available for review. Our study suggests that considerable genetic diversity underlies type 2A, 2B, and 2M VWD; that candidate mutations are still being identified; and that genetic analysis is helpful when there is uncertainty as to the nature of a patient's type 2 VWD diagnosis.

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Hemoglobin Degradation Not Responsible for P3/P4 Peaks in Bio-Rad Variant II HPLC

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Glycated hemoglobin percent (A1c%) is used in the diagnosis and management of diabetes. Some A1c% methods can be adversely affected by the presence of hemoglobin (Hb) variants and Hb degradation in the sample. We use the Bio-Rad Variant II Turbo ion-exchange high-performance liquid chromatography (HPLC) method. According to the manufacturer's instructions, the presence of unidentified P3 or P4 peaks higher than 10% may indicate degradation and a new specimen must be requested. However, in our experience, repeat testing of fresh specimens rarely confirms degradation and P3 and P4 peaks often correspond to infrequent Hb variants. In our study, we sought to confirm that uncommon Hb variants may be misinterpreted as degraded Hb on the Bio-Rad Variant II Turbo instrument. To investigate the possibility that Hb variants are eluting at the P3/P4 peak retention time, we evaluated all specimens submitted to the laboratory for A1c% analysis from December 2011 to January 2012 that contained P3 and/or P4 peaks >10%. These samples were submitted to a reference laboratory for Hb variant phenotyping analysis. To validate the manufacturer's degradation claim, 2 different specimens representing low and high A1c% concentrations and showing P3 and P4 <10% were analyzed after incubation at –30°C, 4°C, and at room temperature (these temperatures represent our current storage conditions). A1c, P3, and P4% were measured initially and daily for a period of 5 days. A total of 9 samples were submitted to a reference laboratory. All of them (9/9) confirmed the presence of infrequent Hb variants, including Hb J (4/9), Hb Anamosa (1/9), Hb Le Lamentin (2/9), Hb Candem (1/9), and Hb Austin (1/9). A1c, P3 and P4 % values on specimens incubated at –30°C, 4°C, or RT remained stable within the analytical imprecision of the method. Infrequent Hb variants are misinterpreted as degraded Hb by Bio-Rad Variant II Turbo instrument. Acknowledgment of the presence of an infrequent unknown Hb rather than degradation may be required. In addition, unnecessary re-collection of specimens may be avoided.

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Detecting Acute HIV Infection Using a Fourth-Generation Immunoassay: A Case Report

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Diagnosing patients during acute HIV infection is critically important because it is during this phase that patients are most infectious, but have mild clinical symptoms and often test negative for HIV antibodies. Indeed, the window period for antibody detection is approximately 3 to 5 weeks after infection. Accordingly, replication of virus during this period is typically based on detection of viral RNA and p24 antigen in blood. The performance of the serologic methods for the diagnosis of HIV infection has continuously improved throughout the past 2 decades. Many laboratories are currently using the fourth-generation immunoassays which allow the simultaneous detection of p24 antigen and HIV antibodies, reducing the seroconversion window and thus detecting acute infections. We report a case of acute HIV infection in a 29-year-old homosexual male who presented with a 4-week history of sore throat, fever, diffuse maculopapular rash and diarrhea. Physical exam showed lymphadenopathy, hepatosplenomegaly and oral candidiasis. Laboratory tests revealed pancytopenia and mildly elevated liver enzymes. The patient's sample tested positive on the Abbott Architect HIV Ag/Ab Combo assay (fourth generation immunoassay). However, the same sample tested negative on the Siemens Avidia Centaur HIV 1/2/O Enhanced and the OraQuick Advance Rapid HIV-1/2 Antibody assays, both of which detect the presence of HIV antibodies but not antigens. Because of the discrepant results and for further confirmation, an HIV-1 Western blot and HIV-1 Viral load were performed. The Western blot was negative, but the HIV-1 viral load was >10,000,000 copies/mL. The patient was diagnosed with an acute retroviral syndrome and antiretroviral therapy was initiated. In order to characterize and confirm the positivity of the Architect HIV Ag/Ab Combo assay, an HIV-1 p24 antigen was performed and was found to be positive; indicating that the positive result on the Architect was due to the presence of the HIV p24 antigen. This case illustrates the benefits of the fourth-generation HIV immunoassays as an initial screening assay to identify individuals with acute HIV infection and to reduce the risk of missing unsuspected primary infections.

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Plasma vs Serum Samples for Analysis of Free Light Chains

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Assays for serum free light chains (FLC) have been shown to be a sensitive method for the detection of plasma cell myeloma and other lymphoproliferative diseases, with the ability to detect as little as 1 mg/L of free light chains. Measurement of serum FLC is also used to monitor response to therapy and to predict prognosis. Serum is the typical sample type used for measurement of FLC; however, analysis of plasma has potential practical advantages compared to serum. In this study, we compared FLC analysis (Freelite, The Binding Site, Birmingham, England) on Roche Diagnostics modular P analyzers using 3 different specimen types: serum, EDTA-plasma, and lithium heparin plasma separator tubes. These were analyzed on statistical software (EP evaluator, Data Innovations). When compared to serum, EDTA-plasma gave comparable results for FLC κ (average error index –0.64), FLC λ (–0.17), and κ/λ ratio (–0.42). Lithium-heparin specimens were comparable to serum for FLC λ (–0.38); however, these specimens gave higher quantitative values for FLC κ (6.10) and the κ/λ ratio (7.29). Comparison of FLC analysis using either serum and lithium-heparin plasma for patients with multiple myeloma indicated that the differences in results were clinically significant. These results are similar to those described by Velthuis et al[1] using a different FLC assay. These findings suggest that using an EDTA tube for measurement of FLC is acceptable whereas use of lithium heparin plasma separator tubes produces clinically significant differences and should not be used for measurement of serum FLC. The ability to use plasma specimens has practical advantages for FLC assay automation and avoidance of interference from microclots during analysis.

[1]Velthuis H, et al. N Latex FLC: new monoclonal high-performance assays for the determination of free light chain kappa and lambda. *Clin Chem Lab Med.* 2011;49:1323-1332.

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Comparison of Hemoglobin A_{1c} Measurements of Samples With Elevated Fetal Hemoglobin by Three Commercial Assays

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HbA_{1c} is one of the most ordered tests to monitor glycemic status for diabetic patients and has recently been recommended as a criterion for diagnosis of diabetes mellitus (≥6.5%). The requirement for obtaining accurate $\%HbA_{1c}$ values has been stricter. However, elevated HbF may be asymptomatic while affecting HbA_{1c} results, thus adversely impacting glycemic control strategies. Reports of HbF interference on commercially available assays are limited. The widely used Tosoh G8, Bio-Rad D-10, and Ortho Clinical VITROS 5,1 FS assays were evaluated herein. Seven whole blood samples with %HbF spanning 0.8%-38.4% and constant $\%HbA_{1c}$ were prepared by mixing a specimen containing 98% HbF, and 0% HbA with another one containing 0.8% HbF, 4.6% HbA_{1c} in various proportions. The mixtures should all contain the same $\%HbA_{1c}$. The G8 showed little or no difference in $\%HbA_{1c}$ value between these mixtures and was determined to be an appropriate comparative method for the other 2 assays. The $\%HbA_{1c}$ values of additional 30 whole blood mixtures containing varying amounts of %HbF were measured by the 3 assays. The D-10 did not show significant differences from the G8 values as long as HbF was below 12.5%, above which there was an increasing positive bias up to +1% HbA_{1c}%. On the other hand, the VITROS showed significant negative bias increasing up to –1.5% HbA_{1c}. Both G8 and D-10 can identify the condition of elevated HbF, whereas the VITROS cannot. As the accuracy of HbA1c is determined by patients' hemoglobinopathy/HbF status and the testing methodology used, clinicians should have knowledge of both in order to correctly interpret HbA1c results and enhance management of diabetic patients.

