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.

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Immunophenotypic Study of Giant Platelets by Multiparameter Flow Cytometry
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The immunophenotypic profile of giant platelets in a setting of myeloid neoplasms is not well established. The CD45/sight light scatter properties of giant platelets may be somewhat similar to those of blasts, which could potentially lead to an inaccurate estimation of blasts by flow cytometry. Therefore, the need to identify the immunophenotypic spectrum of platelets is essential. Our aim was to study the normal and aberrant immunophenotype of platelets.

Consecutive cases of peripheral blood specimens (n = 13) from patients with giant platelets identified on peripheral blood smears were studied for immunophenotypic profile of platelets, using 4- or 8-color flow cytometry. In 3 patients, there were myeloid neoplasms: 2 cases of acute myeloid leukemia and 1 case of primary myelofibrosis (PMF). The remaining patients had reactive changes or lymphoid neoplasms.

Giant platelets were identified by weak to borderline expression of CD45 and low forward and side light scatters. The giant platelet count ranged from 0.5% to 6.1%. In all cases, platelets strongly expressed CD36 and CD61. No expression of CD117 or HLA-DR was observed in platelets. In 5 cases, a variant immunophenotype was encountered. The abnormalities included expression of CD34 (2/13), CD13 (2/13), CD33 (4/13), and CD71 (1/13). One case (PMF) had moderately strong expression of CD34. The other variant markers had weak staining. The case of PMF had also a small population of circulating myeloblasts, distinctive from giant platelets despite CD34 expression in giant platelets.

Our study demonstrates that some myelomonocytic markers and rarely CD34 can be seen in giant platelets. While this can be potentially confusing, a combination of strong expression of CD61 and CD36, lack of expression of CD117 and HLA-DR, and very weak CD45 combined with low forward light scatter allows discrimination between giant platelets and blasts. Additional studies are needed to determine whether abnormal platelet immunophenotype is associated with myeloid neoplasms.

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Validation and Implementation of an Algorithm for Reporting the Automated Absolute Neutrophil Count (ANC) From Selected Flagged Specimens
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Many chemotherapeutic protocols require a recent ANC before initiation of treatment. Samples from hematology/oncology clinics submitted for ANC are frequently flagged by automated cell counters because of abnormal cells. These samples are submitted for microscopic review, despite the fact that the ANC derived from the automated differential may be unaffected and accurate. The consequence is prolonged wait times for patients and clinical staff in hematology-oncology clinics.

We determined whether a set of non-ANC–specific flags can be safely ignored when reporting only the ANC, allowing for the use of the readily available automated differential. We also ascertained the utilization patterns and effects on turnaround times of a new ordering algorithm allowing clinician to specify that the ANC is the primary clinical concern and laboratory staff to discriminate between ANC-specific and non-ANC–specific flags.

Four flags on the Sysmex XE/XT automated hematology analyzers (Sysmex, Kobe, Japan) were hypothesized to be non-ANC

specific and, therefore, safely can be ignored when reporting the ANC from an automated differential. We validated this hypothesis with 518 samples flagged by at least 1 of 9 white cell flags for which an automated and manual differential were available. Subsequently, a new order set indicating that the ANC was the primary clinical interest was created and the new flagging algorithm was incorporated. Utilization patterns and turnaround times for this new order set were prospectively recorded.

The correlation of automated and manual ANCs for samples flagged by only non-ANC–specific flags was 96.9%, with 1 clinically significant discrepant sample of 296 (0.3%). Clinicians ordered the new ANC-specific order set for 16.3% of patients seen in the hematology-oncology clinic. Reporting the automated ANC when only non-ANC–specific flags were present led to a reduction of the review rate by 60%. Turnaround times for samples on which only non-ANC–specific flags were present improved from 115 minutes to 15.4 minutes.

We have validated the selective application of flagging criteria to samples in which the primary clinical interest is the ANC. Clinicians in a hematology-oncology clinic order the ANC-only option for a significant percentage of their patients. Turnaround times for the majority of ANC samples can be decreased significantly by such an algorithm.

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Protective Effects of Lactoferrin in Methicillin-Resistant *Staphylococcus aureus*–Induced SIRS

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Systemic inflammatory response syndrome (SIRS) involves uncontrolled production of proinflammatory and anti-inflammatory mediators, often leading to sepsis and multiorgan failure. Bacterial-induced SIRS represents increasing health risks, especially in the presence of rising incidences of nosocomial infection with methicillin-resistant *Staphylococcus aureus* (MRSA). Prophylactic treatments for bacterial-induced development of SIRS have yielded limited clinical success. Lactoferrin (LF), an iron-binding glycoprotein, possesses multiple immune modulatory effects, including the ability to suppress cytokine production from mitogen-activated T cells and macrophages, and represents a potential agent for the prevention and/or treatment of SIRS. In the present study, the effects of intravenous lactoferrin were assessed in a mouse MRSA peritonitis SIRS model.

Mice were pretreated intravenously with lactoferrin, 2 or 6 hours before intraperitoneal infection with MRSA. Comparisons were made with control treatment with phosphate-buffered saline. Outcomes measured were serum interleukin (IL)-2, IL-6, interferon γ, and tumor necrosis factor (TNF)-α, blood colony-forming unit (CFU), and organs (lung, liver, kidneys, and spleen) CFU. Survival curves were performed for 72 hours after infection. In vitro analysis of cytokine/chemokine production were conducted on macrophages, an innate leukocyte responder to bacterial infection, pretreated with lactoferrin (2-6 hours) followed by exposure to MRSA.

Pretreating mice with lactoferrin 2 hours before MRSA infection led to significantly decreased serum IL-2 and IL-6 levels at 24 hours. Host survival was increased with 75% of mice surviving after 72 hours compared with only 25% of mice in the control group. These effects were not observed in mice treated with lactoferrin 6 hours before MRSA challenge. There were no significant differences in between blood or organ CFU groups at that time after infection. Lactoferrin pretreated macrophages (2-6 hours) led to lowered production of chemokines (MIP-1a, MIP-2, and MCP-1) on exposure to MRSA, but there were no significant changes in production of IL-6 or TNF-α.

Treatment of mice with lactoferrin before MRSA challenge results in increased survival and reduction of SIRS manifesting to deleterious sepsis. Lactoferrin did not exhibit direct bacteriostatic or bactericidal properties; thus, the protective effects are likely due to its ability to subdue the cytokine storm created in response to pathogenic assault. These findings suggest that lactoferrin is a prophylactic agent capable of reducing MRSA-induced SIRS and preseptic events.

7

Rapid Detection of Oleander Poisoning Using Dimension Vista Digoxin Immunoassay: Impact on Serum Digoxin Measurement and Digibind Therapy

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Oleander is an ornamental shrub found in abundance in southern parts of the United States, Europe, and Asia. Accidental poisoning, especially in children, may be fatal, but despite toxicity, oleander extract is used in herbal remedies. Currently, fluorescence polarization immunoassay (FPIA) for digoxin is the standard method for rapid detection of oleander, taking advantage of structural similarity between oleandrin (cardioactive toxic component of oleander) and digoxin, but Abbott Laboratories will discontinue this assay at the end of 2010. Therefore, we explored alternative digoxin assays for rapid detection of oleander poisoning.

Aliquots of a drug-free serum pool were supplemented with increasing amounts of pure oleandrin (50 ng/mL to 5 µg/mL) or oleander extract (1, 2.5, and 5.0 µL/mL; extract prepared by suspending 100 mg of dried oleander leaf in 5 mL of ethanol). In addition, aliquots of 2 different serum digoxin pools were supplemented with similar amounts of oleandrin or oleander extract. Then serum digoxin values were measured using 4 distinct digoxin immunoassay methods. Results were compared with values obtained by FPIA.

We observed high apparent digoxin concentrations using the Dimension Vista digoxin assay with values comparable to the FPIA. For example, when an aliquot of drug-free serum was supplemented with oleandrin (5 µg/mL), the apparent digoxin concentration using the Vista assay was 4.43 ng/mL compared with 4.56 ng/mL with the FPIA. In contrast, the ADVIA digoxin assay showed the least cross-reactivity (0.98 ng/mL), followed by the TinaQuant (1.50 ng/mL) and EMIT (3.36 ng/mL). The Dimension Vista assay also demonstrated 30% to 302% falsely elevated digoxin values, dependent on amount of oleandrin or oleander extract present. However, by taking advantage of strong protein binding of oleandrin, it was demonstrated that monitoring free digoxin can essentially eliminate this interference. When oleandrin (20 µg total dose to 25 g mice) or oleander extract (after 1 to 10 dilution, 100 µL gavage volume) was orally delivered to mice, significant apparent digoxin values were observed in serum samples 1 and 2 hours after feeding, using the Dimension Vista assay; the half-life of oleandrin was approximately 2 hours. In addition, Digibind neutralizes digoxin-like cardioactive components of digoxin in vitro, and such an effect can be monitored by demonstrating significant reductions in apparent free digoxin concentrations.

Oleander poisoning can be detected rapidly by using the Dimension Vista digoxin assay. However, this assay is unsuitable for monitoring of digoxin levels in patients exposed to oleander.

9

Impact of Donor Height and Weight Criteria on Young Donor Eligibility

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New height and weight criteria for blood donors ages 16 to 22 years were recently implemented by the 2 largest blood collection organizations in an effort to minimize vasovagal reactions in this high-risk group. These criteria use donor sex, height, and weight to calculate total blood volume (TBV) with a goal of preventing a greater than 15% decrease in TBV following donation. The aim of this study was to determine the impact that implementation of these criteria would have on collections and donor reaction rates for our small university hospital–affiliated blood center.

Donor history questionnaires from all undergraduate campus blood drives during the 2009 calendar year were retrospectively reviewed to determine the total number of collections, the proportion of donors ages 16 to 22 years, and the number of vasovagal reactions. Height, weight, sex, and age at the time of donation were then extracted from donor history questionnaires from the 4 most recent campus blood drives to determine the number of donors that would potentially be deferred with implementation of the new criteria. Since these criteria were developed for a standard 525-mL collection volume, we also investigated whether adjustment for our smaller 475-mL collection volume would decrease donor deferral rates.

Undergraduate campus blood drives represented 21.3% of our annual collections (770/3,616 allogeneic whole blood units) with 80.0% of campus donors age 22 years or younger (616/770). The vasovagal reaction rate was 1.7% (13/770). During the 4 most recent campus blood drives, 80.1% of donors were age 22 years or younger (258/322) with a vasovagal reaction rate of 2.5% (8/322). If new height and weight criteria were implemented, 10.9% of campus donors age 22 years or younger (28/258) would have been deferred, representing an estimated loss of 67 units annually. Criteria-based donor deferral would have prevented 2 of 8 vasovagal reactions. Revision of height and weight criteria with consideration for a 475-mL collection volume would decrease deferral rates to 1.6% (4/258), decreasing the estimated loss to 10 units per year and preventing the same 2 donor reactions through donor deferral.

Implementation of height and weight criteria based on a standard 525-mL collection volume results in an unacceptably high rate of donor deferral negatively impacting our blood supply. If criteria are adjusted for a 475-mL collection volume, donor deferral rates are decreased and an equal number of vasovagal reactions are prevented. Use of a smaller collection volume and adjusted height and weight criteria optimizes young donor deferral rates while maintaining donor safety.

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Increased RBC Hemolysis After Cell Washing With Different Automated Technologies: Clinical Implications for Neonatal Cardiac Surgery Patients

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Before transfusion of packed RBCs (pRBCs) in subsets of pediatric patients undergoing cardiac surgery, the pRBCs are often washed to reduce potassium (K) levels and avoid hyperkalemia. Also, previous studies in patients undergoing ECMO have shown that washed pRBCs have a higher rate of hemolysis than unwashed cells during storage secondary to mechanical manipulation. While the pathologic effects of plasma free hemoglobin (FHb) are well-documented, no published data exist that state acceptable amounts of FHb from washing-related hemolysis. Our purpose for this study was to determine if our blood bank is providing acceptable washed pRBCs for our neonatal patients with minimal amounts of K and FHb from washing-related hemolysis.

Four units of fresh, 4-day-old, type A+ pRBCs stored at 4°C were washed on the Cobe 2991 Model 1 Blood Cell Processor (serial number <2000), Cobe 2991 Model 2 Blood Cell Processor (serial number >2000), or the Fresenius Continuous AutoTransfusion System (CATS). The Cobe systems use discontinuous centrifugal processing, whereas the CATS uses continuous cell separator technology. Aliquots were drawn before washing, and K and hemolysis indices (HIs) were measured with the Beckman Coulter Unicel DxC 800 Synchron Clinical System. (HIs of 0-1, 2-3, 4-5, and >6, respectively, correlate with FHb of <50, 50-99, 100-199, and >200 mg/dL.) K and HI were measured prewash and postwash at 0, 4, 6, 12, and 24 hours. Analysis of variance and the Student paired t test were used to analyze data, and a 2-tailed P value of less than .05 was considered statistically significant.

Although the K level remained low over 24 hours postwash, units washed with the Cobe devices showed a slightly higher increase in K compared with the CATS at all time points, with statistically significant differences at 6, 12, and 24 hours ($P < .04$). With both devices, HIs increased immediately postwash. It is important to note that HIs reflected significantly higher levels of FHb (100-199 mg/dL) with the Cobe Model 1 device compared with the CATS, evident immediately postwash with levels exceeding 200 mg/dL at 24 hours ($P < .035$). There was no statistical difference in HIs between the Cobe Model 2 and CATS.

Our data show a statistical difference in HIs and, thus, FHb using the 2 different technologies for cell washing. The levels of FHb evident immediately after washing using the Cobe Model 1 that we have in our blood bank are likely to be clinically significant in our neonatal patients. Further investigation is warranted to establish standards regarding acceptable levels of FHb after cell washing.

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Aberrant Expression of Two Novel Histone Deubiquitinases in Breast Carcinoma (BC)

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Posttranslational histone modifications have important roles in controlling gene expression. Accordingly, several histone-modifying enzymes, including ubiquitin ligases for histones H2A and H2B, have been described as proto-oncogenes or tumor suppressors. However, it remains to be determined whether histone deubiquitinases are also involved in cancer development. We evaluated the expression of 2 newly identified histone H2A/H2B deubiquitinases, USP12 and USP46, in BC.

Tissue microarrays (TMAs) of BC (containing in situ and invasive carcinoma) and adjacent normal control samples were purchased from US Biomax (catalogue No. BR804). After pathologic

confirmation of the diagnosis in each TMA core, immunohistochemical analysis was performed using custom-generated rabbit polyclonal antibodies against histone deubiquitinases USP12 and USP46, as well as an antibody against ubiquitinated histone H2B (ubH2B) (Dr Oren, Weizmann Institute of Science, Israel). The intensity (0-3+) and percentage of cells staining (0%-100%) were recorded, and an H score (ranging from 0-300) was also derived. The expression of these markers in BC was then compared with that in benign breast epithelium (BBE).

Anti-USP12 and anti-USP46 antibodies produced nuclear and cytoplasmic staining, whereas ubH2B staining was exclusively nuclear. Strong (3+) nuclear USP12 expression was seen in 37% of BCs vs 9% of adjacent BBE ($P = .006$), and the average USP12 H scores in BC were also higher than in BBE (242 vs 206; $P = .03$). On the other hand, the proportion of cases with strong USP46 expression and the average USP46 H-scores in BC and BBE were very close (59% vs 66%; $P = .6$ and 118 vs 139; $P = .11$). Strong ubH2B expression was seen in 28% of BCs vs 9% of adjacent BBE ($P = .058$), and there was a weak correlation between the ubH2B H score and the USP12 H score ($r = 0.210$; $P = .063$), but not with the USP46 H score ($r = 0.07$; $P = .52$).

The expression of all 3 markers in the in situ and invasive carcinomas was very similar. The higher expression of histone deubiquitinase USP12 in BC compared with BBE suggests that histone deubiquitination may have a role in cancer development. Further studies to determine whether USP12 expression in BC is associated with particular clinical, pathologic, or molecular profiles and/or is of any predictive or prognostic value, are needed.

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Cost-Effective Comprehensive *EGFR* Mutational Analysis by a Novel Two-Tiered Method

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In selected patients with non–small cell lung cancer (NSCLC), acquired mutations in exons 18 to 21 of the epidermal growth factor receptor (*EGFR*) predict response to drugs that inhibit tyrosine-kinase activity of the EGFR protein. Deletions in exon 19 and the *L858R* point mutation together account for about 90% of somatic mutations. The remaining 10% of mutations are heterogeneous throughout *EGFR* exons 18 to 21, and data are limited regarding treatment responses associated with these rarer mutations. Many laboratories have implemented *EGFR* mutational analysis, but the testing strategy typically uses a single comprehensive analysis of exons 18 to 21, with relatively insensitive methods and prices often exceeding $1,000.

To allow a cost-effective testing strategy, we have developed and validated 2 levels of EGFR testing. The level 1 test detects deletions in exon 19 by a highly sensitive sizing assay (adapted from Rosell R, Moran T, Queralt C, et al. *N Engl J Med.* 2009;361:958-967) and detects the *L858R* mutation by a rapid and sensitive melting-curve assay with a mutation-specific sensor probe. The level 2 test detects the less common acquired mutations in exons 18 through 21 by DNA sequencing. We perform *EGFR* level 2 testing only if the level 1 test result is negative and the ordering physician has a high suspicion of an *EGFR* mutation. To establish sensitivity, we performed multiple mixing studies using formalin-fixed, paraffin-embedded DNA from lung cancer tissue and plasmids with exon 19 deletions or the *L858R* mutation.

We determined that the sizing assay (exon 19 deletions) could reliably detect mutations down to 1% in a wild-type background and the melting-curve assay (*L858R*), down to 2% in a wild-type background. The level 1 and level 2 tests correctly identified blinded patient specimens from 2 outside institutions.

We have developed and validated a comprehensive 2-tiered strategy for *EGFR* mutational analysis that is robust, sensitive, and cost-effective.

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COLD-PCR Enhanced Melting-Curve Analysis for Closed-Tube Detection of *KRAS* Mutations

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KRAS mutational analysis is the standard of care before initiation of treatments targeting the epidermal growth factor receptor (*EGFR*) in patients with metastatic colorectal cancer. Many laboratories have implemented sensitive tests for *KRAS* mutations, but the methods often require expensive reagents, parallel reactions, multiple steps, or opening of polymerase chain reaction (PCR) tubes. PCR-based methods that require opening tubes are particularly problematic in the clinical setting owing to contamination risks. Herein we report a highly sensitive, rapid, cost-effective, single-reaction, closed-tube strategy to detect all clinically significant mutations in *KRAS* codons 12 and 13 using the Roche LightCycler instrument. The assay detects mutations via PCR–melting-curve analysis using a Cy5.5-labeled sensor probe that straddles codons 12 and 13 (adapted from Nikiforova MN, Lynch RA, Biddinger PW, et al. *J Clin Endocrinol Metab.* 2003;88:2318-2326).

We incorporated coamplification at lower denaturation temperature (COLD) PCR to selectively amplify mutant alleles in a wild-type background, without additional instrument or reagent costs. A fast COLD-PCR cycling program with a critical denaturation temperature (Tc) of 81°C increases the sensitivity of the assay by more than 10-fold for the majority of *KRAS* mutations (G12D, G12C, G12V, and G13D). Mixing studies using wild-type/mutant pairs of cell lines and patient specimens demonstrated that the COLD-PCR enhanced melting-curve assay can reliably detect down to less than 1% mutant DNA in a wild-type background. We showed that the assay is sensitive enough to eliminate the need for tumor enrichment by macrodissection in most cases. *KRAS* mutation status in formalin-fixed, paraffin-embedded tumor specimens was concordant with testing done at outside institutions and by alternative sensitive methods such as allele-specific PCR.

We have developed and validated a simple-to-use, highly sensitive, and robust clinical assay for *KRAS* mutation detection that is the first closed-tube method to combine COLD-PCR with melting-curve analysis.

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Development of a Multiplexed, Real-Time PCR Assay for Detection of *Kingella kingae* and *Staphylococcus aureus* From Synovial Fluid in Septic Arthritis

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Bacterial cultures of synovial fluid are negative in about one third of suspected cases of septic arthritis. While *Staphylococcus aureus* remains the most common cause of septic arthritis in adult and pediatric patients, *Kingella kingae* is increasingly being recognized as a leading cause of septic arthritis in young children younger than 5 years). Typically, *S aureus* is readily isolated using conventional culture techniques. However, initiation of antibiotic therapy before synovial fluid culture and low numbers of organisms are possible causes of false-negative cultures. *K kingae* is a fastidious organism that is rarely recovered from direct culture of synovial fluids. Although the practice of inoculating blood culture bottles with synovial fluid has greatly improved its isolation, reports comparing polymerase chain reaction (PCR) with culture-based detection suggest that recovery with culture methods remains suboptimal and that the incidence of *K kingae* in septic arthritis may be significantly underestimated in young children. Identification of *S aureus* or *K kingae* as the etiologic agent of septic arthritis is important to initiate optimal antibiotic therapy. This is especially important in pediatric population to prevent long-term sequelae.

By using a combination of previously published and laboratory-developed primers and probes, we targeted the *cpn*60 of *K kingae* and *spa* of *S aureus* to develop a multiplexed real-time PCR assay. The BiOstic bacteremia kit was used for DNA extraction. Specificity of the assay was confirmed using 9 distinct bacterial species. Sensitivity of the assay and extraction efficiency were evaluated on DNA isolated from pure bacterial cultures and on spiked synovial fluid samples. The assay was performed on 10 synovial fluid samples from suspected cases of septic arthritis in pediatric patients.

We correctly identified the 2 cases of culture-confirmed *S aureus* arthritis and did not detect *S aureus* using our assay in the remaining 8 culture-negative cases. Nine of the samples were also negative for *K kingae*. However, the assay was positive for *K kingae* in 1 sample that was negative by culture (direct culture without the use of blood culture bottle). Sequencing the amplified product confirmed its identity as the targeted region of the *K kingae cpn60* gene. This finding and previous reports from other institutions suggest that *K kingae* arthritis may be more prevalent than currently appreciated using conventional culture and highlights the usefulness of our real-time PCR assay for the detection of pathogens in joint fluid.

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Indefinite Valgancyclovir Prophylaxis in Lung Transplant Recipients Monitored by Clinical Outcomes and Quantitative Cytomegalovirus Load in Bronchoalveolar Lavage Fluid
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Cytomegalovirus (CMV) infection is a serious cause of morbidity and mortality in lung transplant recipients. Oral valgancyclovir has proven to be an important element of CMV prophylaxis regimens, but the optimal duration of therapy has yet to be established. The incidence of CMV disease, frequency of adverse effects, and risk of resistant infection with an indefinite regimen of valgancyclovir prophylaxis are undetermined. Furthermore, the relationship between CMV load in bronchoalveolar lavage (BAL) fluid and prophylactic regimen, development of CMV disease, transplant rejection, and mortality remains unresolved.

We conducted an observational cohort study of 65 lung transplant recipients at UCSF Medical Center, who underwent transplantation in 2004 or later, with BAL samples available for analysis from July 2009 or later. All patients received indefinite oral valgancyclovir prophylaxis, with discontinuation of medication only on development of adverse effects or resistant infection. Routine or clinically indicated BAL samples were analyzed by CMV shell vial culture methods and quantitative real-time polymerase chain reaction (PCR).

In 20 patients (31%), leukopenia or other adverse effects developed, leading to temporary or permanent discontinuation of valgancyclovir. In 4 (6%) of the lung transplant recipients, clinically significant CMV infection developed, with 2 strains found to be resistant to valgancyclovir. In 8 patients (12%), there was evidence of CMV in the BAL fluid without evidence of active CMV disease; all of these patients had discontinued valgancyclovir prophylaxis or had a history of valgancyclovir-resistant CMV infection. Quantitative PCR measurement of CMV in BAL fluid was significantly more sensitive than detection of CMV by shell vial assay. BAL CMV levels of less than 10,000 copies/mL were correlated with asymptomatic infection, whereas those with more than 100,000 copies/mL were present only in clinically significant CMV disease.

Results thus far indicate that an indefinite regimen of valgancyclovir prophylaxis leads to lower rates of CMV infection than more limited regimens. However, leukopenia and valgancyclovir-resistant CMV infection remain the major risks of indefinite prophylaxis. Elevated levels of CMV in BAL fluid correlated with active CMV infection, but longer-term follow up is necessary to determine whether asymptomatic presence of CMV in BAL fluid predicts subsequent diagnosis of CMV infection, transplant rejection, or mortality.

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Paroxysmal Cold Hemoglobinuria Presenting as Transfusion Reactions
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Paroxysmal cold hemoglobinuria (PCH) is a rare type of autoimmune hemolytic anemia characterized by intravascular hemolysis. The causative autoantibody is a biphasic, polyclonal IgG. A positive Donath-Landsteiner test for the presence of the biphasic antibody is diagnostic of PCH. The disease is predominantly seen in children and is associated with an infection. We report a case of PCH in an 85-year-old woman with squamous cell carcinoma.

The patient came to our attention owing to an unexplained hemolytic transfusion reaction. The patient was admitted with diverticulitis and was given 2 U of leukoreduced RBCs for symptomatic anemia (hemoglobin, 5.7 g/dL). During the transfusion of the second unit, she became febrile. The posttransfusion hemoglobin level was 8.4 g/dL, but the level decreased to 6.5 g/dL 8 hours later without signs of bleeding. Twenty days before this reaction, she was admitted with fever, coagulase-negative *Staphylococcus* line infection, and anemia (hemoglobin, 6.5 g/dL). In the 20 days leading up to the aforementioned transfusion reaction, she required multiple transfusions and experienced 2 transfusion reactions with similar symptoms; however, the decrease in hemoglobin was more gradual after the transfusion. The indirect antiglobulin test (IAT) was negative before the first reaction but subsequently became weakly reactive

without RBC antigen specificity on the day of the second transfusion reaction. The direct antiglobulin test (DAT) was negative before and immediately after the first 2 transfusion reactions. Subsequent testing during the next month revealed a weakly reactive IAT without RBC antigen specificity, weak cold agglutination, a positive DAT (micro-1+ positive for C3 and negative IgG), and no RBC reactivity detected in an eluate prepared from the patient's RBCs. Antibodies to common RBC antigens were excluded with saline, albumin, and FICIN IATs. The DAT was also negative with anti-IgM and anti-IgA reagents. The Donath-Landsteiner test was positive 6 and 21 days after the third transfusion reaction, confirming the diagnosis of PCH. Testing was unable to confirm anti-P specificity. The patient received a total of 15 RBC units during 42 days. Approximately 100 days out from initial presentation, her hemoglobin level had stabilized with prednisone treatment.

PCH should be considered in all patients, regardless of age, with intravascular hemolysis and detection of complement by DAT.

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Evaluation of Four Molecular Methods for Detection of *Clostridium difficile*

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Clostridium difficile infection is a common cause of nosocomial diarrhea. The symptoms can vary from mild diarrhea to toxic megacolon and death. The increase in incidence, severity of the infection, and the emergence of a hypervirulent strain, NAP1, in recent years have highlighted the necessity for accurate and prompt diagnosis of the disease. Although the cell culture cytotoxicity assay is generally considered the "gold standard" in laboratory diagnosis, it is too time-consuming and labor-intensive for routine clinical use. In recent years, most clinical laboratories used enzyme immunoassays (EIAs) for the detection of toxins A and B owing to their rapid turnaround time, ease of use, and relatively low costs. These assays, however, lack sensitivity, prompting clinicians to perform multiple toxin tests to achieve higher sensitivity. Real-time polymerase chain reaction (PCR) assays, detecting the *tcdB* toxin gene, were approved by the US Food and Drug Administration in December 2008.

In this study, 50 specimens are being tested using an EIA method, Premier Toxins A and B (Meridian Bioscience, Cincinnati, OH), and 4 molecular diagnostic assays: Xpert *C difficile* (Cepheid, Sunnyvale, CA), BD GeneOhm Cdiff assay (BD Diagnostics, San Diego, CA), ProGastro *Clostridium difficile* (Gen-Probe Prodesse, Waukesha, WI), and Illumigene *C difficile* (Meridian Bioscience, Cincinnati, OH). Preliminary data suggest a 15% discordance rate between results obtained from EIA and the PCR methods. The discordant results from these cases will be further analyzed using a cell culture cytotoxicity assay and repeated molecular testing.

Factors to be considered in the final decision regarding introduction of a molecular assay for *C difficile* into routine workflow at our institution include the final outcome of this analysis combined with analysis of cost, ease of use, reproducibility, and platforms currently available in our laboratory.

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Measurement of Tranexamic Acid Levels in Cardiopulmonary Bypass Patients Using a Novel Ultra-Performance Liquid Chromatography–Tandem Mass Spectrometry Assay

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Tranexamic acid is a synthetic derivative of the amino acid lysine that possesses potent antifibrinolytic activity. It has been used to decrease postoperative blood loss in patients with cardiopulmonary bypass (CPB) surgery. Despite the available literature describing the safety and effectiveness of tranexamic acid administration in patients undergoing CPB, some patients have side effects such as seizures of unknown cause. The dose for patients undergoing surgeries has been empiric, with patients receiving a bolus dose of tranexamic acid. There are a paucity of data examining the pharmacokinetics of tranexamic acid levels before and after surgery. Therefore, we have developed a novel method for the identification and quantitation of tranexamic acid in human plasma and cerebrospinal fluid using ultraperformance liquid chromatography–tandem mass spectrometry for use in pharmacokinetic studies of tranexamic acid.

After the addition of aminocaproic acid (ACA) as the internal standard, tranexamic acid is extracted from plasma using methanol. Gradient chromatographic separations are performed on a Waters ACQUITY UPLC BEH C18 column using an ammonium acetate–formic acid mobile phase.

Tranexamic acid demonstrated a reproducible elution time of 1.68 minutes, while ACA consistently eluted from the column at 1.35 minutes. Tranexamic acid (precursor ion 158.2 > product ion 95.2) and ACA (precursor ion 132.1 > product ion 114.0) were monitored using positive electrospray ionization in the MRM mode (Waters Quattro Micro) using the hydrogen adduct mass transitions. Within-run (2.8%-3.7%) and between-run (4.4%-6.0%) imprecision was acceptable using drug-free plasma spiked with known low, medium, and high concentrations of tranexamic acid. Recovery was between 89% and 108%, and ion suppression was less than 13%. The tranexamic acid standard curve for plasma displayed a wide analytic measuring range with linearity up to 10 μg/mL and a limit of quantitation at 100 ng/mL.