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Molecular Pathways of Topotecan-Induced Apoptosis in Human Neuroblastoma Cells

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Neuroblastoma (NB), the most common solid extracranial pediatric tumor, is heterogeneous in terms of its biological, genetic, and morphological characteristics. The overall incidence of neuroblastoma is 1 case per 100,000 children in the United States, or approximately 700 newly diagnosed patients per year. Topotecan hydrochloride is a chemotherapeutic agent that is a topoisomerase I inhibitor. It is the water-soluble derivative of camptothecin used for treating ovarian cancer and small cell lung cancer. We explored the possibility of using topotecan for treating neuroblastoma using an in vitro model and also explored the molecular pathway of topotecan induced apoptosis. Dose response and kinetics experiments were done using SK-N-AS Human neuroblastoma cells. Cells, once having reached 50%-60% confluence, were treated with various concentrations of topotecan (0, 0.1, 0.5, 1, and 5 µmol/L) for 12, 24, 36, and 48 hours. The cell death and proliferation were determined by light microscopy, electron microscopy and MTS Cell Proliferation Assay (Promega). Cell cycle and apoptosis were determined by flow cytometry, TUNEL assay, and mitochondrial potential assay. Expression of intracellular proteins of phospho-Akt1/2/3 (pAkt 1/2/3) and phospho-STAT3 (pSTAT3), Bcl2, Bax, BclxL, and cleaved caspase 3 were assessed utilizing immunofluorescence technique. At 36 hours posttreatment, the 0.5-, 1-, and 5-µmol/L concentrations displayed 28%, 46%, and 78% growth inhibition, respectively. Electron microscopy and TUNEL showed cells to undergo apoptosis. Cell cycle analysis showed G1 arrest and sub G1 peak. Mitochondrial potential assay revealed a decrease in potential at 24 hours of drug treatment. Immunofluorescence experiments displayed a decrease in pAKT at 1 and 5 µmol/L concentrations. At 24 hours of topotecan exposure, Bcl2 and BCL xL showed a decrease in its expression and increase in cleaved caspase 3, whereas p-STAT3 appeared to have no change in their intracellular expression levels. In conclusion, these results demonstrate that topotecan induced apoptosis in human neuroblastoma cells by inhibiting phosphorylation of AKT molecular pathway as well as Bcl2 family proteins Bcl2 and BCL xL. These findings encourage the further exploration of topotecan as a potential chemotherapeutic agent against neuroblastoma.

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Development of a Quantitative Assay for Chloroquine, Desethylchloroquine, and Didesethylchloroquine to Investigate a Potential Case of CNS Toxicity

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Neuropsychiatric disturbances are a rare but serious side effect of the antimalarial drug chloroquine (CQ). In response to a potential case of CQ-associated psychosis, a quantitative assay was developed for CQ and 2 of its metabolites, desethylchloroquine (DCQ) and didesethylchloroquine (DDCQ), using the ABSciex 5600 Triple TOF mass spectrometer. The assay was used to quantitate parent and metabolite levels in biological (serum, urine, cerebrospinal fluid) samples from a patient potentially suffering from CQ-associated CNS toxicity and in buffer samples from cellular transport studies done to investigate possible contributing factors to CNS toxicity. A novel quantitative LC-MS assay was developed on an ABSciex TripleTOF 5600 system for CQ, DCQ, and DDCQ with product ion confirmation. Chromatography was performed using a Waters X-Terra C18 3.5 µM, 2.1×100 mm analytical column with a gradient consisting of 0.05% formic acid, 5 mM ammonium formate in H_2O (mobile phase A) and 0.05% formic acid in methanol (mobile phase B). Two deuterium-labeled internal standards were used, chloroquine-d4 and desethylchloroquine-d4. Sample preparation consisted of either a dilution with 10% methanol in H_2O (buffer, CSF, urine) or a protein precipitation with methanol (serum). The buffer assay was validated over a curve range of 5 to 100 nM for CQ and 10 to 100 nM for DCQ and DDCQ. Intraday and interday accuracy and precision of back-calculated curve points for all analytes ranged from 81.7% to 104% (accuracy) and 0.058% to 19.7% (CV). Quality control samples for all analytes demonstrated 104% to 112% (accuracy) and 1.03% to 5.86% (CV). The assay had minimal carryover, crosstalk, and matrix effects. The analytical measurement ranges for urine and serum were 200 to 1,000 nM and 25 to 100 nM, respectively. An assay for quantitating CQ, DCQ, and DDCQ in buffer was validated for parent and metabolites in samples from cellular transport studies conducted to investigate interaction with P-glycoprotein. The buffer assay was also used to quantitate CQ, DCQ, and DDCQ in CSF. CQ, DCQ, and DDCQ were quantitated in serum and urine with matrix-specific sample preparation and calibration curve ranges.

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Development of a High-Resolution Accurate Mass Method for the Multiplexed Monitoring of Antiretroviral Agents in Human Serum

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There are currently several drug classes used for the management of HIV infection. Antiretroviral drugs (ARVs) are categorized based on their mechanism of action, with many of them eliciting their effects as reverse transcriptase and protease inhibitors. While drug therapy has largely been used for the management of HIV load and administered in cases of significantly decreased CD4+ cell count, prevention studies have illustrated that the use of antiretroviral therapies in an HIV-positive individual decreases the chances of retroviral transmission. The goal of this work is to qualitatively screen and confirm the presence of ARV drugs using a multiplexed approach. The analytical method was developed to detect the following ARV agents: amprenavir, atazanavir, darunavir, efavirenz, emtricitabine, indinavir, lamivudine, lopinavir, nelfinavir, nevirapine, ritonavir, saquinavir, tenofovir, and tipranavir. 100 µL of ARV-spiked standards in drug-free human serum (BioRad) were extracted in cold acetonitrile, evaporated to dryness, reconstituted, and separated chromatographically. The chromatographic run began with 60 sec of 5% methanol containing 10 mM ammonium acetate (mobile phase B), followed by a 10-minute ramp to 95% B, at a flow rate of 500 µL/min. Analytes were eluted from a Hypersil Gold 50 × 2.1 mm; 3 µm particle size HPLC column (Thermo Fisher Scientific) during this gradient. Analytes were detected over a 14.9-minute run using the Exactive Orbitrap mass analyzer (Thermo Fisher Scientific). The mass spectrometer method included 2 positive-mode scan events: 1 full scan event with ultra-high resolution (100,000 @ 1Hz) and 1 in-source collision-induced dissociation (SCID) event with enhanced resolution (25,000 @ 4Hz). The analytical method was found to have a limit of detection of less than 20 ng/mL for all ARVs. Positive identification was determined by exact mass analysis at 5 ppm discrimination, analyte retention time, and identification of mass transitions when applicable. Following the development of the multiplexed approach, the method was applied to a subset of HIV

Prevention Trial Network specimens (n = 214), which contained none, one, or multiple ARV drugs. Specimens were screened blindly and results were compared to a reference single reaction monitoring (SRM)-based method. Agreement for detected ARVs was as follows: atazanavir (89%), efavirenz (73%), nevirapine (78%), and ritonavir (100%). These data indicate the ability to screen and subsequently confirm the presence of ARV agents in a single specimen in a high-throughput, multiplexed format.

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Therapeutic Plasma Exchange for Device-Related Hemolysis

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Implantation of a left ventricular assist device (LVAD) is a well-established therapeutic option for patients with end-stage heart failure. Device-related hemolysis remains a clinically relevant complication of several VAD types. Hemolysis is associated with multiple laboratory abnormalities including anemia, increased LDH, and plasma-free hemoglobin. If the hemolysis is severe, it can lead to acute renal failure and dyspnea on exertion. Therapeutic plasma exchange (TPE) has been shown to ameliorate the side effects of hemolysis. We report on a case of LVAD-associated hemolysis in a 22-year-old patient with adriamycin-induced cardiomyopathy status post Ewing sarcoma who was supported with TPE. The patient presented to a community hospital with jaundice, anemia, and progressively worsening dyspnea on exertion and was transferred to our institution for further evaluation and treatment. The patient was transfused 2 units of leukoreduced red blood cells (RBC) for symptomatic anemia (hemoglobin of 7.2 g/dL). The posttransfusion hemoglobin was 8.2 g/dL, but decreased to 6.4 g/dL within 6 hours with laboratory values indicating ongoing hemolysis. Mechanical versus intrinsic RBC defects were investigated and the hemolysis was concluded to be mechanical. Daily TPE was initiated with a short-term goal to reduce the plasma-free hemoglobin to less than 100 g/dL. The long-term goal was to bridge the patient until a heart was available for transplant. A total of 8 TPE were performed; initially 100% plasma was used for the replacement fluid. Eventually this was replaced with a 1:1 ratio of plasma and 5% albumin as the replacement fluid. The plasma-free hemoglobin decreased from 427 mg/dL to a nadir of less than 15 mg/dL and LDH decreased from 18,789 U/L to a nadir of 710 U/L. Device-related hemolysis could increase with the increasing use of LVADs. We report a case of a patient with device-related hemolysis who underwent daily TPE and laboratory results demonstrated temporary improvement after each procedure. This report adds to the existing literature supporting TPE as adjunctive therapy in cases of hemolysis.