This novel method was successfully used to measure tranexamic levels in human plasma and cerebrospinal fluid from CPB patients and is suitable for pharmacokinetic studies.

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Pediatric Bloodstream Infections Caused by *Staphylococcus aureus* of Swine Origin

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Staphylococcus aureus is a major cause of bloodstream infections (BSIs) associated with significant morbidity and mortality, especially in children. Recent reports indicate that the *S aureus* clone of swine origin, multilocus sequence type ST398, has colonized persons with or without exposure to swine farming. ST398 is multidrug resistant due to the use of antibiotics as a premixed food supplement. To date, no human infections have been reported in this country due to ST398. However, we recently identified *S aureus* ST398 as the cause of BSI in 2 of our pediatric patients. We conducted this study to characterize this strain of infection.

During a 6-month period, 6 blood cultures collected from 2 pediatric intensive care unit patients with BSI were analyzed. They underwent PFGE with digestion by 2 restriction endonucleases, *Sma*I

and *Xma*I, which cut within the same DNA recognition sequence but at different sites. *Sma*I and *Xma*I cut at the asterisk sites CCC*GGG and C*CCGGG, respectively. We also included controls for ST398 isolates. To investigate this restriction pattern, a methylation study was conducted using the MethylCode Bisulfite Conversion Kit (Invitrogen, Carlsbad, CA). Polymerase chain reaction primers were used to amplify a relatively GC-rich genomic fragment containing a *Sma*I site.

All isolates were methicillin-susceptible *S aureus* (MSSA) and resistant to clindamycin, erythromycin, and penicillin. All strains were not digested with *Sma*I endonuclease; however, *Xma*I showed clear banding patterns in all 6 isolates and belonged to a single clone of ST398. The methylation study showed all endonuclease sites were methylated.

This is the first report of BSI infections caused by swine-associated MSSA ST398 in the United States. This strain was digestible by *Xma*I, although not by *Sma*I, due to methylation of its DNA. The child and the neonate recovered from their BSIs after treatment with antibiotics. However, the child has had repeated infections from the same swine-associated MSSA ST398. Further investigations regarding the recurrent infections of this ST398 strain are needed.

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Acquired Prothrombin Deficiency Caused by Lupus Anticoagulant

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Lupus anticoagulant (LA) is an IgG that causes in vitro prolongation of phospholipid-dependent tests such as the partial thromboplastin time (PTT) and the dRVVT. In children, LA may follow an infection, is mostly transient, and rarely causes any symptoms. We describe 3 children whose LA caused prothrombin deficiency and bleeding.

Case 1 involved a previously healthy 2.5-year-old boy with influenza B who was given antiviral drugs and antipyretics. Several days into his illness, gross hematuria developed, and he was admitted for evaluation. The results of a CBC count and a renal ultrasound were normal. However, his prothrombin time (PT) was 17.7 seconds (range, 12.6-15.2 seconds), PTT was 114.1 seconds (range, 22.7-36.0 seconds), and LA was detected in his plasma. Fibrinogen, fibrin degradation products, factor VIII, and factor IX results were within the reference range. Owing to the unexplained hematuria, we measured prothrombin activity, which was low at 22%. The presence of prothrombin antibodies was confirmed by an immunoassay (IgG of >100 U and IgM of 30 U). Within several days, the hematuria began to improve, and the PT and PTT shortened. At a follow-up visit several weeks after discharge, he did not have hematuria clinically or microscopically, his PT was normal, and his PTT was less prolonged (52.4 seconds).

Case 2 involved a previously healthy 3-year-old girl who was evaluated for a recent onset of widespread ecchymoses. Personal and family histories were negative for bleeding disorders. She had recently recovered from a viral gastroenteritis 2 weeks earlier, which was followed by the spontaneous bleeding symptoms. A CBC was normal, but the PT and PTT were prolonged at 19.9 and 74.5 seconds, respectively. Fibrinogen, fibrin degradation products, factor V, and factor X results were within normal limits. LA was also detected in her plasma, and her prothrombin activity was 12%. She was followed up closely, and in reevaluation in the clinic 3 months later, her bleeding had

ceased, the PT had normalized (13.1 seconds), the PTT had shortened (39.0 seconds), and LA was not detected in her plasma.

In case 3, a previously healthy 11-year-old boy had a 1-month history of bruising and recurrent epistaxis requiring cauterization. His CBC was normal, and the PT and PTT were prolonged (24 and 93 seconds, respectively). After detecting an LA, we measured his prothrombin activity, which was only 6%. Owing to persistent epistaxis, he received fresh frozen plasma and prothrombin complex concentrate on different occasions, with cessation of bleeding. For the past 8 years, he has been followed up as an outpatient and continues to have a positive LA and low prothrombin activity (range, 28%-31%) but no bleeding complications.

These patients exemplify the rare presentation of an acquired bleeding disorder associated with the presence of LA. In our experience, this phenomenon is more common in children because we have only seen 1 adult with similar findings during the same period in which we diagnosed the 3 cases described herein.

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Challenges Identified With Blood Bank Ordering and Documentation From an Academic Institution Undergoing Transition to an Electronic Health Record

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Our objectives were to identify the most common areas with challenges for blood bank ordering and documentation with the implementation of a hospital-wide electronic health record (EHR) and computerized provider order entry system (CPOE) and to identify the most common underlying causes encountered.

Blood bank–related calls from health care providers and other hospital staff to the information systems (IS) help desk in the first 4 months of use of the new commercial EHR and CPOE system were aggregated from a knowledge bank using specific text search strings. Personal identifiers of patients and staff were removed from the resulting calls, which were then categorized by using a standardized root cause analysis map.

The query resulted in 67 unique phone calls to the IS help desk pertinent to blood transfusions. The calls were categorized into 14 root causes, with some calls in multiple categories. Root causes included inadequate or incorrect user training (15 calls), lack of intuitive interface (14 calls), incompatibility with existing software (7 calls), incorrect documentation requirements (7 calls), ambiguous entry requirements (6 calls), printing issues (6 calls), insufficient program adaptability (6 calls), poor integration into existing workflow (6 calls), incorrect user authorizations (6 calls), poor interface design (3 calls), incorrect procedures (3 calls), a user not repeating back vital information (1 call), a glitch in the programming (1 call), and duplicate procedures (1 call).

The root causes for events resulting in a call to the IS help desk for blood bank–related issues with the use of a new EHR were varied but most commonly arose from issues with user training, system design, and implementation.

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Establishing and Maintaining Quality Clinical Laboratories in a Developing Country Involves All Phases of the Testing Process and Continuous Supervision

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Establishing robust clinical laboratories requires quality assurance (QA) systems. We have developed and deployed a QA system in a developing country laboratory system. The QA system includes laboratory inspections, education, and external QA (EQA) components. The QA system has been used in the National Health Laboratory (NHL) and in 5 zonal referral hospitals in the country of Eritrea. We performed an initial and follow-up inspection using a 54-question checklist. The checklist addressed all phases of the testing process and included questions targeted at specific subsections of the laboratory such as blood banking and microbiology.

The initial inspections showed major quality problems in all 6 laboratories, and striking inconsistencies in practice *within* individual laboratories were most striking. Some trends seen in all of the laboratories were as follows: (1) Blood banking did daily function checks and quality control (QC), with corrective actions in case of "OUT" QCs. (2) Hematology did daily QC but had no acceptable ranges and did not perform *any* corrective action for OUT QCs. (3) Chemistry rarely performed QC or interpreted QC results.

In addition, major problems were identified in all phases of laboratory testing. Some examples are as follows: (1) In the preanalytic phase, 31% of test requests entered into the laboratory information system at the NHL had a manual entry error, and in 23% of laboratory requests, the physician's name was absent or illegible. (2) In the analytic phase, because of poor workflow, turnaround times (TATs) for creatinine levels and CBCs were 31 and 15 hours, respectively, both more than 50-fold the minimum TATs of 27 and 16 minutes, respectively. In the postanalytic phase, 60% of results with more than a 2-day TAT were never retrieved.

Our interventions included establishment of EQA, educational sessions, in-laboratory visits, and other targeted interventions. Performance on the EQA scheme showed the same inconsistencies with that in the initial inspection. The worst performance was chemistry, with the percentage of EQA results considered acceptable ranging from 88% to 40% across the 6 laboratories. The educational sessions were based on a short QA manual written for developing country settings. Two quizzes, with 30 questions each, were used for preassessment and swapped for the postassessment. The pretest and posttest scores were 70% (SD 11.2%) and 80% (SD 13.5%), respectively. Additional site visits with a focus on QC and EQA interpretation and targeted interventions, such as weekly forwarding of unretrieved results to physicians and audits of manual data entry, were instituted. Follow-up inspections will be performed to monitor improvement.

Establishing and maintaining quality clinical laboratories in developing countries requires oversight. Interventions based on education alone without monitoring are not likely to be effective.

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Implementation of the PCA3 Urine Test: An Additional Tool to Aid in Prostate Cancer Diagnostic Decisions
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The test for the prostate cancer gene 3 (PCA3) expression detects a noncoding prostate-specific messenger RNA (mRNA) that is overexpressed in prostate cancer cells. In contrast, prostate-specific antigen (PSA) gene expression is similar in cancer and benign cells. The PCA3 test was created to aid in managing patients with elevated PSA levels and prior negative prostate biopsy results,

ie, to help predict whether cancer is likely and if repeated prostate biopsy may be indicated. The PCA3 score measures the ratio of PCA3 to PSA mRNA concentrations in postprostatic massage urine by transcription-mediated amplification; the reported cutoff for a positive test is 35×10^{-3} or more. Our objectives were to validate the PCA3 assay for clinical use in our institution and to evaluate its ease of implementation and cost-effectiveness.

During 13 months, 54 samples were collected from 50 men. Clinical samples (n = 33) were paired; one sample was sent to a reference laboratory for clinical testing, and the paired specimen was tested in-house. Analyte-specific reagents (Gen-Probe) were used to perform the PCA3 assay in triplicate. Results were compared among the paired samples.

In samples tested in the reference laboratory, the mean ± SD PCA3 score was $19.5 \pm 14.9 \times 10^{-3}$, and 5 of 33 were positive. In the validation testing we performed, the mean ± SD PCA3 score was $30.4 \pm 41.8 \times 10^{-3}$, and 9 of 33 were positive. For individual in-house samples, average intra-assay coefficients of variation (CVs) of triplicate patient samples for PCA3 and PSA analytes were 19.6% and 17.8%, respectively. PCA3 score positivity was in agreement in 26 (79%) of 33 samples. Discordant samples fell into 3 categories. Two samples at the reference laboratory were reported as "inconclusive," although we were able to obtain a PCA3 score. Three samples had PCA3 scores within 10% of the cutoff. The remaining 2 samples were discordant, although PCA3 scores we performed correlated with clinical outcomes in those cases. Although the test is easy to perform, numerous calibrators and controls are necessary, contributing to reagent expense; thus, batch sizes of 25 or more were needed to price the test competitively.

The PCA3 assay is a relatively new clinical test that some urologists are using to aid in evaluating men for prostate cancer. The test can be easily performed on existing Direct Tube Sampling/APTIMA instrumentation and is cost-effective when samples are batched in groups of 25 samples or more. Because the observed and previously reported CVs of this assay are more than 10%, we propose not using the stringent cutoff of 35×10^{-3} to deem a test result "positive" or "negative." Rather, we suggest creating an indeterminate range that centers around a ratio of 35×10^{-3} to facilitate prostate cancer risk assessment and stratification.

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A Case of Delayed Hemolytic Reaction Caused by Rh-DVa
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A 49-year-old man with HIV/AIDS complicated by multicentric Castleman disease, membranous nephropathy, Kaposi sarcoma, and pancytopenia was admitted for worsening anasarca and hypotension. One unit of "B Rh+" RBCs was transfused for management of anasarca and for a hemoglobin level of 7.6 g/dL. On the 8-day posttransfusion, he was found to be icteric, his total bilirubin rose from 2.1 mg/dL at transfusion to 16.3 mg/dL on day 8, and his urine, which was negative for bilirubin at admission, turned positive on day 8. His hemoglobin level dropped from 9.1 g/dL (day 7) to 7.3 g/dL (day 8), and schistocytes were seen on his peripheral blood smear. A delayed hemolytic reaction workup was initiated.

Front and back typing, polyethylene glycol indirect antiglobulin tube test (PEG-IAT), red cell antibody gel card, and red cell phenotyping were done. The transfused unit was crossmatch compatible.

His blood group was confirmed to be B, Rh+ based on 4+ agglutination with anti-B, no reaction with anti-A, 3+ with anti-D, 3+ reaction of his serum with A cells, and no reaction of his serum with B cells. A reagent red cell antibody panel done using PEG-IAT showed a 2+ reaction of his serum with D+ cells; anti-Le[a], however, could not be ruled out. An alternative red cell antigen panel using a gel card ruled out anti-Le[a]. Eluate from his red cells showed a 4+ agglutination with D+ cells of the second panel and confirmed the presence of anti-D antibody. A direct antiglobulin test done on his red cells was positive with polyspecific antisera and anti-IgG antihuman globulin serum (AHG), but it was negative with anti-C3 AHG. Eluate from patient's cells further showed alloanti-D reactive with all D+ red cells including DIIIa and DIIIc at PEG-IAT; however, it did not react with partial DVa and DVI cells. Red cell phenotyping was done, and his reticulocytes were positive for DVI; however, DVa antigen typing could not be done for the lack of availability of antisera.

Based on the testing done on the cells and plasma, this represents a delayed hemolytic reaction due to alloanti-D in the setting of a possible partial Rh-DVa. DVa is a rare subcategory of partial-D antigens with cells that are D[w]+. The antigen DVa, when present, is carried with Rhce in Africans and RhCe in Caucasians. It has variable strength/reactivity and, to the best of our knowledge, has never been reported to be associated with hemolytic reactions. In our opinion, DVa+ transfusion recipients should get crossmatch-compatible RhD– red cells or DVa+ blood units (rare), while blood from DVa+ donors should be considered RhD+.

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Comparison of Two Methodologies for *KRAS* Mutation Testing in Colorectal Cancer

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Binding of a ligand to the epidermal growth factor receptor (EGFR) stimulates various intracellular signaling pathways, resulting in cell cycle progression, proliferation, angiogenesis, and apoptosis inhibition. Drugs targeting EGFR are approved for patients with colorectal cancer, the second most common cause of cancer death in the United States. *KRAS* (Kirsten RNA-associated rat sarcoma 2 virus) is involved in signaling pathways, including *MAPK* and *RAS/RAF,* and mutations in this gene result in constitutive activation of these pathways, independent of EGFR activation. Of all human tumors, 30% harbor mutations in *KRAS*, with around 40% of patients with metastatic colon cancer having mutations in this gene. Seven mutations in codons 12 and 13 comprise around 95% of the observed mutations, rendering monoclonal antibodies against EGFR (eg, cetuximab and panitumumab) useless in treatment of this disease.

Our objective was to compare *KRAS* mutation testing by 2 methods on DNA extracted from colorectal tumors. DNA was extracted from the tissue of 18 colorectal tumors. For DNA sequencing, the DNA was amplified by polymerase chain reaction (PCR), M13 tails were added, and then the DNA was sequenced by capillary electrophoresis (Life Technologies, Foster City, CA) specifically for the detection of any mutations in codons 12 and 13 of *KRAS*. By using the Autogenomics Infiniti assay (Carlsbad, CA), the DNA was amplified by PCR, fluorescently labeled using analyte-specific primer extension, hybridized to a microarray, and then scanned. The signal from the microarray was detected and analyzed for 20 *KRAS* mutations in codons 12, 13, and 61. The results from the two methods were then compared.

Of 18 colorectal tumor samples tested, 17 were concordant between the 2 methods for the mutations that were detected in both assays. In the discrepant case, a mutation in codon 13 was found by the Autogenomics Infiniti assay that was not found by DNA sequencing, and we are investigating the cause of this. Mutations in codon 61 were not included in the DNA sequencing method, and with the Autogenomics Infiniti assay, we found codon 61 mutations in 2 of 18 cases. Our studies are ongoing.

We observed good concordance between the Autogenomics Infiniti assay and DNA sequencing methods for the detection of *KRAS* mutations in colorectal tumor samples. This study indicates the usefulness of the Autogenomics Infiniti method in a clinical laboratory setting where DNA sequencing capabilities do not exist.

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Sepsis Masquerading as an Acute Transfusion Reaction

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Acute transfusion reactions (ATRs) present with an array of symptoms ranging from urticaria to shock. The most severe reactions, hemolytic, anaphylactic, and transfusion-related acute lung injury (TRALI), may present with overlapping symptoms. Making the determination that a true ATR occurred and classifying it correctly can be challenging and underscore the role of clinical pathologists, who are often more familiar with them than is a patient's physician. We present a case that exemplifies this situation.

A 23-year-old woman with lupus nephritis receiving immunosuppressants presented to the emergency department (ED) with headache, nausea, vomiting, and malaise. Her workup revealed a hematocrit of 12%, and she was given 2 U of packed RBCs (PRBCs). Less than 30 minutes later, she became increasingly hypotensive and hypoxic (oxygen saturation decreased from 100% to 85%). Owing to the timing of the symptoms, the clinical team suspected a hemolytic transfusion reaction, which was excluded by a negative direct antiglobulin test and lack of plasma hemoglobin. However, the pathology resident noted that the posttransfusion chest radiograph had new interstitial edema and that her CBC count showed a drop in the leukocyte count, which led us to suspect TRALI.

We consulted with the ED physicians as to the proper supportive care for the possibility of TRALI. Unfortunately, the patient experienced cardiac arrest and was transferred to the intensive care unit. Then, intracellular bacteria were noted in her blood smear and the question of contaminated PRBC units was raised by the ED physician, who did not expect an infection owing to the absence of fever. However, Gram stain and cultures of both PRBC units were negative. In addition, a skin rash present initially, became bullous and hemorrhagic and was found to have gram-variable rods.

On consultation with her physicians, we pointed out that her first CBC in the ED had shown leukopenia with 60% bands, toxic granulation, Döhle bodies, and vacuolation of the neutrophils. Since she had received broad-spectrum antibiotics without prior blood cultures, the blood from the EDTA tube used for the CBC was cultured and identified *Escherichia coli*. Despite full support, she remained hypotensive and, ultimately, died 24 hours after admission. Postmortem examination showed no anatomic cause of death, and sepsis remained the most likely explanation for her clinical picture.

This case is an example of the difficulty in discerning an ATR from underlying medical conditions and the opportunity for clinical

pathologists to collaborate with the clinical teams to reach the proper diagnosis to treat accordingly.

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Functional Protein S Levels in Pregnant Women

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Protein S (PS) is a vitamin K–dependent cofactor for activated protein C. Together, these inactivate the procoagulant factors, Va and VIIIa. In the plasma of healthy people, about 40% of PS exists in the "free" form, while the remainder is protein-bound. Only the free form can interact with activated protein C. Numerous studies have shown that congenital and acquired PS deficiencies are associated with recurrent thromboses. During normal pregnancy, a transient decrease in free PS activity is known to occur, which may or may not reflect true deficiency. However, there is little agreement in how PS levels change during the phases of pregnancy. Current reference ranges for PS levels are based on healthy nonpregnant donors and may not be applicable to the pregnant population. The aim of our study was to determine how PS levels change during the trimesters of pregnancy and to see if reference ranges for each trimester could be established for this unique patient population.

The Laboratory Data Repository was searched to identify all pregnant and nonpregnant females at University Hospital who had PS activity testing between January 2008 and December 2009. A retrospective chart review was performed to identify pregnant vs nonpregnant females. Patients with a history of thrombosis were excluded. Estimated gestational age at the time of testing was established by first trimester ultrasound report. Functional PS levels were measured using a clot-based assay on a Diagnostica Stago instrument. Mean PS levels were calculated for nonpregnant females and for each trimester. Additional statistical analysis is ongoing.

An initial cohort of 255 pregnant and nonpregnant females was identified; 46 were excluded owing to a history of thrombosis. Of the remaining patients, 29 were in the first trimester, 32 in the second trimester, 30 in the third trimester, and 118 were not pregnant. The mean PS activity level in healthy, nonpregnant females was 83 (SD, 17; SEM, 1.57). Mean PS activity levels by trimester were as follows: first trimester, 56 (SD, 17; SEM, 1.57); second trimester, 48 (SD, 18; SEM, 3.18); and third trimester, 40 (SD, 17; SEM, 3.10). Compared with nonpregnant females, PS levels were on average 25% less, 40% less, and 50% less in the first, second, and third trimesters of pregnancy, respectively.

Functional PS levels fall progressively during pregnancy. However, there is significant overlap with the reference ranges for nonpregnant patients; therefore, separate reference ranges for pregnant patients cannot be reliably established. This phenomenon complicates screening for congenital PS deficiency during pregnancy because patients with congenital deficiency may have similar functional PS levels. Testing for a definitive diagnosis of PS deficiency should be delayed until after the postpartum period.

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Correlation Between Symmetric Dimethylarginine, Creatinine, Estimated GFR, and Cystatin C

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Symmetric dimethylarginine (SDMA) is an isomer of the endogenous nitric oxidase synthase inhibitor, asymmetric dimethylarginine. SDMA is synthesized by type 2 protein arginine methyltransferase from L-arginine residues. SDMA has a relatively stable production rate and is eliminated exclusively by renal excretion. Because of these features, the potential use of SDMA as an endogenous renal function marker has been proposed. The aim of this study was to evaluate the potential clinical usefulness of SDMA as a renal biomarker by comparing SDMA with 3 other conventional renal biomarkers—creatinine, estimated glomerular filtration rate (eGFR), and cystatin C (CysC)—in a small cohort of patients.

We randomly selected 98 adult patients (older than 18 years) who had been tested for creatinine and eGFR (creatinine, alkaline picrate method standardized against ID-MS, Roche Modular P; eGFR, MDRD equation) at our laboratory and showed an eGFR of less than 120 mL/min. These cases represented a wide range of eGFR (>60 mL/min, 12 cases; 30-60 mL/min, 20 cases; and <30 mL/min, 66 cases). The lithium heparin plasma samples were then tested for CysC (DAKO immunoturbidimetric assay, Roche Modular P) and SDMA (ELISA, IDEXX Labs). The results were analyzed by least-squares linear regression.

Statistically significant correlations were observed between SDMA and all 3 biomarkers. The Pearson correlation coefficient (r) was 0.63 between SDMA and creatinine, –0.71 between SDMA and eGFR, and 0.64 between SDMA and CysC ($P < .0001$ for all 3 pairs).

Our results showed that SDMA had a strong correlation with eGFR and good correlation with creatinine and CysC in a randomized cohort with a wide range of renal function. These results support that SDMA has potential clinical value as an endogenous marker for renal function. Further studies are needed to evaluate if SDMA offers better sensitivity and/or specificity than currently available renal function biomarkers in general or in specific patient populations.

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Lymphocyte Subset Quantitation by Flow Cytometry: Analytical Insights for the Clinical Laboratory

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Lymphocyte subset quantitation (CD45 total lymphocytes and T, B, and natural killer [NK] cells) by flow cytometry is widely used for the diagnosis and monitoring of patients with HIV and primary and secondary immunodeficiencies. Our objective was to evaluate the analytic performance of a lymphocyte subset quantitative flow cytometry assay in a high-throughput specialized immunology laboratory.

This assay was performed using a US Food and Drug Administration–approved kit (6-color Multitest kit, BD BioSciences). Data from January 2007 to December 2009 were retrospectively analyzed (n = 4,341). The analytic variability in the lymphocyte subsets during the 3 years for the observed and calculated cell counts was compared. The observed cell counts were obtained from the flow cytometric analysis for total lymphocytes (CD45), total T cells (CD3), helper T cells (CD4), cytotoxic T cells (CD8), B cells (CD19), and NK cells (CD16/56). Calculated results were obtained

as follows: (a) CD3 = CD4 + CD8; (b) CD45 = CD3 + CD19 + CD16/56; (c) CD19 = CD45 – (CD3 + CD16/56); and (d) CD16/56 = CD45 – (CD3 + CD19). The percentage difference between the calculated and observed results was derived as follows: 100 × (calculated – observed)/observed. The percentage differences were compared between the 3 years using the Kruskal-Wallis test (P values <.01 considered significant). All analyses were performed using SAS version 9 or JMP version 8.

The observed counts for CD45 were significantly greater than the calculated values with no change during the 3-year period. The observed total CD3 count was greater than the calculated value, while the CD4 and CD8 observed counts were less than the calculated results. During the 3-year period, the median difference approached zero, indicating improved concordance between the observed and calculated results. The interquartile ranges for these subsets also decreased, suggestive of reduced analytic variability over time (P < .0001). The CD19 and CD16/56 observed counts were less than the calculated values, and the median and interquartile ranges did not change over time (P, not significant).

The observed CD45 count was greater owing to the unintentional inclusion of monocytes within the lymphocyte gate, which also results in the CD19 and CD16/56 observed counts being lower than the calculated counts. Conversely, the observed CD3 count was greater owing to the exclusion of the small CD4+/CD8+ and/or CD4–/CD8– populations. For certain cell subsets, there was an improvement in analytic performance during the 3 years, which is likely related to improvement in operator skill in identifying and gating lymphocyte subpopulations.

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Successful Transfusion of hr^B+ Red Blood Cells Into a Patient With an Anti-hr^B Antibody
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Anti-hr^B (Rh31) is an anti-e–like antibody that recognizes a high-incidence epitope of the e antigen. The clinical significance of anti-hr^B is unclear. Two cases of hemolytic disease of the newborn associated with anti-hr^B were reported, although both mothers also had other clinically significant antibodies. In addition, 1 case of warm autoimmune hemolytic anemia was attributed to an "hr^B-like" antibody. Finally, there are no published cases of hemolytic transfusion reactions caused by anti-hr^B. Nevertheless, some sources advise that all antibodies in the Rh system should be regarded as potentially clinically significant and recommend transfusion of antigen-negative RBCs for all patients with Rh system antibodies. Owing to the rarity of such units, obtaining compatible blood for patients with anti-hr^B is difficult and potentially dangerous, sometimes requiring coordination with centers abroad, where infectious disease screening protocols differ from those in the United States. Therefore, additional information about the clinical significance of anti-hr^B would enable blood banks to determine whether these complicated, costly, and possibly hazardous logistical maneuvers are warranted.

We present the case of a 7-year-old boy with sickle cell disease who required transfusion of packed RBCs before removal of a biliary stent that had been placed during an episode of choledocholithiasis. Prior antibody screening showed anti-hr^B and anti-K; current antibody screening revealed a "new" anti-E, presumably the result of transfusion with C–/e–/E+hr^B– RBCs. Because anti-E is clearly

associated with hemolytic transfusion reactions, identification of this antibody necessitated administration of E– RBCs, ie, E–/e+/hr^B+ or (very rare) E–e+hr^B– RBCs. Our patient received 1 unit of E–/K–/C–/Fy^b–/s–/HbS– packed RBCs before stent removal. He had no signs or symptoms of a transfusion reaction or other clinical deterioration. However, the anti-hr^B in his plasma changed from below detectable before transfusion to weakly reactive by PEG IAT following transfusion.

Based on our review of the literature, this is the first reported case of transfusion of hr^B+ RBCs into a patient with an anti-hr^B. The absence of overt adverse effects in this case may influence clinical decisions regarding the management of patients with this antibody.

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Detection of an IgE Monoclonal Protein in a Patient With Previously Treated Multiple Myeloma
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In multiple myeloma (MM) and monoclonal gammopathy of uncertain significance, the most common secreted monoclonal protein is IgG, while IgD (2% of all MM cases) and IgE (approximately 40 described cases in the literature) are the rarest. A paraprotein detected during screening with urine and serum protein electrophoresis is further characterized by urine or serum immunofixation electrophoresis, usually measuring only the most common immunoglobulins. Testing guidelines for IgE and D are not well characterized. We report a case in which the follow-up of a patient initially diagnosed and treated for free κ light chain MM resulted in the diagnosis of an IgE-type myeloma.

Serum protein electrophoresis was performed on a liquid-based system, urine protein electrophoresis and serum and urine immunofixation were performed on a gel-based system, and serum free light chain studies were performed using nephelometry. Initial diagnosis of free κ light chain MM in this patient, then a 69-year-old man, was based on an M spike of 2.9 g/dL of monoclonal free κ chain in serum and a bone marrow biopsy finding of approximately 90% plasma cells (CD138+, κ light chain restricted). During early follow-up, negative paraprotein detection on serial serum and urine protein electrophoresis correlated with excellent clinical response to initial chemotherapy. However, approximately 11 months after the initial diagnosis, an M spike of 0.29 g/dL was detected on a follow-up serum protein electrophoresis with no paraprotein on urine protein electrophoresis. The presence of a solitary free κ monoclonal population on a follow-up serum immunofixation study in the absence of any monoclonal gammopathy in the urine protein electrophoresis led to additional immunofixation studies for IgE and IgD. This resulted in the detection of a monoclonal population with IgE specificity. This case report outlines a sequence of tests that indicates further testing for IgE and IgD.

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Evaluation of a Pneumococcal Rapid Latex Agglutination Test for Screening Positive Blood Cultures
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Bacteremia caused by *Streptococcus pneumoniae* is associated with high morbidity and mortality. Rapid latex agglutination tests are very useful because they allow early presumptive identification of pneumococci in blood cultures growing gram-positive cocci in pairs and chains. However, one known limitation of rapid latex agglutination tests is occasional cross-reactivity with other bacteria including other *Streptococcus* spp. In many cases, isolation of viridans streptococci or *Enterococcus* spp from blood cultures may not be clinically significant, so a false-positive result in the rapid latex agglutination test could potentially lead to initiation of unnecessary antibiotic therapy.

To evaluate the performance of the Wellcogen *S pneumoniae* rapid latex agglutination test (Remel, Lenexa, KS), we reviewed results of all blood cultures growing gram-positive cocci for a 14-month period. During this period, 144 grew gram-positive cocci in pairs and chains, and these were screened by the rapid pneumococcal latex agglutination test. Only 2 false-positive tests and 1 false-negative test were reported, resulting in a sensitivity of 97.3% and a specificity of 98.1%. Both false-positive results were subsequently identified as viridans streptococci. In this study, the rapid latex agglutination test performed very well and provided an early presumptive identification of *S pneumoniae* in positive blood cultures.