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Circulating PTH Is Not Significantly Correlated to Total 25-OH Vitamin D Concentrations Greater Than 10 ng/mL in a Healthy Pediatric Population

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The objective of this study was to examine the relationship between 25-OH vitamin D concentrations and intact parathyroid hormone (PTH) in a healthy pediatric population. Concentrations of 25-OH Vitamin D (Diasorin Liaison), intact PTH, creatinine (both Roche Cobas 6000), and ionized calcium (Radiometer ABL 800) were determined in 179 serum specimens (average age 10.5 years, range 2 months-21 years) received at Saint Louis Children's Hospital from June of 2011 to January of 2012. Ten samples from patients in renal failure were identified due to significant elevation of both creatinine and PTH and excluded. Four samples with equivocal elevations of either creatinine or PTH were also excluded following chart review that revealed a diagnosis of renal disease. The average concentrations of vitamin D, ionized calcium, creatinine, and PTH in the final cohort of 165 specimens were 28.5 ng/mL (71.3 mmol/L), 5.01 mg/dL (1.25 mmol/L), 0.50 mg/dL (44.2 μmol/L), and 30.9 ng/L, respectively. Fifty-nine percent of samples had a vitamin D concentration less than 30 ng/mL and the distribution of vitamin D concentrations in this population agreed with a much larger NHANES study. Regression analysis of all specimens (linear least squares) showed a significant ($r = 0.26$, $P = .0006$) inverse relationship between PTH and 25-OH vitamin D. When specimens with vitamin D concentrations ≤ 10 ng/mL (n = 9) were excluded there was no significant relationship ($r = 0.14$, $P = .07$) between PTH and 25-OH vitamin D. Specimens were further stratified according to 25-OH vitamin D concentration into groups containing 11 to 20, 21 to 30, 31 to 40, and 41 to 50 ng/mL 25-OH vitamin D. There was no significant difference in PTH concentrations between cohorts as assessed by single factor ANOVA. There was a statistically significant difference in PTH values between cohorts with 25-OH vitamin D concentrations ≤ 10 ng/mL and > 10 ng/mL ($P = 8.1\times10^{-9}$, ANOVA). Two specimens had PTH concentrations above the normal range (14-72 ng/L) and both contained less than 10 ng/mL 25-OH vitamin D. The statistical analysis was limited by the small number of specimens with 25-OH vitamin D ≤ 10 ng/mL. This study demonstrates that PTH concentrations in a healthy population from birth to 21 years of age do not rise significantly until 25-OH vitamin D concentrations fall below 10 ng/mL and suggests that defining vitamin D sufficiency based on the concentration that suppresses PTH in cohorts that include renal failure patients may overestimate the concentration at which individuals should be considered deficient.

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Antibody to Diego Can Cause Severe Hemolysis, Yet Is Not Detected by Standard Antibody Screening

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Of the 2 most common antigens in the Diego blood group, Di_b is ubiquitous, Di_a however, is rare in people of European descent (1/10,000). The prevalence of Di_a varies in Native Americans from near 0% in the north (Inuit) to 54% in Brazil (Kainganges). Anti-Di_a reactions have been reported to cause hemolytic disease of the newborn and acute hemolytic transfusion reactions. We report 2 cases of anti-Di_a, one of which resulted in a marked hemolytic reaction. Patient 1 is a 74-year-old male who is RBC transfusion dependent. After receiving 34 RBC units over 2 years without incident, he experienced a significant transfusion reaction after receiving 75 mL of RBCs. Symptoms consisted of nausea, back pain, chills and rigor. The reaction workup revealed 4+ hemolysis in the posttransfusion specimen, a negative DAT, correct blood type, and a negative clerical check. Since the pretransfusion antibody screen was negative, the transfused RBC unit had been electronically cross-matched. A posttransfusion antibody ID panel was reactive on a Di_a-positive cell. All other antigens on the panel were ruled out. The reactive unit

was confirmed to be Di_a-positive using anti-Di_a plasma. The patient was restricted to IgG-crossmatched units for future transfusions and has received multiple RBC transfusions since without incident. Our anti-Di_a acute hemolytic transfusion reaction is the first reported since 1979. Patient 2 is a 27-year-old male who has received 14 RBC units over the past 6 years without significant reactions. The patient's antibody screen became positive, and the antibody workup revealed the presence of both anti-E and anti-Di_a antibodies. Had he not had the anti-E in addition to the anti-Di_a, it is likely his anti-Di_a would have not been detected, which could have led to a significant hemolytic reaction had he received Di_a positive blood. An institution in southern Texas with a 20% Mexican American donor population reported a Di_a antigen rate of 2.6% and an anti-Di_a rate of 1.3%. Our local donor population is 33% Hispanic and 6% Native American. It is expected that our community expresses higher rates of Di_a and anti-Di_a than those reported in south Texas. Due to its scarcity in donor populations, Di_a is not usually present on RBC antibody screens. This leads to potential problems in regions where the donor population has a higher prevalence of Di_a, and therefore a higher rate of alloimmunization in the transfused population. Although the prospect of including a Di_a-positive cell in routine antibody screens would be costly, we propose that this option should be investigated further in patient populations such as ours with a higher prevalence of Di_a.

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Assessment of Common Chemistry Analyte Stability and Specimen Rejection Criteria for Unprocessed Bloods

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The University of Chicago Medical Center Clinical Chemistry laboratories provide laboratory testing services for several outreach programs, which transport specimens to the laboratory via courier service. Published literature has shown varying data on the stability of common analytes (stored at different temperatures) and exist mostly on centrifuged serum/plasma rather than unprocessed whole blood specimens. For visiting nurse outreach programs, where bloods are drawn and remained unprocessed until courier pick-up and delivery to the central laboratory, it is important to establish acceptability criteria for maximum transport-receipt time for these unprocessed specimens. To establish these criteria, whole blood samples from 12 apparently healthy volunteers were collected in heparinized plasma separator tubes and left at room temperature, either stationary or subject to constant agitation (to simulate movement of transport in a vehicle) for up to 8 hours. We also separately spiked exogenous vancomycin, amikacin, tobramycin, and gentamicin at therapeutic concentrations into a volunteer blood specimen; this blood was left at varying times up to 8 hours at room temperature before processing. Twenty-eight common chemistries and 4 drugs were interrogated at the following time points for centrifugation and measurements: 0, 1, 2, 4, and 6 (agitated samples) or 8 hours (stationary samples) after collection. The following tests were stable (did not show clinically significant changes) up to 6 hours in uncentrifuged bloods: BUN, creat, Na, K, Cl, tCO2, Ca, Mg, P, Uric, TBil, DBil, Chol, HDL, trig, total protein, albumin, prealbumin, iron, UIBC, amylase, lipase, ALT, ALP, GGT, vancomycin, amikacin, tobramycin, and gentamicin. However, several analytes showed time-dependent changes: Gluc decreases by ~ 5%/hour; AST increases by ~ 5%/hour, LDH by ~ 20%/hour. In summary, we were able to establish acceptable transport times of 6 hours for most analytes and instituted a policy to append the following disclaimers for Gluc and LDH: (a) Gluc decreases by 5% per hour and may be significantly falsely decreased for unprocessed blood after 2 hours; (b) LDH increases by more than 10% per hour and may be significantly falsely increased for unprocessed blood after 2 hours.

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CSF and Blood Biomarkers for Alzheimer's Disease

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Alzheimer's disease (AD) is recognized as the most common dementia in the elderly, affecting almost half of all people in the United States over the age of 85 years. Despite its prevalence, population-wide diagnostic screening tests for AD are not available and current therapies are initiated only after clinical diagnosis, when substantial cell loss in vulnerable brain regions has already occurred. Our goal is to develop a noninvasive, high throughput screening assay for Alzheimer's disease that reflects the neuropathologic changes occurring as the disease unfolds. We have developed a first-generation tau protein blood test that detects elevated tau in the plasma of 19.2% of patients who are demented (n = 26) and 0% of age-matched control subjects (n = 28) and a TDP43 assay that detects abnormal protein in the plasma of an additional 12.5% of patients who are demented (n = 16) and 0% of control subjects (n = 16). We are currently working on increasing assay sensitivity by multiplexing this test with measurements of amyloid protein and are also using amplification techniques to improve signal detection. A robust diagnostic test for Alzheimer's will be beneficial for monitoring response to new pharmacologic therapies, will help screen individuals at risk for the disease and may help clinicians initiate treatment prior to severe neuronal loss.

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Genotype/Phenotype Relationship in a Dyslipidemic Population Undergoing LDL Apheresis

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Familial hypercholesterolemia (FH) is an autosomal dominant disease caused primarily by mutations in the *LDLR*, *PCSK9*, or *APOB* genes. The FH phenotype is characterized by extremely elevated low-density lipoprotein cholesterol (LDL-C) and associated with premature cardiovascular disease and atherosclerosis. In phenotypically defined FH patients ~75% have a causative genetic variant, yet a subset remain unresponsive to lipid-lowering therapies and require LDL apheresis treatment. This study examined the genotype/phenotype relationship in patients with dyslipidemia undergoing routine LDL apheresis and characterized the lipoproteins via novel methods. *LDLR*, *APOB*, and *PCKS9* genes were analyzed in 7 LDL apheresis patients by: (1) sequencing the promoter, all 18 exons and exon-intron flanking regions of *LDLR*; (2) multiplex ligation-dependent probe analysis (MLPA) of *LDLR* for large rearrangements; (3) sequencing all 12 exons and exon-intron flanking

regions of *PCSK9*; and (4) targeted fluorescent bead-based analysis for p.R3500W and p.R3500Q in *APOB*. Plasma and serum specimens were collected pre- and postapheresis, subjected to lipid fractionation, and analyzed with CDC-certified lipid assays. Lp(a) cholesterol was performed via electrophoresis. Lipoprotein particle concentrations (HDL-P, LDL-P, VLDL-P, IDL-P, total and subclasses) were analyzed by NMR (Bruker-Biospin, 400MHz). Four patients harbor *LDLR* mutations and no mutations were found in *APOB* or *PCSK9* genes. Three patients presented with xanthoma or arcus cornea, all of whom had *LDLR* mutations. Similar reductions in lipoproteins were observed following apheresis in patients with and without mutations: LDL-C (46% vs 57%), apolipoprotein B (61% vs 64%), LDL-P (64% vs. 58%), as well as Lp(a) cholesterol in 2 patients with detectable concentrations. A significant difference in small LDL-P reduction was noted between the 2 groups (69% vs. 53%, $P < .01$). Apheresis did not significantly alter HDL-C, HDL-P, or apolipoprotein A concentrations. The *LDLR* mutation cohort had a more proatherogenic profile preapheresis compared to those without genetic mutations: LDL-C (238 vs 138 mg/dL), apolipoprotein B (191 vs 120 mg/dL), LDL-P (3,268 vs 1,739 nmol/L), small LDL-P (2,806 vs 1,303 nmol/L). Those without mutations had smaller HDL-P (28.1 vs 17.8 μmol/L, $P < .01$). A majority of patients also had elevated triglycerides (mean = 282 mg/dL), suggestive of the familial combined hyperlipidemia (FCH) phenotype. LDL apheresis effectively removes apolipoprotein B-containing particles, including Lp(a), regardless of the cause of dyslipidemia. Individuals with *LDLR* mutations tended to present with lipid deposits and had a significantly greater number of proatherogenic lipoproteins including LDL-P, small LDL-P, and apolipoprotein B.

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Optimum Diagnostic Methods for Evaluating for Circulating Neoplastic T-Lymphocytes in Patients With Cutaneous T Cell Lymphoma

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We routinely review peripheral blood smears (PBS) from patients with cutaneous T-cell lymphoma (CTCL) for involvement by circulating neoplastic T lymphocytes (CNTL), with additional testing by multicolor flow cytometry (FC) and T-cell receptor gene rearrangement studies by PCR (TPCR) less frequently ordered by clinicians. The objective of this study is to assess the utilization of these newer diagnostic tests and potential increased sensitivity over morphology alone. PBS results from April 2009 to February 2012 were gathered from pathology data records using free text key words in the clinical history and the last name of the 2 primary dermatologists ordering these evaluations. Concurrent FC and TPCR results, if available, were also obtained from the pathology data records. 43 PBSs ordered for evaluation for the presence of CNTL, representing 35 patients, were obtained from the pathology data records. Diagnostic testing practices were heterogeneous, with concurrent FC ordered on 51.1% of cases, concurrent TPCR on 13.9%, and 13.9% of cases with all 3 modalities; TPCR was never ordered in isolation and was always ordered with concurrent FC. PBS morphology was positive for Sézary cells in 18.6% of cases, FC was positive for an abnormal T-lymphocyte population in 11.6% of cases, and TPCR was positive in 2.3% of cases. In cases where the PBS was considered "suspicious" for involvement (25.5% of all cases), FC was ordered 54.5% of the time, with negative FC results in 45.4% of suspicious cases and positive FC results in 9.1% of suspicious cases. When peripheral blood involvement was positively identified by PBS, the results were confirmed by FC in 37.5% of cases, were refuted in 12.5% of cases, and FC was not ordered in the remaining 50% of cases. FC increased the clinical stage (either by increasing the count to >1,000 atypical T-lymphocytes/L, or a positive flow result following a negative PBS) in 18.8% of cases in which concurrent FC was ordered. Test ordering practices by dermatologists at our institution for diagnostic assessment for CNTL in patients with CTCL is heterogeneous. In cases suspicious by morphology, FC was only ordered half of the time. FC would appear to serve an important role in staging patients by increasing, decreasing, or confirming clinical stage when ordered in addition to PBS morphology, a method which is based on subjective morphologic findings and has significant inter-observer variability. While this is a limited retrospective study, the data suggest that FC may be a more useful and objective method to evaluate for CNTL.

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CD14 in 8-Color Flow Cytometry Distinguishes Monocytes From Rare Cells of Large B-Cell Lymphoma and Hairy Cell Leukemia

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Multicolor flow cytometry has become an important tool to evaluate blood, marrow, and lymph nodes for clonal B cells. With traditional gating strategies of CD45 vs side scatter, large B cells and hairy cells may be located within or near gates for monocytes that have high background staining for CD20 and κ and λ immunoglobulin light chains. Since large B cells may be partially lost during tissue processing and since hairy cells may be infrequent when aspirated from a fibrotic marrow, there may be difficulty finding the infrequent-to-rare clonal B cells intermingled among numerous monocytes. We hypothesized that adding CD14 to an 8-color flow cytometry panel would facilitate discriminating monocytes from rare clonal B cells of large B-cell lymphoma and hairy cell leukemia. We created an 8-color antibody panel: λ FITC, κ PE, CD14 PerCP, CD5 PE-Cy7, CD10 APC, CD20 APC-H7, CD19 V450, and CD45 V500. Gating strategies included removing air bubbles (light scatter versus time), debris (forward versus side scatter), cell doublets (light scatter height vs width), granulocytes (CD45 vs side scatter), and monocytes (CD14 versus side scatter). CD19- and CD20-positive B cells were then isolated, and clonality was assessed in subsets based on cell size (forward scatter) and immunofluorescence intensity for various antigens: CD5, CD10, CD19, CD20, and CD45. This panel has been used on thousands of clinical specimens. We identified a series of cases of large B-cell lymphoma and hairy cell leukemia where this strategy was particularly effective in finding rare clonal B cells. Addition of CD14 to the 8-color B-cell panel enhanced our ability to distinguish monocytes from infrequent-to-rare clonal B cells in large B-cell lymphoma and hairy cell leukemia where the clonal B cells overlap with monocytes in traditional gating strategies and where the neoplastic cells may be partially lost during tissue processing or difficult to aspirate from a fibrotic marrow.

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Optimum Diagnostic Methods for Monitoring Minimal Residual Disease in Bone Marrow Biopsies Collected From Myeloma Patients

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Multiple myeloma (MM) is a disease characterized by neoplastic plasma cells, an M-spike, lytic lesions, and other end-organ damage. New innovations in treatment, including marrow transplant, have improved patient survival and increased test volumes. Our institution is a large tertiary center that evaluates hundreds of marrow biopsies from MM patients each year. Numerous tests are performed on serum and urine, predominantly protein studies, and multiple tests are performed on the marrow, including morphology, flow cytometry, immunohistochemistry (IHC), and cytogenetics. As the goal of MM patient treatment is often to control disease rather than cure, our objective was to identify the most effective and practical strategy to evaluate MM patients for minimal residual disease (MRD). We retrospectively reviewed 96 marrow biopsies and data corresponding to 34 patients at various stages in their treatment, including after autologous marrow transplant. In a majority of patients, serum and urine studies, including electrophoresis, immunofixation, and free light chain were performed a week or less prior to biopsy. Biopsies were analyzed by 8 color flow cytometry, morphology, and IHC. Of the 96 patient biopsies reviewed, MRD was present in 79.1% by any modality. Protein studies were positive in 73.9%, IHC in 42.7%, and flow cytometry in 37.5%. Of those showing MRD, the most likely positive tests to rule-in MRD were protein studies by SPEP/UPEP/IFIX, which was positive in 97.2% of cases. IHC of the trephine biopsy was positive for MRD in 53.9% of cases. Lastly, 8 color flow cytometry identified an abnormal monotypic plasma cell population in 47.3% of cases. In only 3 instances was MRD identified by marrow biopsy in the absence of a detectable M-spike, including 2 cases by IHC and 1 case by flow cytometry. There are numerous tests that can be performed to assess for clonal plasma cell populations in MM patients. As new technologies have been developed, including IHC, flow cytometry, and free light chain assays, the number of tests ordered has increased each year. However, in a majority of situations at our institution, the presence of an M-spike is known within a week prior to biopsy. While this is a limited study, our initial data support that new management strategies are necessary for triaging ancillary testing of MM patients after diagnosis has been established and treatment commenced. We will present a novel algorithm to guide clinicians and pathologists with stepwise testing and discuss a prospective study at our institution to confirm our retrospective findings.