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Peripheral Blood Eosinophilia as a Clue to the Diagnosis of an Occult *Coccidioides* Infection

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A 70-year-old man from Arizona presented to Vanderbilt University Medical Center for evaluation as a candidate for radical prostatectomy. His medical history was significant for prostate cancer, coronary artery disease, and a 2-year history of cough that had been previously diagnosed in Arizona as new-onset asthma. Initial laboratory values showed macrocytic anemia. Further examination of the peripheral blood smear demonstrated profound eosinophilia, which on differential was found to be 16.5% of leukocytes. A computed tomography scan done as part of the workup to rule out metastatic prostate cancer revealed multiple, bilateral pulmonary nodules. They ranged in size from 1 to 9 mm with concomitant bulky mediastinal and hilar lymphadenopathy. Serology demonstrated the presence of *Coccidioides* IgG antibody and was negative for IgM antibody. A bronchoscopy with biopsy of the nodules and a paratracheal lymph node was negative for organisms and a neoplastic process. However, a week later, fungal cultures of the lymph node aspirate grew *Coccidioides immitis*.

Coccidioides species are found in the soil of the southwest United States. *Coccidioides* species cause a spectrum of disease that ranges from subclinical respiratory infection to the uncommon widespread systemic infection, coccidioidomycosis. Systemic sites include the skin, bones, and cerebrospinal fluid. Pulmonary nodules are a rare, but well-known complication that occurs in about 4% of patients with a respiratory infection. They present a diagnostic quandary in patients with cancer because of their resemblance to pulmonary metastases on imaging studies. Diagnosis is made with culture and serology. Treatment for symptomatic disease includes antifungal medications (Galgiani JN. *Coccidioides* species. In: *Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases.* 7th ed. Mandell GL, Bennett JE, Dolin R, eds. Philadelphia, PA: Churchill Livingstone Elsevier; 2010:3333-3344).

Peripheral blood eosinophilia is known to occur in patients with *Coccidioides* infection (Ward CM, Zuromskis PJ, Ives DV, et al. A case of fever, eosinophilia, and pneumonia. *Allergy Asthma Proc.* 2007;28:382-387). A previous case report suggested that peripheral blood eosinophilia may portend the presence of disseminated disease (Harley WB, Blazer MB. Disseminated coccidioidomycosis associated with extreme eosinophilia. *Clin Infect Dis.* 1994;18:627-629). This case highlights the importance of the lesser known clinical finding of profound eosinophilia in the diagnosis of *C immitis* infection.

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Prediction of Immunoassay Cross-Reactivity Using Chemoinformatic Methods

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Immunoassays are widely used in laboratory medicine for a variety of applications, including drug-of-abuse screening and endocrinology assays. False-positives on immunoassays can occur when the assay antibodies cross-react with molecules structurally similar to the target compound. In this study, we evaluated several computational approaches to predict immunoassay cross-reactivity.

The first type of method used molecular similarity algorithms to quantitate how similar a molecule is to the target molecule of the assay. Molecules with lower similarity would be predicted to be less likely to cross-react. The second approach uses quantitative structure-activity relationship analysis. With this approach, cross-reactivity is gathered on a data set of compounds. Models are then developed for the relationship between cross-reactivity and structural features of compounds. The models can then be applied to prediction of molecules that have not yet been tested. The third approach uses the 3-dimensional technique known as docking. Although the structures of immunoassay antibodies have not, to our knowledge, been reported, there are published studies of antibody-drug structures for some of the target molecules used in clinical immunoassays, including cocaine, digoxin, estradiol, morphine, and phencyclidine. We used these structures as surrogates for modeling how immunoassay antibodies might interact with analytes. Our best success so far in predicting cross-reactivity has been with 2-dimensional similarity. By using this technique for data sets derived from 30 drug-of-abuse and therapeutic drug monitoring assays, the receiver operating characteristic curves for predicting cross-reactivity have an average area under the curve of 0.9125 (range, 0.623 to 1.000).

The computational methods used are amenable to screening of large databases of drugs, drug metabolites, and endogenous compounds and may be useful for identifying cross-reacting molecules that would be unsuspected and not yet tested.

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Osmolality: What's in the Gap?

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The osmolal gap is routinely calculated for patients admitted to emergency departments (EDs) with symptoms of toxic concentrations of osmotically active substances, such as ethylene glycol, methanol, and isopropanol. The difference between the measured and calculated osmolality is the osmolal gap (OG). A recent study suggested the OG has increased.

We determined the OG range for healthy subjects and hospitalized patients. Serum samples were collected from 126 healthy subjects. The osmolality and glucose, sodium, and blood urea nitrogen (BUN) levels were measured. ED records at a tertiary referral center were searched to identify patients for whom a serum osmolality measurement was ordered in 1998 (n = 157) and 2007-2009 (n = 117). Cases were eligible if serum sodium, BUN, glucose, and osmolality were measured simultaneously. Cases in which ethylene glycol, isopropanol, and methanol were detected were excluded. Serum osmolality was calculated using 2 equations: (1) the general osmolality equation, Osm = 2(Na+) + Glucose/18 + BUN/2.8 + EtOH/4.6, and (2) the Glasser et al and Pappas et al equation, Osm = 1.86(Na+) + Glucose/18 + BUN/2.8 + 9 + EtOH/4.6, adding a general correction for ethanol (EtOH), dividing by 4.6. The MDRD equation was used to assess the glomerular filtration rate.

The healthy population had an OG ranging from –8 to 11 and 3 to 22 using equations 1 and 2, respectively. In 1998, 1 positive ethylene glycol sample was excluded (n = 156); OG ranges were –11 to 19 and –1 to 32, respectively. In 2007-2009, 7 total positive volatile screens were excluded (n = 110); OG ranges observed were –2 to 39 and 7 to 47, respectively. Renal insufficiency, present in 14% and 35% of the 1998 and 2007-2009 ED patients, respectively, had no effect on the overall OG ranges. With equation 2 for the 2007-2009 data set, the OG ranged from 11 to 53 and 6 to 44 for patients with and without a positive EtOH screen, respectively. Thus, EtOH caused a 12% increase in the OG range even after correcting for its presence.

The OG depends on the equation used to calculate serum osmolality. Physicians need to know the correct reference interval for the OG. Our study has shown that EDs have become more selective in the practice of ordering osmolality measurements, as evidenced by a 67% reduction in orders from 1998 to 2008. We found the OG reference interval, using equation 1 (–8 to 11) is still consistent with the general rule of –10 to 10 gap for a healthy person. Use of equation 2 requires use of a higher range: 3 to 22. The apparent increase in the OG observed in ED patients is likely due to more selective ordering.

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An Evidentiary Rules–Based Approach to Reducing Unnecessary Lab Testing

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Laboratory testing is an integral part of modern medical practice. Currently, more than 200,000 clinical laboratories conduct more than 7 billion tests that account for $50.6 to $66 billion (2.3%-3%) of national health care spending and influence the majority of patient care decisions. Hospitals are typically reimbursed for managing inpatients through a fixed fee based on a patient's medical condition. Unnecessary laboratory tests add to a hospital's expenses without leading to improved patient care. Although the cost of an individual laboratory test is small, systematic elimination of unnecessary tests can result in considerable cost savings. Herein we describe an evidentiary-based automated "rules" approach designed to reduce redundant testing in an inpatient population while maintaining patient outcomes.

Tests were targeted by review and analysis of more than 3 years' of laboratory ordering and result data by an expert panel. Rules were then generated and approved by the medical executive committee of the hospital in which the rules were first introduced (Penn Presbyterian Medical Center, a 344-bed community hospital of the University of Pennsylvania Health System) to establish a local standard of practice before implementation. Briefly, these rules were written to trigger when an order was placed in the laboratory information system (LIS) but subsequently via the hospital information system's order entry module to be collected with a "routine" priority. When the order crosses the interface to the LIS, the appropriate rules are implemented through the "Discern Expert" module in the laboratory's Cerner Classic Pathnet 3.06 LIS (Cerner, Kansas City, MO). After 6 months of close monitoring in the community hospital, where they were shown to do no harm, the rules were approved by the medical board of the 772-bed tertiary care flagship hospital. The impact of the rules on ordering practices and patient outcomes was monitored for 6 months and compared with month-matched data from the previous year. All patients for whom repetitive testing was indicated were exempted from the rules by design based on location, test, or attending physician (eg, troponin I testing).

Compared with previous ordering practices, overall test ordering was reduced by approximately 60% for phosphate, more than 50% for glucose and chloride, approximately 45% for magnesium, approximately 30% for albumin, and approximately 25% for calcium with no reported deleterious effects on patient outcomes.

A marked reduction in the number of repetitively ordered tests was effected in a large teaching hospital through logically developed rules. Nevertheless, ordering practices were unchanged by the implementation of the rules, demonstrating the need for their continuing application.

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Determination of Performance and Optimization of Delta Check Policies to Detect Specimen Mislabeling Using Simulation Studies

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The *delta check*, or real-time monitoring of changes in individual patient laboratory values, is an integral part of quality control procedures in the clinical laboratory. Originally developed to detect instrument failures, delta checks are now most useful for identifying mislabeled specimens. Real-time delta comparisons can be automated in laboratory information systems, but the subsequent confirmation must be performed manually. Thorough investigation of all potentially mislabeled specimens would be time-prohibitive, so thresholds are set to limit false-positive rates, or the proportion of "failed" delta checks for properly labeled specimens (eg, predialysis and postdialysis creatinine levels that are normally quite disparate). The tradeoff between sensitivity and specificity for delta checks has not been well characterized. Furthermore, there is no consensus on how to select analytes for delta checking to detect mislabeled specimens, and there are no established methods for selecting delta cutoffs. Quantitative analysis of delta check test performance is greatly limited by the low frequency of verified mislabeling events. Therefore, we used historic laboratory data to simulate specimen mislabeling.

Simulation studies were performed using the statistical language "R." Analytes included in the study were components of the basic metabolic panel or commonly used tests of liver function. Simulations conducted to mimic mislabels occurring on a ward identified total protein, albumin, alkaline phosphatase, creatinine, and blood urea nitrogen as having an area under the curve (AUC) greater than 0.8 in predicting mislabeling. Simulations of cross-ward mislabels identified a similar subset of analytes, and the AUC increased to more than 0.9 for all but creatinine. By applying our current laboratory delta check parameters on this data set, we calculated specificities ranging from 96% to 100% and sensitivities ranging from 3% to 43% for detecting potentially mislabeled specimens. Ongoing studies include the prospective application of revised delta check rules, as well as the identification of methods to detect "false-positive" delta check violations.

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Implementation of an Automatic Specimen Loss Metric at a Large Academic Medical Center

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The ability to quantify key components of the preanalytic phase of laboratory testing is essential for continuous, positive progression in the clinical laboratory. Among the most significant of measures is the number of specimens lost by the testing institution, defined here as the specimen loss rate. The current study presents a model of implementing a quality control metric centered on quantifying the current specimen loss rate.

The definition of specimen loss used in this study encompassed physically lost specimens as well as "perfectly good" specimens that, owing to laboratory error, were no longer adequate for testing. By using an automated report initiated from the laboratory information system and run in the statistical language "R," we monitored various laboratory activities, including cancelled and credited tests and the subset of cancelled tests attributed to specimen loss. A retrospective analysis of the specimen loss rate during a 3-month period was determined to be 0.25%. Because the accuracy of this metric is directly dependent on selection of the correct cancellation codes by the staff, we investigated the current use of available cancellation codes as a way to assign a level of confidence in the determined rate. We found a total of 83 codes available for use, many with vague or generic interpretations and several with a high degree of overlap, making accurate code assignment unnecessarily difficult if not virtually impossible. The cancellation code, although seemingly unimportant when compared with typical laboratory results, becomes a part of the medical record, and, as such, the misclassification of test cancellations results in an inaccurate medical record and the inability to effectively monitor laboratory operations. A pilot project that standardized around a subset of 10 codes yielded increased accuracy of code use (determined by the number of cancelled tests determined to have been incorrectly assigned before and after code restructuring) and increased confidence in the accuracy of the metric assigned to monitor specimen loss (postpilot = 0.13%) and other preanalytic components of the laboratory.

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Supraphysiologic Doses of Ascorbic Acid (Vitamin C) Can Effectively Prevent Bone Loss Caused by Ovarian Failure

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Postmenopausal osteoporosis is a global health problem and the leading cause of morbidity in elderly people. Epidemiologic data demonstrate that most of women's bone loss occurs in late perimenopause and early menopause. During this time, while levels of estrogen are relatively stable, there are dramatic increases in the levels of the pituitary hormone, follicle-stimulating hormone (FSH). Based on the hypothesis that FSH contributes to perimenopausal bone loss, we demonstrated that FSH directly stimulates bone degradation by augmenting the osteoclastogenesis.

Animals engineered to be genetically deficient in FSH or its receptor were protected from the bone loss accompanying estrogen deficiency. We recently focused on the pathophysiologic mechanisms whereby FSH exerts its proresorptive effects. We have shown that FSH rapidly induces the production of the inflammatory and proosteoclastic cytokine tumor necrosis factor (TNF) in murine bone marrow monocytes and granulocytes. We have extended these observations to human monocytes and now show that FSH directly stimulates TNF production in human osteoclast precursors and in differentiating osteoclasts. The mechanism whereby TNF was able to augment osteoclast formation was subsequently investigated. TNF induced the activation of NF-κB, JNK, p38 MAPK, and ERK pathways; these same pathways were previously found to be stimulated by FSH, suggesting that FSH-induced TNF production enhances FSH-induced signaling. Because studies in other tissue types have suggested that NF-κB inhibition leads to apoptosis after TNF application, we examined if NF-κB inhibition would prove a plausible drug target.

Ascorbic acid (vitamin C) was identified in the literature as a nontoxic, low-cost drug inhibitor of IKK (the kinase upstream of NF-κB). To test whether ascorbic acid could prevent the bone loss caused by ovarian failure, we ovariectomized animals and treated them with and without ascorbic acid. We report that daily treatment with supraphysiologic doses of ascorbic acid prevents trabecular bone loss caused by estrogen deficiency (ovarian failure). These results suggest that high FSH levels drive TNF-mediated osteoclastogenesis and that inhibiting IKK with high doses of ascorbic acid can prevent the early menopausal bone loss caused by estrogen deficiency.

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Total 25-Hydroxyvitamin D Method Comparison Between Two Commercially Available Automated Immunoassays

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Vitamin D deficiency is common among adults and children worldwide and has been linked to growth retardation and skeletal deformities in children and risk of hip fractures, osteopenia, and osteomalacia later in life. Furthermore, recent evidence suggests that vitamin D may have a role in decreasing the risk of chronic illnesses. Renewed interest in this vitamin has led to the development of high-throughput methods to measure 25-hydroxyvitamin D (25-OHD), which is the major circulating metabolite. Although methods exist that can distinguish D_2 (plant sources) from the more active metabolite D_3 (cutaneous UV-B irradiation), current immunoassay platforms

measure both forms and report total 25-OHD. While standardization and harmonization of 25-OHD methods is likely to occur in the near future, current studies that address possible assay performance differences and how they may affect medical decisions, especially at the generally accepted less than 20-ng/mL cutoff for vitamin D deficiency, are urgently needed. Our objective was to compare method performance of 2 commercially available automated platforms that measure total 25-OHD.