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Clonal Vbeta by Flow Cytometry in CD10-Positive T Cells of Angioimmunoblastic T-Cell Lymphoma

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Angioimmunoblastic T-cell lymphoma (AITL) is a mature T-cell lymphoma with unique lymph node histology including arborizing high endothelial venules, increased follicular dendritic cell meshworks, and a polymorphous cellular infiltrate of EBV-positive B immunoblasts and neoplastic, CD4-positive, follicular helper T cells (FHT). Early disease with floridly reactive germinal centers may be difficult to diagnose, as the neoplastic T cells represent a minor population. By flow cytometry, the neoplastic T cells in AITL often have an aberrant immunophenotype including coexpression of CD10, although weak CD10 may be seen, also, in reactive follicular helper T cells. An aberrant immunophenotype by flow cytometry is often associated with clonality assessed by other methods including Southern blot or PCR for T-cell receptor gene rearrangements. Analysis of T-cell receptor beta chain (Vbeta) by flow cytometry (FCVbeta) has been previously described to document clonality among atypical T cells in blood. In this study, we hypothesized that FCVbeta would be an accurate, rapid, and easy method to demonstrate clonality among CD10-positive T cells in AITL. We identified 4 cases of AITL with abnormal T cells characterized by flow cytometry in our laboratory over the last 3 years. These 4 cases were from blood, bone marrow, or lymph node. Each case was analyzed by flow cytometry for T-cell antigens, for CD10, and for 24 variable regions of the T-cell receptor beta chain (FCVbeta). Concurrent morphology and ancillary studies were also performed. In all 4 cases (100%) flow cytometry detected aberrant, CD10-positive, T-cells. In 2 of these cases, the CD10-positive T cells were restricted for Vbeta 8, and in the remaining 2 cases, the CD10-positive T cells lacked the alpha-beta T-cell receptor. While this is a limited series of a rare lymphoma, our experience demonstrates that FCVbeta is an accurate, relatively easy, and novel method to demonstrate the clonality of CD10-postive T cells in AITL. These results provide further evidence that the CD10-positive T cells in AITL are the clonal neoplastic cells.

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Identification of Models to Predict Early Sepsis in Medical ICU Patients

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Sepsis is a life-threatening condition characterized by systemic inflammatory response syndrome (SIRS) with a documented infection. Rapid diagnosis and initiation of early goal directed therapy significantly reduces mortality. Identification of patients early in the sepsis pathobiological process is difficult because clinical symptoms overlap with SIRS. No single biochemical or clinical marker can accurately identify early sepsis. A retrospective cohort study was conducted to identify sepsis prediction models which combine inflammatory biomarkers, demographics, and routine lab values from Medical ICU patients in early stages of sepsis. Residual plasma was collected from 202 patients up to 2 days prior (days –1 and –2) and on the day that SIRS criteria were met (day 0). TNFα, IL-6, IL-10, CRP, and LBP were quantitated on the Siemens Immulite (Siemens Healthcare Diagnostics Inc). Demographics and routine laboratory values were obtained from patient medical records. Diagnoses were adjudicated by 2 ICU physicians as follows: SIRS (n = 109), sepsis (n = 93; severity: sepsis, 11; severe sepsis, 42; and septic shock, 39). ROC analysis was used to generate areas under the curve (AUC) and 1-way ANOVA was used to compare sepsis severities. Sepsis prediction models were determined by multivariable logistical regression analysis and their predictive ability was evaluated by Akaike's information criterion (AIC). On days –1 and 0, concentrations of all 5 inflammatory biomarkers, individually, were significantly ($P < .001$) different among patients with SIRS and septic shock. CRP was the best single predictor of sepsis (all severities) on day –2 (n = 61, 48% septic), day –1 (n = 173, 46% septic) and day 0 (n = 182, 45% septic); AUC and LR+ were 0.83 and 23.8, 0.73 and 28.8, and 0.76 and 35.7 for days –2, –1 and 0, respectively. A model including CRP, LPB, IL-6, patient origin, and glucose from day –2 best predicted sepsis

within 7 days (AUC = 0.92, LR+ = 32.8, AIC = 22.8, $P < .001$). On day –1, a panel of CRP, IL-6, and temperature best predicted patients who would develop sepsis in up to 6 days (AUC = 0.78, LR+ = 48.8, AIC = 42.8, $P < .001$). On the day of SIRS, the best model to predict sepsis within 5 days consisted of CRP, WBC, platelets, temperature, systolic BP, and glucose (AUC = 0.88, LR+ = 48.8, AIC = 75.9). Logistical regression models consisting of plasma inflammatory biomarker concentrations, demographics, and laboratory values can accurately predict sepsis in the early stages of the pathobiological process. Utilization of such sepsis prediction models may allow for earlier implementation of goal-directed therapy and reduce sepsis related mortality among ICU patients.

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Limitations of Lipemic Interference Studies as Illustrated With the Roche Elecsys Parathyroid Hormone Immunoassay

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We report a case of a 51-year-old female patient referred for endocrine consultation for hypertriglyceridemia and possible vitamin D deficiency. Initial laboratory values were: triglycerides (TG) of 4,100 mg/dL, total Ca 9.2 mg/dL, PTH of 30 pg/mL, and vitamin D of 9.0 ng/mL. Due to the extremely high TG, rechecks were performed on an ultracentrifuged plasma, which showed an elevated PTH of 173 pg/mL, suggesting TG as a potential interferent. Serial dilution of this patient's lipemic sample showed a recovery of 157 pg/mL from a 20-fold diluted specimen. The Roche Elecsys PTH assay is a 2-site, electrochemiluminescent immunoassay and the package insert indicates that no lipemic interference is observed at "<1,500 mg/dL Intralipid." We found this information is not actionable because "intralipid unit" is imputed from the labeled stock concentration (eg, 20 g%); hence is not quantifiable, nor is it the same as analyzer-determined parameters like lipemic index or TG (mg/dL), measured on the Roche modular analyzer. Therefore, we conducted extensive lipemic interference studies using lipids derived from intralipid and a high TG patient pool, respectively. We observed no analytically significant interferences using Intralipid up to 4,000 mg/dL of TG for the low (20 pg/mL) PTH pool, while a 15% decrease in the high (350 pg/mL) PTH pool was observed. The correlation equation between TG and lipemic index for this intralipid-spiked set was: [TG] = 2.4 [L-Index] + 52 ($r^2 = 0.99$). In contrast, using patient-derived lipids, a positive bias of 15% was observed in high PTH (327 pg/mL) pool at TG between 1,200-4,300 mg/dL; the correlation equation was [TG] = 4.5 [L-Index] + 66 ($r^2 = 0.99$). Since these interference studies showed little-to-no significant interferences up to 4,000 mg/dL TG, while this patient showed significant negative bias at TG > 400 mg/dL, we conclude that lipemia per se is unlikely the cause of the falsely decreased PTH value in this patient. Our results are consistent with the hypothesis that an unknown interfering substance that is associated with the lipid phase caused the under-recovery of the PTH value in this patient. This case highlights the current, nonactionable information regarding the limitations of lipemic interferences found in many FDA-approved clinical assays. Since these studies are typically performed using manufactured lipids derived from Intralipid, the recommendations may not be translatable to an individual lipemic patient sample. In this patient, removing the lipids by ultracentrifugation prevented the misdiagnosis of the patient, where an actual elevated PTH level is consistent with the clinical suspicion of secondary hyperparathyroidism.

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Concomitant Deletions of *IKZF1, CDKN2A,* and *CDKN2B* in Pediatric B-Acute Lymphoblastic Leukemia

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Pediatric B-acute lymphoblastic leukemia (B-ALL) generally carries a favorable prognosis with cure rates of approximately 80%. However, pediatric B-ALL with normal cytogenetics represents an intermediate risk group that presents a challenge for clinical management. Deletion of the *IKZF1* (IKAROS) gene, present in approximately 10% of pediatric B-ALL with normal cytogenetics, is an independent predictor of leukemic relapse after adjusting for age, leukocyte count at presentation and presence of minimal residual disease. Several authors have proposed that *IKZF1* abnormalities, in and of themselves, are insufficient to produce leukemogenesis. They suggest that the development of a leukemic phenotype in B-cells may require simultaneous abnormalities of genes involved in pathways involved in B-lymphocyte development (including *IKZF1*) and cell-cycle regulation (eg, *CDKN2A, CDKN2B*). *CDKN2A* and *CDKN2B* abnormalities are known to occur in approximately 1% to 20% of B-ALL. Though *CDKN2A*, *CDKN2B,* and *IKZF1* deletions have been independently described in pediatric B-ALL, the prevalence of concomitant deletions of these genes in pediatric B-ALL has not been examined. We evaluated the prevalence of concomitant deletions of *CDKN2A* and *CDKN2B* among 9 cases of karyotypically normal, pediatric B-ALL with *IKZF1* deletions using a commercially available multiplex ligation probe assay (MLPA) kit (MRC, Holland). Six cases had concomitant deletions of *CDKN2A* (67%) and 5 cases had deletions of *CDKN2B* (56%). Interestingly, 5 cases with *CDKN2A* deletions also had deletions of *CDKN2B*. This preliminary study suggests that concomitant deletions of *IKZF1*, *CDKN2A* and *CDKN2B* are a common occurrence in this subgroup of patients with B-ALL. Given the loss of tumor-suppressor activity of *CDKN2A* and *CDKN2B*, the pathological and clinical implications of these findings warrant further studies.

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How Does Donor's Sex, Age, Weight and G-CSF Dose Affect the Yield of Peripheral Hematopoietic Progenitor Cells?

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Many allogeneic donors enrolled in the National Marrow Donation Program (NMDP) are donating hemopoietic progenitor cells (HPC) from peripheral blood after mobilization with granulocyte colony-stimulating factor (G-CSF). Filgrastim is commonly used in HPC mobilization and the dose varies in different programs. In our institution, we use 10 μg/kg of filgrastim but round up to a full dose of 600 μg, 780 μg, 900 μg, etc. To determine whether donor factors have an impact on the yield of HPC, a retrospective study was performed with 60 healthy HPC donors aged 19 to 59 years (34 M and 26 F) and 10 autologous donors aged 19 to 65 years (1 M and 9 F). There was a strong correlation between the number of CD34+ cells immediately prior to leukocyte apheresis (pre-CD34) and final products collected in both allogenic healthy donors ($P < .001$; $r = 0.84$) and autologous donors ($P < .01$; $r = 0.95$). Thus, we used pre-CD34 as an indicator to examine which factors were associated with

good HPC yield in healthy donors. First, we found that the female donors had a lower pre-CD34 than that of male donors (51.5 ± 5.4/μL vs 84 ± 10.2/μL; $P < .01$). Younger female donors (<20 y/o) had the lowest pre-CD34 (40.7 ± 5.3/μL). Next we divided the donors into 3 groups based on their pre-CD34: excellent (pre-CD34 > 107/μL), good (38-107/μL), and poor (<38/μL).The donors with excellent yield of pre-CD34 had higher body weight (average 96.5 kg vs 78 kg in good and 71 kg in poor producer groups; $P < .01$). The high pre-CD34 yield is not just due to high blood volume in this group because their pre-CD34 was also the highest after adjusted by weight (1.7/μL/kg vs 0.8/μL/kg in good and 0.4/μL/kg in poor producers; $P < .001$). Interestingly, donors with lower weight (<83kg) received higher dose of filgrastim (11.4 μg/kg) than that of donors with weight of 83-110kg (10.7 μg/kg) or >110 kg (8.8 μg/kg) ($P < .01$). These findings indicate that donors with higher body weight respond to filgrastim mobilization better and have high yield of HSCs. The dose of filgrastim could be decreased to avoid unnecessary side effects since the total dose of filgrastim is also high. Donors with lower body weight may require higher doses of filgrastim in order to obtain an appropriate number of HSCs.

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FilmArray RP vs xTAG RVP for the Detection of Respiratory Viruses: A Comparative Analysis

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Rapid diagnostic detection of respiratory pathogens is useful for differentiation of bacterial vs viral infections, and implementation of an accurate treatment plan. Molecular methods for diagnosing respiratory pathogens have been recognized as a preferred methodology. Recently, multiple new testing methodologies have been approved for respiratory viral testing, yet few reports are available on the FilmArray RP. The aim of our study was to perform a comparative analysis of 2 commonly utilized molecular methodologies: FilmArray RP (automated nested multiplex PCR) versus xTAG RVP (luminex technology). A total of 48 frozen patient samples that had previously been tested using xTAG RVP were tested using FilmArray RP, as well as 21 ZeptoMetrix respiratory panel samples. The patient samples consisted of 37 nasopharyngeal (NP) swabs and 11 bronchoalveolar lavage (BAL) specimens. Our results revealed an overall concordance between platforms of 93.8%. Only 3 NP samples yielded discordant results (2 adenovirus and 1 influenza type A were not detected by FilmArray RP). The rate of concordance in the BAL samples and ZeptoMetrix was 100%. Our conclusion is that FilmArray RP methodology is rapid and less labor intensive, needing approximately 10 minutes hands-on time and yielding results in just over 1 hour. However, a drawback of the FilmArray is that 1 sample can be processed at a time. In contrast, the xTAG RVP methodology involves 3 hours hands-on time and yields results in 6 hours, although samples can be batched together. In summary, the FilmArray RP testing method is a quick, user-friendly method of pathogen recognition and presents a favorable alternative to the xTAG RVP, especially during non–flu season, when batching of samples will significantly increase turnaround time.

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Validation of a Diagnostic Algorithm for Improved Assessment of Apolipoprotein B Dyslipoproteinemias Compared to Classical Fredrickson Phenotyping

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Conventional approaches to lipid screening focus on quantitation of plasma lipids and are largely ineffective at identifying and characterizing the major apolipoprotein B (apoB) dyslipoproteinemias, many of which are genetic in origin. Fredrickson phenotyping historically utilized and defined 6 dyslipidemic phenotypes, all of which involve apoB-containing particles. Method complexities prevent use in the routine clinical laboratory. This study validated an algorithm for diagnosis of all 6 Fredrickson phenotypes and apoB dyslipoproteinemias using only plasma apoB, triglycerides (TG) and total cholesterol (TC). Retrospective data analysis was performed of adult lipoprotein metabolism profiles, which included analysis of TC, TG, beta-quant LDL-C, HDL-C, VLDL-C, VLDL-TG, beta-VLDL-C, beta-VLDL-TG, apoB, chylomicron-C, chylomicron-TG, Lp(a)-C, IDL, and LpX. Standard reference assays including ultracentrifugation, lipid precipitation, fractionation, and electrophoresis were used for assigning Fredrickson phenotypes. Interpretations from 9,153 unique subjects were compared with the apoB/TC/TG diagnostic algorithm. Profiles consisted of 4,824 males and 4,335 females with a median age of 54 years (range 18-98). A total of 6,320 patients (68.6%) had normal apoB (<120 g/dL) and normal TG (<133 mg/dL) based on the algorithm; concordance with the phenotyping was 99.6%. Normal apoB with increased TG due to increased VLDL (TG:apoB<10 and TC:apoB<6.2) was observed in 1,752 patients (19%), corresponding to a Type IV phenotype (familial hypertriglyceridemia); concordance was 44.1%. Normal apoB with increased chylomicrons and VLDL remnants (TG:apoB<10 and TC:apoB>6.2; Type III/familial dysbetalipoproteinemia) was noted in 105 patients (1.1%) with only 10.8% concordance. HyperapoB-hyperTG was observed in 387 patients (3.1%) due to increased VLDL and LDL (Type IIb/familial combined hypercholesterolemia) while 183 patients (1.99%) had hyperapoB-normoTG due to increased LDL (Type IIa/familial hypercholesterolemia), each having 21.8% and 35.7% concordance, respectively. There were 528 (5.7%) patients with hyperchylomicronemia and VLDL (Type V, 87.3% concordance) and 21 (0.23%) patients with only hyperchylomicronemia (Type I/lipoprotein lipase or apoC-II deficiency, 76.5% concordance). LpX prevented assignment of a phenotype in 14 subjects. Accurate identification and diagnosis of a specific apoB dyslipoproteinemia helps define the appropriate treatment modality, assessment of cardiovascular risk, and overall need for lipid-lowering therapies. A significant amount of discordance was observed between Fredrickson phenotyping and the algorithm, suggesting further refinement is needed in the algorithm to account for hypertriglyceridemia due to elevated VLDL and remnant chylomicrons.