Clinical plasma specimens that were destined for 25-OHD measurement by DiaSorin Liaison (ARUP) were divided in aliquots and measured by Immunodiagnostic Systems IDS iSYS (Emory). Intra-assay and interassay precision values were determined using respective assay controls and 25-OHD$_2$- and D$_3$- spiked 7% bovine serum albumin (BSA). Method comparisons were assessed on 215 plasma samples and BSA spiked with varying concentrations of purified 25-OHD$_2$ or D$_3$.

IDS interassay precision values using accompanying assay controls were 19.5% (6.5 ng/mL), 12.4% (27.3 ng/mL), and 15.9% (64.9 ng/mL), while DiaSorin intra-assay precision values were 3.5% (10.9 ng/mL), 5.0% (40.4 ng/mL), and 3.4% (69.2 ng/mL). Initial evaluations between the IDS (y) and the DiaSorin (x) methods on plasma samples yielded a correlation of y = 0.76x + 6.32; r^2 = 0.59. Furthermore, 25-OHD$_2$ recovery for both assays was lower than expected. The 2 assays were in 83.3% concordance in identifying patients with or without vitamin D deficiency according to the less than 20-ng/mL cutoff.

The IDS assay exhibits good performance characteristics regarding assay precision and acceptable correlation with the DiaSorin assay. The fair concordance between the 2 assays further underscores the importance of standardization and harmonization of 25-OHD assays for the correct identification of vitamin D deficiency.

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New Technology in Testing Bacterial Contamination of Platelets
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One of the largest infectious disease risks in transfusion medicine occurs in bacterial contamination of platelet products. Current studies suggest that contamination occurs in up to 1:3,000 units collected by accidental inclusion of donor skin flora in the sample or by collection of products from patients with asymptomatic bacteremia. If bacterial concentrations in contaminated platelet units are low at the time of collection, they are not reliably detectable by available test methods in samples drawn at that time. With apheresis platelets, a small sample drawn from the donor unit is cultured and observed over time, thereby enabling detection of a contaminated unit before transfusion. In the case of random donor platelet units (RDPs), however, the size of the donor unit is such that removal of the necessary amount of fluid to culture the product would drastically deplete the unit size, and, thus, culture is not usually done.

In our institution, RDPs are tested for bacteria with a urine dipstick before pooling and transfusion. This method is reliable only when a large portion of the component is sufficiently contaminated. A newly available technology, Verax Pan Genera Detection (PGD), detects the presence of conserved antigens lipoteichoic acid and lipopolysaccharide found on aerobic and anaerobic gram-positive and gram-negative bacteria, respectively. Therefore, our purposes for this study were to evaluate the efficacy of PGD technology in detecting bacterially contaminated RDPs and to compare outcomes with the currently used urine dipstick method in our blood bank.

We conducted daily testing of all RDPs pooled for transfusion with the urine dipstick and PGD methods simultaneously. Positive units by either test were plated for culture on sheep's blood agar. Preliminary results on 342 pooled RDPs showed that all units were negative by the urine dipstick method. Three units were positive by PGD technology for gram-positive organisms, but all were negative on follow-up culture. Repeated testing by PGD technology revealed negative results. More samples are being collected to further elucidate the efficacy of the new technology within our blood bank.

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False-Positive HIV and HCV Serology Due to Nonspecific Antibody Binding in a Patient With Polyclonal Hypergammaglobulinemia
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Current enzyme immunoassays (EIAs) for detection of antibodies to HIV and hepatitis C virus (HCV) are very sensitive and specific. However, false-positive reactions can occur by a number of mechanisms, including interference from heterophil antibodies and nonspecific antibody binding. Our objective was to determine the cause of falsely reactive HIV and HCV serology in the same patient.

The subject was a 69-year-old man with chronic alcoholic hepatitis (CAH) and cirrhosis referred for liver transplant evaluation, including infectious diseases serology. HIV serology was performed using the Advia Centaur HIV 1/O/2 Enhanced Assay (Bayer HealthCare) and was repeatedly reactive (index, >50). HIV-1 Western blot (WB; Bio-Rad) was performed but was negative with no bands detected. The HIV assay was repeated with a different serum tube and was again positive, but a repeated WB remained negative. HCV serology was performed using the Advia Centaur HCV Assay and was also repeatedly reactive (index, 1.39). However, a negative HCV RIBA (Chiron) confirmed the absence of HCV antibodies. Serum samples were also negative for HIV with the OraQuick Advance HIV-1/2 (OraSure) and HIVAB HIV-1/HIV-2 EIA (Abbott) assays and negative for HCV with the Architect Anti-HCV EIA (Abbott) assay. When the serum was treated with the nonspecific antibody binding tube (Scantibodies), which blocks nonspecific antibodies, the Advia Centaur HIV and HCV assays both returned negative results. This indicated that these 2 assays were both falsely reactive owing to nonspecific antibody binding. Serum protein electrophoresis and immunofixation revealed a polyclonal hypergammaglobulinemia (PHG) pattern consistent with a chronic inflammatory state, likely due to polyclonal B-cell proliferation induced by CAH. The PHG in this patient seems to be the source of the nonspecific antibody binding interference that was specific only to the Advia Centaur HIV and HCV assays but none of the other assays.

PHG has previously been shown to cause false-positive HCV EIA results in children with autoimmune hepatitis and false-positive HIV EIA results in acute malaria infections in younger people in Africa. Fortunately, this type of reaction seems rare; however, nonspecific antibody binding should be considered a source of potential false-positive immunoassay results in patients with known PHG or conditions associated with PHG. Therefore, clinicians and laboratory professionals should be aware of this phenomenon.

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Positive B-Cell Flow Cytometric Crossmatch Correlates With Microcirculation Injury in Late Kidney Allograft

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Anti-HLA antibody-mediated microcirculation injury (MI) is the major cause of late kidney transplant failure. While the presence of anti-HLA antibodies detected by T-cell flow cytometric crossmatch (FCXM) has been reported to inversely correlate with kidney graft survival, the clinical significance of positive B-cell FCXM (B+ FCXM) is still a matter of debate, particularly in long-term graft outcomes. In this study, we investigated the impact of B+ FCXM on the survival and MI of kidney allografts.

A total of 196 of 598 patients who received kidney or kidney/pancreas transplants at the University of Chicago Medical Center between January 1998 and December 2003 were randomly selected. Pretransplant serum samples were tested for the presence of HLA antibodies using Luminex assays. The presence of anti-HLA antibodies was evaluated using complement-dependent cytotoxicity (CDC) crossmatch (XM) and/or T- and B-cell FCXM methods. All patients were negative for T- and B-cell AHG-CDC XM and T-cell FCXM. Of the patients, 27 (13.8%) patients were B+ FCXM; 16 patients in the B+ FCXM group had early (<1 year posttransplant) and late (>1 year posttransplant) biopsies, while 95 and 87 patients in the B– FCXM control group had early and late biopsies. Of 16 patients, 10 (63%) and 5 (31%) B+ FCXM patients with late biopsies had a diagnosis of MI and positive C4d staining. In contrast, only 5 (31%) and 0 (0%) B+ FCXM patients with early biopsies had a diagnosis of MI ($P = .33$) and C4d+ ($P = 1$). A strong association was found between B+ FCXM and MI ($P = .0095$; relative risk [RR], 2.26; 95% confidence interval [CI], 1.36~3.77)/C4d+ ($P = .03$; RR, 1.31; 95% CI, 0.94~1.84) in late biopsies but not in early biopsies (MI, $P = .33$ and C4d+, $P = 1$). In addition, patients with MI, C4d+, and B+ FCXM were more prone to lose their grafts than patients with B– FCXM (MST, 5.92 and 8.81 years, respectively). Moreover, patients with MI had shorter graft survival time (MST, 5.8 years) than patients without MI (MST, 11.2 years). Of 27 B+ FCXM patients, 12 (44%) had HLA antibodies and 6 (22%) had donor-specific antibodies (DSA). All patients with DSA (n = 6) showed MI lesions in their late biopsies.

Prospective studies are needed to evaluate whether posttransplantation monitoring of DSA is clinically useful in identifying patients at risk for early graft loss. These results revealed a strong association among B+ FCXM, C4d+, MI, and reduced graft survival in late kidney allografts.

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Impact of Preformed, Donor-Specific, Anti-HLA Antibodies on Combined Liver Kidney Transplant Outcome

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Presence of HLA antibodies, particularly donor-specific antibodies (DSA), in kidney transplant recipients is a known risk factor for antibody-mediated rejection and is routinely tested. Antibody-mediated rejection (except ABO incompatibility) is rare in liver graft, which may also protect a concomitant kidney graft from humoral rejection. The exact mechanism of this protection is unknown. There

are a few single-case reports showing that protection may not be complete and kidney can also be rejected in combined liver-kidney transplant (CLKT). However, anti-HLA antibodies are not routinely screened in CLKT. We aimed to retrospectively study the preformed HLA antibodies in a CLKT case series and to examine how the presence of HLA antibodies or DSA affects the survival of kidney grafts in CLKT patients.

Frozen pretransplantation serum samples from cadaveric kidney transplant (133 randomly selected from a total of 338 between 1998 and 2003) and CLKT recipients (23 available of a total 55 between 1992 and 2009) at the University of Chicago Medical Center were retrieved. These were tested for HLA antibodies using a solid-phase Luminex platform. Clinical data were collected, and Kaplan-Meier survival statistics and univariate Cox regression analysis were performed.

Of 23 CLKT cases, 9 (39%) had pretransplantation anti-HLA antibodies, of which 7 (30%) had DSA, all with mean fluorescence intensity (MFI) more than 1,000. Among the kidney cases, 44 (33.1%) of 133 had pretransplant antibodies, of which 18 (13.5%) had detectable DSA with only 13 (<10%) MFI more than 1,000. There was no significant association between HLA antibodies and DSA with allograft survival in CLKT ($P = .892$ and $P = .400$, respectively) and the kidney group ($P = .598$ and $P = .472$, respectively). The 5-year survival for CLKT patients was 37.47% vs 62.74% for kidney patients, but the difference was not significant ($P = .098$).

Although the CLKT sample was small, pretransplantation DSA antibodies do not appear to impact long-term kidney survival in CLKT recipients. The DSA in the kidney-only group was generally weak because patients who had stronger DSA may not have received the transplant owing to a positive crossmatch, a contraindication for kidney transplants. The CLKT patients have higher mortality rate compared with kidney patients, shown by the Kaplan-Meier analysis, similar to the trend in national UNOS survival data, possibly due to other comorbidities and recurrent liver disease.

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Determination of 5-Oxoproline and Other γ-Glutamyl Cycle Metabolites in Acute and Chronic Acetaminophen Intoxication Using LC-MS/TOF

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This study sought to determine whether 5-oxoproline accumulation was associated with anion gap metabolic acidosis in short- and long-term exposure to acetaminophen using LC-MS/TOF. The study also sought to determine if other metabolites of the γ-glutamyl cycle can be detected.

In 2 cases of acetaminophen exposure, serum and urine samples were analyzed using the Agilent liquid chromotaograph (LC) time-of-flight mass spectrometer (LC1200-MS/TOF 6230). The chromatograms obtained were analyzed using Agilent's MassHunter Qualitative Analysis and Quantitative Analysis software to determine the presence and levels of 5-oxoproline and other metabolites of γ-glutamyl cycle that may be elevated in oxoprolinuria.

Two patients had a widened anion gap metabolic acidosis following acetaminophen ingestion. The first patient had confirmed acute acetaminophen overdose (371 µg/mL) from ingestion of Vicodin. Her acidosis (AG, 24) was accompanied by hepatic injury (peak alanine aminotransferase level, 8,045 U/L; aspartate

aminotransferase level, 8,026 U/L). The second patient complained of chronic abdominal pain and vomiting that had been ongoing for 3 months. She reported long-term ingestion of acetaminophen and carisoprodol. Her serum acetaminophen level at admission was within the therapeutic range (3 μg/mL), and her liver function test results remained normal throughout observation. Her initial AG was 22.

By using LC-MS/TOF, 5-oxoproline was detected in the urine sample of the first patient (0.1 mmol 5-oxoproline/mmol creatinine) and was also positively identified in the serum sample (35.8 mmol/L 5-oxoproline) and urine sample (0.65 mmol 5-oxoproline/mmol creatinine) of the second patient. In addition, test data indicated the possible presence of γ-glutamylcysteine in the samples.

Oxoprolinuria was associated with a widened anion gap metabolic acidosis in 2 patients with acetaminophen overexposure, one short-term and the other long-term. Moreover, γ-glutamylcysteine, a precursor of 5-oxoproline and the initial metabolite overproduced in the γ-glutamyl cycle in episodes of glutathione depletion, was also detected. 5-Oxoproline (pyroglutamic acid) may explain the clinical presentation of acidosis early in acetaminophen toxicity independent of frank hepatic failure.

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Investigating Possible Causes of Discrepant Creatinine Results: Discordance in Patients With Renal Insufficiency
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Monthly correlation analyses at Johns Hopkins Hospital have revealed a subgroup of patients whose creatinine results do not correlate between the Nova EZ CHEM Point-of-Care (POC) Creatinine Meter and an enzymatic central laboratory method. This ongoing investigation aimed to determine the frequency of such discrepancies and explore possible causes by further examination of the patient population in question.

We tested 150 heparinized whole blood samples from hospital inpatients with the EZ CHEM POC Creatinine Meter and compared the results with results from an automated end-point creatininase enzymatic method (Roche Hitachi Modular) and an isotope dilution mass spectrometry (IDMS) reference method. When compared with the IDMS method, the enzymatic method correlated well (y = 1.032x + 2.320; r^2 = 0.987), while POC results did not correlate as well (y = 0.777x + 0.643; r^2 = 0.791). Thirty-four samples (from 28 people) had creatinine results that differed between the POC and automated enzymatic methods by more than 0.5 mg/dL (22.7% of all samples). Of these samples, 68% had enzymatic creatinine results more than 2.0 mg/dL. The mean EZ CHEM creatinine value in this group of patients was 4.20 mg/dL, compared with 1.37 mg/dL in an age-, sex-, and race-matched control group. Patient age, sex, race, hematocrit, arterial pH, arterial pO₂, estimated glomerular filtration rate (eGFR), bilirubin, creatine, currently prescribed medications, and diagnosis were all documented and compared between groups. No differences in blood pH, pO₂, bilirubin, creatine, or currently prescribed drugs were noted between the 2 groups.

Correcting POC creatinine values for varying hematocrit levels and differences between plasma and whole blood determinations did not explain the noted discrepancies. However, 68% of patients with discrepant results had eGFR values less than 30 mL/min/1.73m², compared with 7% of the control group. Of patients whose results did not correlate between the POC instrument and the automated method, 50% had some form of renal insufficiency, compared with only 21% of patients in the control group. Overall, discrepancies

were observed in patients with elevated creatinine values (>2.0 mg/dL). Patients with creatinine values in this range commonly have renal insufficiency, which may have a role in the lack of agreement between these 2 instruments.

After investigating likely interfering substances, imprecision of the POC meter at higher creatinine levels seems to be the primary source of the discrepancies observed. POC creatinine levels should be interpreted with caution in this population.