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Large Granular Lymphocytic Leukemia: A Flow Cytometry Immunophenotypic Analysis of 77 Cases With a Correlation to TCR Clonality Study

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The diagnosis of T-cell large granular lymphocytic leukemia (T-LGL) is challenging due to its indistinct border with reactive conditions. Detection of an aberrant immunophenotype and/or T-cell monoclonality are considered useful, but it is unclear how phenotype

and genotype correlate. We reviewed the immunophenotype from 82 patients with cytopenia and LGL expansions assessed by flow cytometry using bone marrow aspirate during 1/2008-11/2011, and correlated the findings with TCRγ/TCRβ clonality studies by PCR and other laboratory parameters (ANC, ALC, WBC, Hb, Plt). The panel of antibodies included CD2, CD3, CD4, CD5, CD7, CD8, CD16, CD56, CD57, CD94, TCR-αβ, and TCR-γδ. Cases with monoclonal TCR gene rearrangement (group 1) were compared to those with a polyclonal/oligoclonal pattern (group 2). The threshold for aberrancy is set at ≥20% of cells for each marker. TCR gene rearrangement was monoclonal in 62 cases (group 1); oligoclonal or polyclonal in 15 cases (group 2). Twenty-two (29%) monoclonal cases displayed aberrant expression in 1 marker (loss or diminished CD5, CD2, or CD7) and 19 (24%) cases in 2 markers (CD5, CD2, or CD7). Four polyclonal/oligoclonal cases showed aberrant expression. Loss or diminished CD5 expression was significantly associated with monoclonal TCR (29/46 vs 1/7; $P = .034$) and more profound neutropenia [median ANC 0.9 (0.02-6.36) $\times 10^9$/L vs 1.715 (0.02 - 8.21) $\times 10^9$/L; $P = .0329$]. In contrast, loss or diminished CD2 or CD7 expression (12/50 vs 1/11, $P = .429$; 19/56 vs 3/11, $P = .364$, respectively) were equally seen in both group 1 and group 2. CD94 overexpression is also correlated with monoclonal TCR (33/56 vs 3/13, $P = .0299$) gene rearrangement. In conclusion, loss or diminished CD5 and CD94 overexpression can effectively identify LGL leukemia patients. Lack of demonstrable monoclonal TCR gene arrangement does not exclude the diagnosis of T-LGL.

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Implementation of a Quantitative hCG Assay to Screen for Pregnancy Adversely Affected the Predictive Value of the Screen

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Rapid testing of human chorionic gonadotropin (hCG) in serum is frequently desired in healthcare settings to identify a possible pregnancy and allow for clinical decisions in favor of protecting a fetus. While the gold standard for pregnancy testing is the serum quantitative hCG test, it has a longer turnaround time (TAT) than most of the point-of-care qualitative serum or urine tests. In order to provide more rapid TAT, some laboratories offer a serum qualitative assay in addition to the serum quantitative test. However, the qualitative assays are less sensitive for diagnosing a pregnancy compared to serum quantitative methods. Additionally, we determined that using automation and auto-verification, our TAT for negative hCG serum quantitative tests was similar to the TAT for our qualitative manual method. Positives were reported in about twice the amount of time (limited AMR and dilution). In July 2011, we eliminated the serum qualitative Wampole Clearview hCG point-of-care test kit in favor of the more sensitive serum total β-hCG on the Beckman DxI, and utilized qualitative reporting to maintain the rapid TAT (ie, did not dilute the positives). The quantitative screen is performed by measurement of β-hCG but is reported only as negative (<5 mIU/mL) or positive (≥5 mIU/mL). When we compared data from the 6 months prior to the switch (Jan-Jun 2011) with the 6 months after (Jul-Dec 2011), we found that the percentage of positive results increased from a mean of 4% to 12%. Upon chart review of patients that had tested positive (n = 136), we found that our false-positive rate increased from 20% to 50% and positive predictive value decreased from 85% to 48%. Positive qualitative screens that also had quantitative β-hCG measurements (n = 23) had a 9% false-positive rate based on the quantitative result. Positive screens that also had quantitative β-hCG measurements ordered (n = 72) had a 28% false-positive rate upon retesting. However, an additional 26% were true positives below the detection limit of the qualitative Wampole kit, which suggests that these pregnancies may have gone undetected using the previous method. These data indicate that while the quantitative assay is more sensitive, it is not as specific for diagnosing pregnancy as the qualitative test and a dramatic increase in the false-positive rate appears to be leading to increased costs of confirmatory testing (including ultrasounds in some cases).

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Racial and Gender Disparities in cTnI Testing: Findings From Two Hospitals

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Acute myocardial infarction (MI) is a major cause of death worldwide. Cardiac troponins I and T (cTnI and cTnT) are established as preferred biomarkers for early diagnosis of MI in patients with a recent onset of chest pain. The guidelines recommend at least 2 serial measurement of cTnI or cTnT within the first 24 hours after onset of symptoms of myocardial necrosis. The specific objective of this study was to determine whether there were racial and gender disparities in cTnI testing at 2 Atlanta hospitals (*A* and *B*). We performed a retrospective analysis of the data obtained for all patients tested for cTnI for a period of 10 years from 2002 to 2011 for both hospitals: 116,259 patients from hospital *A*, and 75,535 patients from hospital *B*. All analyses were performed with SAS version 9.2 (SAS Institute) for the following groups: blacks (*B*), whites (*W*), females (*F*), males (*M*), black females (*BF*), black males (*BM*), white females (*WF*), and white males (*WM*). On average, each patient had 2.09 cTnI tests done per admission in both hospitals in the span of 10 years. Interestingly, in 2009 there was an increase of cTnI tests per admission in both hospitals: 2.27 in *A* and 2.19 in *B*. In 2011, in hospital *A,* cTnI measurements per admission were the following: *B* – 1.97; *W* – 1.99; *F* – 1.89; *M* – 2.08; *BF* – 1.89; *BM* – 2.09; *WF* – 1.89; *WM* – 2.07. In 2011, in hospital *B,* measurements per admission were the following: *B* – 2; *W* – 2.25; *F* – 2.02; *M* – 2.15; *BF* – 1.99; *BM* – 2.09; *WF* – 2.17; *WM* – 2.3. In 2011, in hospital *A*, for patients tested positive on cTnI upon admission the percentage of patients receiving subsequent cTnI tests (≥2 tests) was the following: *B* – 15.4%; *W* – 16.5%; *F* – 13.4%; *M* – 18.9%; *BF* – 12.2%; *BM* – 20.4%; *WF* – 14.8%; *WM* – 18%. In 2011, in hospital *B*, for patients tested positive upon admission the percent of patients receiving subsequent cTnI tests (≥2 tests) was the following: *B* – 17.6%; *W* – 20.2%; *F* – 15.5%; *M* – 21.3%; *BF* – 14.7%; *BM* – 21.5%; *WF* – 19.2%; *WM* – 20.8%. Our study demonstrates both racial and gender discrepancies among patients receiving cTnI testing in these 2 hospitals. Significantly less cTnI testing was done for females compared to males ($P < .0001$), and for blacks compared to whites ($P < .005$). The same trend continues for same groups of patients tested cTnI positive upon admission. A multicenter study is being conducted in order to include additional data from other hospitals located in various geographic regions of the country in an effort to reduce the selection biases present in this initial retrospective analysis.

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Cathinone-Induced Cardiac Injury and Mortality in Patients Presenting to an Emergency Department

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Coronary artery disease is the leading cause of death worldwide and most patients die from complications of atherosclerosis. The objective of this study was to determine the association between the levels of biochemical markers and the risk of inducing myocardial infarction (MI) among khat chewers and nonchewers. 152 patients with myocardial infarction (MI) admitted to cardiac and intensive care units (CCU/ICU) all are in Al-Thawora Hospital, the largest academic hospital in the Yemen. History of chewing khat, hypertension, diabetes mellitus, hyperlipidemia and cigarette smoking, demographic characteristics were recorded, blood samples were drawn from each patient for cardiac enzymes and other markers. A total of 75% of all admitted patients to the emergency room were khat chewers, 25% were non–khat chewers. ST-elevation (STEMI) with higher troponine, CK, CK.MB than non-ST elevation (NSTEMI) (1.52 ± 1.00 vs 0.87 ± 0.57, $P < .0001$), (1900 [IQR: 1254.24-314.75] vs 870 [IQR: 539-1901.4], $P< .0001$), (212.8 [IQR: 120.25-338.5] vs 112.5 [IQR: 73.45-199.75], $P < .0001$), respectively. Mortality rate represents 5% and was higher among STEMI than NSTEMI (6% vs 4%). Mortality was twice higher among khat chewers as compared to non–khat chewers (6.1% vs 2.7%) respectively. Cardiac complications were more frequent among khat chewers (complete heart block [CHB], acute mitral regurgitation [AMR], reinfarction, ventricular fibrillation [VF], heart failure [HF]), whereas cardiogenic shock and atrial fibrillation (AF) were more frequent among non–khat chewers. The findings of this study show that khat chewers are more than 2-fold likely to develop cardiac complications than non–khat chewers. Therefore, chewing khat could be a risk factor for the development of MI with subsequent worsening health outcome and mortality.