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Urinary Estrogens and Estrogen Metabolites in Women With Pulmonary Hypertension
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Pulmonary arterial hypertension (PAH) is a devastating disease of the smallest pulmonary arteries. The greatest known risk factor for PAH is sex, with a 4:1 ratio of females/males. We recently reported that females with PAH have an abnormal 2-OH1/2:16-αOHE1 ratio in the urine, as measured by enzyme-linked immunosorbent assay (ELISA) (Austin ED, Cogan JD, West JD, et al. *Eur Respir J.* 2009;34:1093-1099).

The determination of estrogen metabolite levels has traditionally involved ELISA, which is restricted to available antibodies and does not measure all metabolites. Owing to these limitations, we developed a gas chromatography–mass spectrometry (GC-MS) method to quantitate simultaneously in urine the parent estrogens, estrone (E1) and estradiol (E2), and the catechol estrogens, as well as methoxy estrogens and metabolites in the 16α pathway, including estriol (E3).

We optimized pH and temperature conditions for the hydrolysis and examined ethoximation and oximation to protect E1. Extraction methods were investigated using liquid-liquid partitioning into organic solvents such as acetonitrile, diethylether, dichloromethane, isopropanol, and ethyl acetate. Chromatographic separation of each group of compounds was optimized to achieve complete resolution of all analytes producing common ion fragments in the mass spectrometer. By using this method, we analyzed urine samples from healthy women matched by age with patients with PAH.

We determined the levels of parent compounds and metabolites and found that the parent hormones E1, E2, and E3 comprised the predominant fractions in all women (each >10%). Catechol and methoxy estrogens were also detected, but at lower quantities, with the exception of 2-OHE1, which was also more than 10%. Patients with PAH had a reduced ratio of 2-OH1/2:16-αOHE1 compared with control subjects.

GC-MS provides a more detailed method of evaluating urinary estrogens compared with traditional ELISA testing. Alterations in urinary estrogen metabolite ratios suggest differences in estrogen metabolism in patients with PAH, which may contribute to disease pathogenesis and explain the profound sex disparity in this devastating disease. The urinary estrogen metabolic profile may serve as a biomarker for people at risk for PAH.

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Evaluation of a Rapid Real-Time PCR Assay for KPC-Producing Gram-Negative Pathogens Directly From Blood Culture Bottles

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Klebsiella pneumoniae carbapenemase (KPC)-producing gram-negative bacilli (GNB) are endemic in New York City hospitals and are causing serious infections on national and global levels. Their rapid detection from positive blood cultures can impact therapeutic and infection control decisions. This study evaluates the performance of a rapid real-time (RT) polymerase chain reaction (PCR) assay to detect KPC-producing GNB directly from blood culture bottles.

DNA was extracted from 393 blood culture bottles with GNB collected from January 2009 to February 2010, using the BioRobot EZ1 automated system (Qiagen, Valencia, CA). RT-PCR was performed on a SmartCycler (Cepheid, Sunnyvale, CA), using primers and fluorescently labeled probes that target a plasmid sequence common to the currently known KPC genotypes. PCR results were compared with the results of culture-based methods as a "gold standard." Culture confirmation of carbapenemase production included imipenem, meropenem, and ertapenem resistance by automated and manual methods. Pulsed-field gel electrophoresis (PFGE) of selected isolates was also performed.

Of 393 blood culture bottles tested, 323 (82.2%) were positive for enteric GNB. KPC-producing pathogens, detected in 28 (8.7%) of 323 bottles, included 23 (82%) *K pneumoniae,* 4 (14%) *Escherichia coli,* and 1 (4%) *Enterobacter cloacae.* In comparison with culture, the sensitivity, specificity, positive predictive value, and negative predictive value of the PCR assay were 90%, 99%, 96%, and 99%, respectively. Of 323, 4 strains (1.2%) gave discordant results. Two *K pneumoniae* isolates and 1 *E coli* isolate were KPC false-negative, suggesting carbapenemase production by some other mechanism. The remaining isolate, an *E coli,* was false-positive by KPC PCR. However, subsequent blood cultures from the same patient continued to show *E coli* that was KPC-positive, while demonstrating varying susceptibility to carbapenems ranging from sensitive to resistant. Further analysis of the original and later *E coli* isolates by PFGE suggested that the carbapenem resistance evolved in a single *E coli* strain.

Our results demonstrate that the RT-PCR test is rapid and accurate for detecting carbapenem-resistant GNB pathogens directly from blood culture bottles. This PCR assay decreased the time to detection of carbapenem-resistant GNB pathogens from 72 hours by culture-based methods to 4 hours, which can impact the selection of an optimal therapeutic regimen.

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New Rapid In Situ Hybridization Technique to Identify Epstein-Barr Virus Infections in Tissues: A Single Institution's Preliminary Experience

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Epstein-Barr virus (EBV) is an oncogenic virus that is widely distributed throughout the world, with most people having been infected by the time of adulthood. Most immunocompetent people have little trouble once the initial infection resolves. However, in a subset of people, an infection-related neoplasm like Burkitt lymphoma and Hodgkin lymphoma will develop. Immunosuppressed people are even more at risk for developing neoplasms, particularly EBV-driven lymphoproliferative disorders, because their immune systems cannot control the virus. The detection of an EBV infection in tissue can be made by using immunohistochemical and in situ hybridization techniques. The problem with immunohistochemical staining is low sensitivity (false-negatives) and high background staining. Currently available in situ hybridization techniques eliminate the problem of background staining but often require 1 or 2 days to provide a result. The ability to provide same-day results could dramatically improve turnaround times. The objective of this study was to compare the use of a new rapid in situ hybridization (RISH) staining technique with the current method used in our immunohistochemistry laboratory.

The RISH technique uses a digoxigenin-labeled DNA probe for the Epstein-Barr encoded RNA (EBER) 1+2. This probe is then labeled with an unconjugated antidigoxigenin antibody, and the reaction is visualized with the addition of a chromogen. Our laboratory's current method is an automated technique performed on a Ventana BenchMark Automated Stainer using the Ventana INFORM EBER probe (Ventana, Tucson, AZ). The RISH technique takes approximately 3 hours from start to finish compared with 6 hours for our automated technique. We initially obtained 6 paraffin-embedded, formalin-fixed specimens that had been previously stained for EBER by our automated method.

The cases included negative (1 case), rare scattered positive (1 case), and diffusely positive (4 cases) specimens. There was 100% concordance between the 2 methods. Even the degree of positive cells was concordant in the case that had only scattered rare positive cells. Our results show that RISH staining technique is faster, taking half the time of our currently available method, and significantly faster than manual methods and accurately determines EBV infection in the tissues.

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Pediatric Reference Intervals for IGF-1, IGFBP-3, and Growth Hormone Considering Age, Gender, Pubertal Maturation, Height, and Body Mass Index

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Pediatric reference intervals (RIs) for insulin-like growth factor-1 (IGF-1), insulin-like growth factor binding protein-3 (IGFBP-3), and human growth hormone (HGH) can be useful for assessment of disorders of stature. Pathologic conditions include acromegaly, dwarfism, hypopituitarism, growth hormone deficiency, and multiple endocrine neoplasms. For conditions due to deficiencies, a younger age of onset can lead to a greater loss of stature. It is important to have RIs from as close to birth as possible because early clinical intervention can preserve stature.

Our study analyzed the data for 2,866 healthy children who were 6 months through 17 years old. An equal number of boys and girls from each year of life were included. All analytes were measured by chemiluminescent immunoassays with an IMMULITE 2000 analyzer. We did not include children younger than 7 years for HGH RIs. We partitioned our data by age and sex. Our groups for boys and girls were 6 to 24 months, 2 to 3 years, 4 to 5 years, 6 to 7 years, 8 to 9 years, 10 to 11 years, 12 to 13 years, 14 to 15 years, and 16 to 17 years.

We found a significant increase in IGF-1 for boys that continued from 4 to 5 years up through 8 to 9 years and also for girls 10 to 11 years up to 12 to 13 years. IGF-1 peaks at an earlier age in girls, and RIs for girls were higher for each age. We also established RIs for boys and girls in each Tanner stage. Girls in Tanner stage 3 had the highest peak for IGF-1. IGF-1 RIs decreased and reached a plateau at age 14 to 15 years and Tanner stage 4 for both sexes. When the less than third and more than 97th percentiles for height

were eliminated for each age, we saw a change in the RIs for IGF-1 and IGFBP-3 but not for HGH. RIs for HGH trend higher for higher body mass indices (BMIs) and lower for lower BMIs. RIs for IGF-1 and IGFBP-3 for subjects with BMIs less than the 25th percentile were significantly lower than those from subjects with BMIs more than the 75th percentile.

When using IGF-1, IGFBP-3, and HGH for clinical assessment, one should consider the child's pubertal maturation and, more important, their BMI, rather than just age and sex.

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Multiplex Cytokine Analysis for the Differentiation of SIRS and Sepsis

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Sepsis is a major cause of mortality in critically ill patients and is challenging to differentiate from systemic inflammatory response syndrome (SIRS). SIRS is identified by the presence of 2 or more of the following characteristics: abnormal body temperature, WBC count, respiratory rate, and blood pressure. Sepsis is SIRS in the presence of a documented infection. Rapid identification and treatment of septic patients with antibiotics significantly reduces morbidity and mortality; patients with SIRS do not require antimicrobial therapy. Thus it is desirable to have a rapid and reliable method for detecting sepsis in patients with SIRS. Studies of single biomarkers, including procalcitonin, for sepsis have been relatively unsuccessful, likely owing to the complex pathobiology of the disease. Sepsis may be best assessed through a panel of biomarkers. Our objective was to identify a panel of biomarkers that accurately detects sepsis in patients with SIRS.

We used 64 leftover plasma samples collected from intensive care unit patients on the first day they had SIRS (identified through an automated electronic medical record scan). Of these patients, 26 had culture-confirmed sepsis and 38 no bacterial infection 3 days before or after specimen collection. Concentrations of 8 cytokines, interleukin (IL)-1β, IL-6, IL-8, IL-10, MCP-1, granulocyte-macrophage colony-stimulating factor (GM-CSF), tumor necrosis factor (TNF)-α, and IFN-γ, were determined by simultaneous multiplex analysis on the Luminex platform.

Receiver operating characteristic curves generated for each cytokine gave areas under the curve of 0.58 ($P = .26$) for IL-1β; 0.74 ($P = .001$) for IL-6; 0.57 ($P = .34$) for IL-8; 0.64 ($P = .05$) for IL-10; 0.62 ($P = .10$) for GM-CSF; 0.70 ($P = .009$) for MCP-1; 0.60 ($P = .18$) for TNF-α; and 0.53 ($P = .71$) for IFN-γ. Further analysis was restricted to analytes with significant ($P < .05$) curves. Cutoffs were determined at a sensitivity of 70% for all markers based on published data for single sepsis biomarkers in clinical use. For IL-6, IL-10, and MCP-1, cutoffs were 63, 15, and 642 pg/mL, respectively. At 70% sensitivity, the specificity, positive predictive value (PPV), and negative predictive value (NPV) were 70%, 72%, and 67% for IL-6; 68%, 71%, and 66% for IL-10; and 76%, 76%, and 68% for MCP-1. When combined, the sensitivity, specificity, PPV, and NPV were 46%, 84%, 67%, and 69% and 85%, 43%, 51%, and 80% for 3 positive markers (IL-6, IL-10, and MCP-1) and at least 1 positive marker (IL-6, IL-10, or MCP-1), respectively.

The diagnostic usefulness of IL-6, IL-10, and MCP-1 alone is similar to data on the US Food and Drug Administration–approved marker procalcitonin in distinguishing sepsis from SIRS. Combining multiple markers improves specificity at the expense of sensitivity.

Future work will combine additional markers like procalcitonin and clinical indicators such as neutrophil counts.

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A One-Year Retrospective Review of the Use of QuantiFERON-TB Gold In Tube at the University of Alabama at Birmingham

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The University of Alabama at Birmingham (UAB) is a large academic institution employing approximately 7,000 people. The Occupational Safety and Health Administration requires all health care workers to be screened annually for latent tuberculosis infection (LTBI) with a tuberculin skin test (TST). However, the TST is subject to problems in administration and interpretation. The test also can have false-positives due to infection by a nontuberculous mycobacterium or previous administration of bacille Calmette-Guérin (BCG) vaccine. The latter is an important problem at academic centers, where a significant portion of the house staff is foreign born and had the BCG vaccine. Determining true LTBI is crucial for patient protection because a course of isoniazid (INH) decreases the risk of active disease in health care workers. It is also important for health care workers because INH is not an entirely harmless medication. The QuantiFERON-TB Gold In Tube (QFT) test is a relatively new whole blood test for diagnosing *Mycobacterium tuberculosis* infection. The test is not affected by BCG immunization or infection by most environmental mycobacteria.

UAB brought QFT in house in April 2008 to screen the large number of health care workers who have a positive TST. In 2009, 147 QFT tests were performed on 68 females and 76 males; in 3 patients, the sex was unknown. The average age of the patients was 36 years (range, 12-82 years; median, 33 years). Positive, negative, and indeterminate results were found in 26.5% (39), 70.0% (103), and 3.4% (5) of the tests, respectively. The reason for ordering the test was not available for 6.1% (9) of the tests. Surprisingly, employee health ordered only 34.7% (51) of the tests. The test was ordered on all UAB workers who had a positive TST and refused INH treatment. Of the 16 (31%) workers who had a positive QFT, only 6 (38%) agreed to take INH. The majority of QFT tests (50.3% [n = 74]) were ordered on patients who were not UAB health care workers but were hospitalized patients with suspected clinical tuberculosis (58% [n = 43]) and patients at outpatient clinics undergoing screening for LTBI (42% [n = 31]). The main reason for the use of QFT vs the TST in the latter population was convenience, ie, not having the patient travel to a health care worker to have the TST read at 48 to 72 hours. Student health ordered 7% (10) of the tests.

The use of QFT is increasing in hospitalized patients and in the outpatient clinics. Unfortunately, the additional use of QFT in UAB health care workers with a positive TST does not increase the acceptance of INH therapy as much as was expected.

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Assessment of Prasugrel Antiplatelet P2Y12 Receptor Therapy in a Clopidogrel Nonresponder

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The clinical laboratory is increasingly positioned to have a critical role in the assessment of platelet function in the setting of antiplatelet therapy. There is typically a complete loss of arachidonic acid–induced platelet aggregation in patients responsive to aspirin. In patients receiving aspirin and an adenosine diphosphate (ADP) P2Y12 receptor antagonist such as clopidogrel or prasugrel, assessing the patient's response to the ADP receptor antagonist is more difficult, particularly in the absence of pretreatment baseline studies. In this increasingly common situation, a derived percent inhibition (DPI) must be used in lieu of a directly measured percent inhibition (MPI) of platelet function following drug initiation. The VerifyNow platelet function device, for example, references the response to ADP to that obtained with thrombin receptor activation peptide (TRAP) to arrive at a DPI.

Following the recent availability of prasugrel at our institution, comprehensive laboratory evaluation of primary hemostasis was requested for a post–coronary artery stent patient receiving aspirin and clopidogrel, for whom the VerifyNow device reported a P2Y12 DPI of 0%. Following a full light transmission aggregometry (LTA) study that identified abnormalities limited solely to those anticipated from effective aspirin therapy, anti-P2Y12 treatment was changed from clopidogrel to prasugrel. Repeated LTA permitted comparison of the true MPI with a variety of surrogate DPI values obtained in the postprasugrel study by ratioing peak light transmittance changes in response to ADP (2, 5, 8, 10, or 20 µmol/L) over responses to TRAP (10 µmol/L), collagen (2, 5, or 20 µg/mL), or the calcium ionophore A23187 (25 µmol/L). Similar ratios at 6' postpeak were calculated to assess partial disaggregation. Postprasugrel initiation, the VerifyNow reported a DPI of 67%, similar to the MPI of 72% calculated from the device's proprietary ADP response units. By LTA, unsatisfactory agreement between DPI and MPI was observed using any concentration of ADP referenced to TRAP or to 2 to 5 µg/mL collagen. In contrast, excellent agreement was seen using 20 µmol/L ADP referenced to 20 µg/mL collagen: DPI values for peak and postpeak aggregation were 33% and 62%, respectively, compared with MPI values of 30% and 61%, respectively; in the preprasugrel sample, the DPI was appropriately quite low, at less than 10%. More definitive assessment of the potential usefulness for this approach in patients receiving combined aspirin and ADP P2Y12 receptor antagonist therapy will, of course, require extensive studies, optimally including clinical outcome results.

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Pathologist Review as a Strategy to Improve the Diagnostic Accuracy of Body Fluid Analysis in the Hematology Laboratory
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At our institution, cerebrospinal and serous cavity fluids are often submitted to multiple laboratories for analysis, including the hematology and cytology laboratories. In the hematology laboratory, a hematology technologist evaluates air-dried, Wright-Giemsa–stained, cytocentrifuged preparations. Rarely, the technologist identifies cells "suspicious" for malignancy. In the past, no formal process of pathologist review of body fluid samples originating from hematology existed in our laboratory, despite practice guideline recommendations. In contrast, in the cytology laboratory, a cytotechnologist and cytopathologist evaluate alcohol-fixed, Papanicolaou-stained slide preparations.

During a 9-month-long study evaluating the diagnostic concordance between hematology and cytology fluid samples, we asked our hematology technologists to set aside samples containing suspected malignant cells for pathologist review. Of the 62 flagged samples that were reviewed (review rate, ~1%), 57 (92%) were also evaluated by cytology, with concordant findings in 46 of 57 cases (81%). Of the 11 discordant cases (19%), 9 (16%) were interpreted as atypical or suspicious for malignancy by hematology, usually owing to the presence of reactive mesothelial cells or macrophages, and were correctly interpreted as benign by cytology. Of the 57 samples, 2 (4%) were interpreted as suspicious for malignancy by hematology and were incorrectly interpreted as benign or mildly atypical by cytology; both cases (Burkitt lymphoma and metastatic rhabdomyosarcoma) contained small round blue cells that were better visualized on Wright-Giemsa–stained preparations. We subsequently initiated formal pathologist review of all body fluid samples suspected to contain malignant cells after preliminary evaluation by the hematology technologist, and all flagged cases now have a pathologist's diagnostic interpretation.

Concordance rates were evaluated for 10 months following implementation, during which time 169 body fluid cases were flagged for pathologist review (review rate, ~3%). Of the 169 cases, 106 (62.7%) had a concurrent cytology specimen, with a concordance rate of 91.5% (97/106), an increase from the previous rate of 81%. Of the 9 discordant cases (8.5%), the hematology sample revealed abnormalities not identified in the cytology specimen in 7 (6.6%) of 106 cases, all of which were hematolymphoid malignancies. The cytology specimen revealed abnormalities not identified in the hematology sample in 2 (1.9%) of 106 cases; both were cases of metastatic carcinoma. Reasons for the discordant cases included sampling error in a paucicellular sample, compromised cytomorphology owing to sample degradation, technologist or pathologist error in interpretation, and differences in sample processing and staining.

Pathologist review of hematology body fluid samples improves diagnostic accuracy, particularly by avoiding overdiagnosis of malignancy in samples containing reactive cell populations and by enhanced recognition of hematolymphoid malignancies in air-dried samples.

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Chronic Inflammatory Demyelinating Polyradiculoneuropathy Refractory to Multiple Treatment Modalities Responds to Rituximab
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Our objective was to describe the dramatic therapeutic effect of rituximab in a patient with chronic inflammatory demyelinating polyradiculoneuropathy (CIDP) that was refractory to multiple therapies. Sparse case reports describe effects that range from remission to a decreased requirement of other therapies to no benefit.

Briefly, this 60-year-old woman had bilateral lower extremity weakness that gradually worsened to require a walker in addition to bilateral upper extremity weakness, numbness in her fingers and toes, and paresthesias in her face and head. Electromyography of the right first dorsal interosseous and abductor pollicis brevis muscles was normal. Nerve conduction studies of the right median and ulnar nerves revealed abnormally decreased motor nerve conduction velocities and absent sensory responses.

No monoclonal antibody was incriminated. Serum quantitative IgM, IgA, and IgG levels were decreased, and neither serum nor urine monoclonal proteins were detected. Motor neuropathy autoantibody

testing was repeatedly negative at multiple laboratories, including negative results for antibodies against myelin associated glycoprotein, sulfoglucuronyl paragloboside, gangliosides (GM1, GM2, GM3, GD1a, GD1b, GA1, GT1b, GQ1b, and GD3), sulfatide, neuronal nuclear antigen-1 (ANNA-1 or Hu), acetylcholine receptor, and β-tubulin among others. Sural nerve biopsy showed an increased rate of myelin remodeling, indicating a demyelinating neuropathy, and small perivascular inflammatory infiltrates suggested the diagnosis of CIDP.

During the course of 6 years, she poorly tolerated and/or received minimal to no long-term benefit from IVIG, prednisone, methotrexate, cyclophosphamide, mycophenolate, and more than 200 plasmapheresis treatments. Before receiving rituximab, plasmapheresis was the only modality that reliably was tolerated and that gave a moderate degree of symptomatic relief. After receiving rituximab (900 mg) over 4 consecutive weeks, her symptoms dramatically improved. She has been in remission for approximately 3 years. Even though no monoclonal protein or autoantibody was detected, rituximab stopped production of the putative antibody. Our case adds to the argument that a randomized clinical trial is needed to measure the efficacy of rituximab for CIDP, as rituximab may prove to be cost-effective.

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The Stability of Multiple Myeloma Markers in Serum
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Serum chemistry tests, serum protein immunofixation, and separation studies are routinely used in multiple myeloma diagnosis and monitoring of treatment. Esoteric testing is required to perform much of this monitoring and may not be locally available. In many cases, a patient's blood sample can be drawn and centrifuged and the resulting serum sample sent to a remote facility for analysis. Thus, a common concern of clinicians and clinical laboratory professionals alike is the stability of myeloma markers when specimens are subjected to different temperatures and time periods before analysis.

The stability of common markers involved in the investigation of multiple myeloma (IgG, IgM, IgA, free λ and κ light chain, β₂-microglobulin [B2M], total protein [TP], lactate dehydrogenase [LDH], and C-reactive protein [CRP]) at different storage temperatures was investigated. A serum sample was pooled from different patients with multiple myeloma with representative monoclonal proteins from different patients with IgG, IgA, and IgM heavy chains, as well as κ and λ light chains. This pooled sample was divided into aliquots and stored at room temperature (RT) or 4°C, and 3 replicate measurements were taken at different time points spanning a 1-month period. A parallel pooled serum sample was divided into aliquots, stored at –80°C, and assayed in a similar manner over 2 months. Samples at the 3 different storage temperatures were also subjected to capillary zone SPEP and IFE (Sebia) analysis in a 3-week period.

Analytes were deemed stable if the mean of the 3 replicates was within 10% of their initial concentration. In general, very little visual change was observed at RT, 4°C, or –80°C stored samples by SPEP or IFE analysis. All analytes in the frozen aliquots were stable for at least 2 months. The IgG, IgA, IgM, TP, albumin, and CRP analytes were stable at RT and 4°C for the 4-week period. B2M was stable over the measurement period at 4°C but for only 3 weeks at RT. LDH was stable at RT but was stable only for 1 week at 4°C. It is interesting that free λ light chain was stable for 1 week at RT and 4°C, but free κ was stable only for 72 hours under these conditions.

Analysis of serum samples within 3 days of phlebotomy avoids significant effects on the measurement of common myeloma markers under RT, 4°C, and –80°C storage conditions. The effects of transport and storage conditions on specific markers should be considered in the interpretation of results produced from specimens with more extended preanalytic periods.

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Evaluation of the Efficacy of Utilizing Total Serum Pseudocholinesterase Activity as a Screening Tool for Pseudocholinesterase Deficiency
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Pseudocholinesterase (PCHE) is an enzyme with no known physiologic role that inactivates choline ester paralytic agents such as succinylcholine (SC) used to assist intubation. Prolonged paralysis following SC dosage may occur in people with inherited variant forms of PCHE. PCHE activity assays are available, but enzyme activity can be temporarily decreased by numerous factors and provides nondefinitive information about a person's sensitivity to paralytic agents. Determining PCHE activity in the presence of the inhibitor dibucaine can be used to calculate a dibucaine number (DN), which can improve the identification of SC-sensitive people. Few clinical laboratories perform dibucaine inhibition testing, leaving clinicians with no established rapid, preoperative screening tool. Our objective was to determine if PCHE activity alone might be useful for screening for SC-sensitive PCHE variants by correlating PCHE activity by phenotype to identify upper activity limits of potential deleterious PCHE phenotypes.

We examined results from 2,768 physician-ordered, deidentified PCHE phenotype tests (PCHE activity, DN, and phenotype). PCHE phenotypes were organized into 1 of 4 categories representing known phenotypic response to SC (normal sensitivity [U, n = 1,033; PCHE range, 41-14,189 U/L]; slightly sensitive [UA, UF, US, n = 763; PCHE range, 7-8,475 U/L]; somewhat sensitive [FF, AF, FS, n = 129; PCHE range, 399-6,925 U/L], and severely sensitive [A, S, AS, n = 438; PCHE, 32-5,800 U/L]).

We excluded 405 results from the data set secondary to noncanonical DN values. In the remaining data, 99% of severely sensitive people exhibited PCHE activity less than 3,000 U/L, while 99.1% of "normal" people had an activity above this limit. Likewise, 65% of somewhat sensitive and 37% of slightly sensitive people had activities below this cutoff. This approach was not useful in screening for somewhat sensitive (99% below a 6,800-U/L cutoff) and slightly sensitive (99% below a 7,800-U/L cutoff) people as only 32% and 13% of the normal U samples exhibited PCHE activity above these respective cutoffs.

These data suggest that while total PCHE activity cannot be used to identify slightly sensitive or somewhat sensitive people, it may have potential as a screening tool for identifying potentially severely sensitive people.

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Applicability of the MDRD Equation in Estimating the Glomerular Filtration Rate Among the Elderly Population
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The Modification of Diet in Renal Disease (MDRD) equation is widely used for the estimation of the glomerular filtration rate (eGFR) from serum creatinine concentrations. However, this equation has not been validated in elderly people (age >70 years), leading some to question whether eGFR values should be reported in this population. To address this, a retrospective study was conducted to determine the accuracy of the MDRD eGFR equation compared with GFR measured by iothalamate clearance in the elderly population and the nonelderly population.

The study population consisted of patients who had undergone renal function assessment by simultaneous measurements of serum creatinine (IDMS-traceable method) and renal iothalamate clearance at our institution between July 2007 and February 2010 (n = 3,828). Dialysis patients, transplant recipients, and nephrectomized transplant donors were excluded from the study. The remaining patients underwent GFR testing for staging of chronic kidney disease (CKD; n = 3,232) or as potential kidney transplant donors (n = 596). Of the patients, 16.9% (n = 647) were 70 years or older. Patients were classified by CKD stage using iothalamate GFR (mL/min/1.73 m^2): less than 15 (stage 5), 15 to 29 (stage 4), 30 to 59 (stage 3), or more than 60 (stage 1/2). CKD staging of patients by measured and calculated GFR (MDRD equation) was then compared for patients older or younger than 70 years.

Among patients younger than 70 years, the percentages for whom eGFR fell within the same GFR classification was 70.1%, 67.2%, 70.2%, and 90.5% for CKD stages 5 through 1/2, respectively, while the concordance rates for patients older than 70 years were 43.3%, 65.8%, 78.2%, and 75.3%, respectively. The average percentage differences between eGFR and iothalamate measurements were 39.8%, 6.8%, 8.7%, and –6.5%, respectively, for patients younger than 70 years, while the percentage differences for patients older than 70 years were 48.9%, 20.5%, –0.7%, and –0.2%, respectively. The percentages of patients younger than 70 years for whom the eGFR fell within 30% of the measured GFR were 57%, 72.5%, 74.4%, and 73.4% for CKD stages 5 through 1/2, respectively, and 48.3%, 68.3%, 79.5%, and 76.7%, respectively, for patients older than 70 years.

We conclude that the concordance for CKD classification between the calculated and the measured GFR is comparable in patients older and younger than 70 years for stages 4 and higher. For patients older than 70 years and a true GFR less than 30 mL/min/1.73 m^2, the MDRD-estimated GFR significantly overestimates GFR, and other direct measures of GFR may be necessary to make a firm assessment of renal function, especially to differentiate between stages 4 and 5 CKD.

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Medical Students' Impressions of a New Elective Course in Laboratory Medicine

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Laboratory medicine (LM) is more than a specialty. While all physicians order laboratory tests while caring for their patients, time to teach LM during medical school is limited, leaving many physicians not fully prepared to use the clinical laboratory efficiently. We started an elective 1-week (20-hour) course in LM to second year students in our new curriculum. We report the students' responses to several questions asked at the beginning and end of each week. They answered them anonymously on sheets provided by the coursemaster.

A total of 43 students (6 groups of 3-12) have participated since August 2008. The curriculum consisted of case-based discussions of the most appropriate tests to be ordered and how to interpret the results. On the first day of the week, approximately 60% of the students believed that the course would be important for their clerkships and/or career. Yet, fewer than 80% had heard of LM, and among them, 57% had only learned of it through medical school lectures. At the end of the week, when asked what they learned from the course, 37% mentioned that they now understood the importance of thinking through the clinical question before ordering a test; 16% said that they learned of the consultative role of LM specialists, including laboratory staff; and the rest gave a variety of answers. In terms of what surprised them the most in the course, 27% said that it was the myriad of clinical laboratory tests available considering 71% believed at the beginning of the course that there were only 500 or fewer tests available; 24% were surprised by the *actual* role of LM specialists. To the question "How do you think you will use this knowledge in the future?" 45% responded that they will use it to curb unnecessary ordering of tests during their future careers and/or clerkships. Examples of other answers included aid in the choice of a specialty and assay technical details. More than half of the students were open to the choice of a specialty, while some had very specific ideas.

This initial attempt at exposing medical students to LM has achieved the overall goal of making them realize that LM is not merely the ordering of clinical laboratory tests. The open questions posed to them led to a wide range of specific answers that are difficult to categorize. However, it provided us the opportunity to hear what they had to say without being prompted by multiple choices. Since 56% of the students did not have a specialty in mind, this course also provides us with a recruiting tool for careers in LM. The impact of such a course in the future of LM and overall clinical practice is unknown.