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Laboratory-Based Guidance Toward Successful Therapy of Plastic Bronchitis

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Plastic bronchitis is a rare condition characterized by the production of large, rubbery, branching bronchial casts composed of inflammatory cells and proteinaceous material that can lead to extensive airway obstruction. Our plastic bronchitis patient was a 10-year-old boy with an unremarkable past medical history, who presented every 1-2 months in severe respiratory distress despite conventional supportive therapy and recurrent emergent bronchoscopies for cast removal. The presence of fibrin/fibrinogen in samples of the patient's casts was demonstrated by immunofluorescence microscopy and by staining formalin-fixed paraffin-embedded tissue with phosphotungstic acid hematoxylin (PTAH), and the presence of mucin was confirmed with periodic acid-Schiff (PAS) staining. The present study was undertaken to assess the potential utility of delivering a fibrinolytic agent to the patient's airway. Samples of the casts were incubated in vitro at 37°C for 0, 60, and 120 minutes in 1 mg/mL of tissue plasminogen activator (tPA), then weighed and measured. Casts were then incubated for 16 hours at 37°C with concentrations of tPA ranging from 0 to 1.0 mg/mL and 200 mg/mL of N-acetylcysteine, a mucolytic. Following incubation in 1.0 or 0.5 mg/mL of tPA at 0, 60, and 120 minutes, casts were fixed, embedded, and stained with PTAH. Fibrin degradation products in the supernatant were measured at each time point. Casts demonstrated a decrease in size and weight following incubation with tPA after 2 hours, and the effect of tPA on the casts was dose-dependent. The effect on the casts of tPA was increased with the addition of the mucolytic. PTAH staining following tPA digestion showed a decrease in the amount of fibrin within the casts. The supernatants also showed an increase in the amount of fibrin degradation products at each time point. Rare case reports in the literature describe the successful use of aerosolized tPA to treat severe cases, although the mechanism of action of tPA on the bronchial casts has not been investigated. Aerosolized tPA was administered to our patient (5 mg every 12 hours) and, as anticipated from in vitro studies, there was significant improvement of symptoms. Improvement was initially noted after 48 hours, following which the patient has subsequently received tPA treatment for 6 months. During this time, the patient has not required bronchoscopy for cast removal or admission to the intensive care unit due to airway obstruction. In summary, through in vitro studies on a naturally generated bronchial cast, we have developed a laboratory-based rationale supporting the local administration of fibrinolytic agents in the treatment of plastic bronchitis.

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Evaluation of Interference in Siemens ACMIA Tacrolimus Immunoassay

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Tacrolimus is an immunosuppressant with a narrow therapeutic window with an upper therapeutic range of 15 ng/mL. Affinity column mediated immunoassay (ACMIA) has been proposed as a less labor-intensive alternative to the microparticle enzyme immunoassay (MEIA). Tacrolimus activity is localized within red blood cells. The Abbott MEIA uses centrifuged and subsequently lysed red blood cells. In contrast, the ACMIA uses whole blood samples. There have been several reports of interference substances in plasma, such as heterophile antibodies, resulting in artifactual elevations using the ACMIA. This study investigated a potential interference in the ACMIA Siemens Dimension Xpand for measuring tacrolimus concentration in whole blood and to develop an algorithm for detecting inappropriately elevated results. ACMIA (Xpand) and MEIA (Abbott IMx) methods were used to assay tacrolimus in 563 consecutive whole blood samples. Plasma fraction tacrolimus concentration was analyzed by ACMIA on samples when the ACMIA result was ≥2 ng/mL than MEIA. If the plasma tacrolimus activity was ≥ 30% of the original tacrolimus concentration, the original whole blood tacrolimus activity was reported. A Deming regression analysis yielded an equation of ACMIA = 0.936(MEIA) – 1.39 ($r = 0.91$), demonstrating a strong correlation between ACMIA and MEIA. However, apparent interferences were observed in five of 563 cases (0.9%). The mean values for tacrolimus activity on ACMIA and MEIA were 18.9 ng/mL and 7.1 ng/mL, respectively. We developed a clinical algorithm for detecting these interferences. Every sample with a tacrolimus concentration ≥15.0 ng/mL by ACMIA was centrifuged, and the plasma concentration was analyzed by ACMIA. If plasma tacrolimus activity was <30% of the whole blood concentration, the original result was reported. If plasma tacrolimus concentration was ≥30% of the original measurement, the sample was also assayed by MEIA. After implementing this algorithm, 3 of 227 (1.3%) exhibited interference. The average tacrolimus concentrations

for these samples assayed on ACMIA and MEIA were 29.3 ng/mL and 3.9 ng/mL, respectively. Thus, this protocol prevented the report of false critical high concentrations. Implementation of an algorithm to screen high plasma tacrolimus concentrations measured on the Siemens ACMIA can mitigate potential interferences.

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Preanalytical Considerations for BK Virus Quantitation: Impact of Storage Temperature on Viral Titer

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The quantification of BK virus is important in renal transplant patients. A high viral load in the urine often precedes viremia, which places the patient at an increased risk of polyomavirus-associated nephropathy (PVAN). Physicians therefore monitor serial measurements of BKV DNA in the management of their kidney transplant patients. To provide the most accurate and precise results from our BK DNA quantitative assay, we evaluated the effect of preanalytical storage temperatures on BK viral load. We stored aliquots from clinical urine samples at the following temperatures for 48 hours before processing them for the quantitative polymerase chain reaction (PCR): room temperature (RT), +4°C, and –20°C. We additionally processed an aliquot in a manner identical to our original clinical specimen (the "immediate process" [IP] condition). Of the 35 BK-positive clinical specimens tested, 22 had results that fell within our range of quantitation (ROQ), which is 2,500 to 50,000,000 copies/mL. Of these, 10 (45%) showed a decrease in viral load between the IP and all temperature conditions. 4 specimens amplified but remained beneath our ROQ for all study conditions. Of the remaining 18 IP study specimens: 89% of RT, 72% of +4°C, and 61% of –20°C specimens showed a decrease in BK DNA after 48 hours. The average change in log between the IP and the other study conditions was 0.58 (RT), 0.29 (+4°C), and 0.58 (–20°C). Finally, of the 18 specimens with an IP within our ROQ, 10 (56%) were > 0.3 log, and 7 (39%) were > 0.5 log different from the original clinical sample that was amplified in a separate run of the assay. The average change in log between the original patient and the IP specimens was 0.47 log. There is a trend toward decay of BK virus DNA after storing samples at RT for 48 hours, as 89% of specimens displayed a decrease in viral load. However, it is also necessary to consider the magnitude of the change and, on average, the smallest difference in log occurred between IP and +4°C. Therefore, it may be preferable to store specimens for BK quantitative PCR at +4°C, at least for up to 48 hours before processing the sample. This information can assist laboratories that batch BK virus testing and can guide policies regarding the transport of specimens to the laboratory. Larger studies are needed to assess the true stability of the virus, while considering each laboratory's assay variations as well as the possibility of longer and nonstandardized transport conditions.

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Postmortem Peripheral Blood and Liver Fentanyl Concentrations in Medical Examiner Cases: Redistribution and Determination of Fentanyl Toxicity

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We determined the relationship of postmortem (PM) peripheral blood (PB) and liver fentanyl concentrations and whether PM redistribution (PMR) occurs in medical examiner cases in which fentanyl is identified. In 2010-2011, PB and liver tissue were collected at autopsy in 64 cases involving the presence of fentanyl, independent of the type of exposure. Fentanyl was quantitated by GCMS. Mean blood fentanyl concentrations were 6 µg/L (range 2-15 µg/L) in cases with non–drug-related causes of death (other causes, n = 5), 10 µg/L (2-22 µg/L) in deaths from mixed drug toxicity (n = 26) and 16 µg/L (2-56 µg/L) in deaths resulting from fentanyl toxicity (n = 33). Corresponding liver fentanyl concentrations were 38 µg/kg (11-104 µg/kg), 80 µg/kg (6-235 µg/kg), and 104 µg/kg (18-365 µg/kg), respectively. Overall blood and liver fentanyl concentrations showed some correlation (r = 0.67), with the strongest correlation observed in deaths from other causes (r = 0.99); compared with r = 0.61 in mixed drug toxicity and r = 0.70 in fentanyl toxicity. Comparison of PM interval (time of death to autopsy) to the liver/blood ratio showed a decrease in the ratio with time in an exponential manner in cases from fentanyl and mixed drug toxicity but not in cases from other causes. Liver fentanyl concentrations helped define toxicity using a liver therapeutic cutoff concentration of <23 µg/kg and overdose concentration of > 56 µg/kg. Overlap of the liver to peripheral blood fentanyl ratio was observed in the 3 different cause of death groups. Only 5 (7.8%) had fentanyl as the sole drug detected. Multiple drugs were routinely identified in most cases with benzodiazepines identified in over 40%. In conclusion, fentanyl appears to undergo PMR, particularly in cases associated with a longer PM interval from time of death to autopsy. Revised discriminatory liver fentanyl concentrations associated with therapeutic and overdose cases have been described. The overlap in blood fentanyl concentrations observed in the different medical cause of death groups suggests that determining both liver and blood concentrations would be important in some cases of suspected fentanyl toxicity.