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, Eric D. Spitzer, MD, PhD, FASCP. Reviewers are blinded to authors and institutions. Young Investigator Award recipients are granted free registration to the annual meeting, reimbursement for a portion of travel expenses, and the opportunity to present their scientific work before an audience of peers and mentors.

The following abstracts were presented at the 42nd Annual Meeting of the Academy of Clinical Laboratory Physicians and Scientists, June 7-9, 2007, in San Diego, CA. Authors receiving a 2007 Young Investigator Award are marked with an asterisk (*).

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

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A 2-Year Study of Patient Safety Competency Assessment in 29 Clinical Laboratories.

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Competency assessment is a critical component of laboratory operations and is mandated by the Clinical Laboratory Improvement Amendments. However, there have been no previous reports on methods for assessing competency in patient safety. In partnership with an online publishing company (Medical Training Solutions, Seattle, WA), we developed and implemented a computer-based tool to assess objectively the performance of 875 laboratory staff from 29 laboratories in patient safety. Web-based tests were administered semiannually for 2 years; the 4 tests contained a total of 40 questions. Of these, 5 questions were primarily about workplace culture, 10 about types of error (eg, cognitive vs noncognitive), 8 about prioritization of projects for patient safety interventions, 13 about specific interventions, and 4 about general patient safety concepts.

Overall, the mean score was 85% (range, 56%-100%). The questions were reliable (KR-20 coefficient, 0.79). When categorized by question type, the mean for workplace culture was 84%; types of error, 86%; prioritization of projects for intervention, 84%; specific interventions, 81%; and general concepts, 88%. Of the questions in the highest quartile (>98% correct), 3 were about types of error, 3 about prioritization of projects for intervention, 3 about specific interventions, 1 about workplace culture, and 1 about general concepts. For example, when asked about an event in which a nurse submitted a specimen with mismatched labels and laboratory staff failed to detect the error and analyzed the specimen, 100% of technologists correctly indicated that an intervention should be directed to the nursing and laboratory staff. Of the questions in the lowest quartile of scores (<72% correct), 6 were about specific interventions, 3 about types of error, 1 about workplace culture, and 1 about prioritization of projects for intervention. For example, 1 question asked, "A 'blame-free' approach is reasonable to apply to which of the following data entry errors?" Only 56% of technologists correctly chose "an error made by an employee when the lab was busy and understaffed"; 1% chose "an error made by an employee who was drunk"; and 39% chose "neither of the above." Of 13 questions about specific interventions, 6 (46%) were in the lowest quartile, suggesting that this may be a relatively difficult topic for laboratory technologists.

Computer-based competency assessments help laboratories identify topics for continuing education in patient safety. This method allows supervisors to monitor individual performance of laboratory staff, to compare the group's performance with that of other laboratories, and to set and monitor quantitative goals for performance improvement.

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Role of Cardiac Marker Screening Panels in the Detection of Acute Myocardial Infarction.

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Although cardiac-specific troponin appears to be the marker of choice for the diagnosis for acute myocardial infarction (AMI), many institutions continue to offer routine clinical screening with creatinine kinase (CK) and troponin. At the Veterans Affairs Puget Sound Healthcare System Seattle Division Hospital, the screening protocol to rule out an AMI includes measurement of total CK, CK-MB fraction, and troponin T within 2 hours of presentation with repeated screening within 10 hours. To determine whether routinely providing total CK and CK-MB provides any additional benefit, all screening panels done between August 2005 and March 2006 at the Veterans

Affairs Puget Sound Healthcare System Seattle Division Hospital were analyzed. During August and September 2005, a total of 1,075 cardiac screening panels were ordered, representing 525 patients.

With negative cutoff values of less than 0.04 ng/mL for troponin T and less than 5.0 ng/mL for CK-MB in males and less than 3.0 ng/mL in females, 535 panels were negative for both markers, 185 were positive for both markers, 129 were positive for CK-MB only, and 226 were positive for troponin T only. Next, sequential panels on the same patient done within 48 hours were compared to identify patients in whom laboratory values changed over time. From August 2005 through March 2006, a total of 19 patients were identified whose results converted from an initially positive CK-MB to positive for both markers. Of these, 4 patients had laboratory values indicative of an AMI with an initially positive CK-MB value that converted to positive for CK-MB and troponin T on repeated testing done within 7 hours of initial testing. All 4 patients were identified as having potential AMIs by elevated troponin T levels on the repeated testing done per our hospital screening protocol.

There appears to be little benefit in routinely offering both creatinine kinase and troponin testing when screening for AMI. Based on this study, we propose initially screening patients with troponin with the reflexive addition of CK-MB only in patients with positive troponin values. This change has the potential to eliminate approximately 3,600 unnecessary CK and CK-MB tests annually at our institution with no apparent impact on the diagnosis of AMI.

7

Measurement of Tissue Inhibitor of Metalloproteinase-1 and Carcinoembryonic Antigen in Plasma From Subjects Undergoing Colonoscopy.

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Tissue inhibitor of metalloproteinase 1 (TIMP-1) is a glycoprotein that has an important role in the growth and spread of cancer. Past studies have demonstrated clinical utility for plasma TIMP-1 as a marker for colorectal cancer. Other studies have found associations between plasma TIMP-1 concentrations and other medical conditions, including other cancers, cardiovascular disease, diabetes mellitus, hepatic fibrosis, renal disease, and increasing age. The purpose of our research was to examine the clinical sensitivity and specificity of TIMP-1 for colorectal cancer in subjects undergoing colonoscopy for any indication.

EDTA plasma samples were collected from 2,003 subjects before colonoscopy, 11 of whom had colorectal cancer. Twelve additional plasma samples were collected from subjects before undergoing colorectal cancer resection. Of 23 subjects with cancer, 15 had stage I or IIA and 8 had stage III or IV disease. TIMP-1 was measured using a 2-step sandwich microplate immunoassay or an ARCHITECT i2000 analyzer and carcinoembryonic antigen (CEA) was measured exclusively on an ARCHITECT i2000. The 95th percentiles for TIMP-1 and CEA in subjects without colorectal cancer were 174 ng/mL and 3.9 ng/mL, respectively. Of 23 samples from subjects with cancer, 6 (26%) were positive for TIMP-1. Of 8 samples from subjects with stage III or IV cancer, 4 (50%) were positive for TIMP-1. Of 23 subjects, 4 (17%) were positive for CEA. Of 8 samples from subjects with stage III or IV cancer , 2 (25%) were positive for CEA. When TIMP-1 and/or CEA were positive, the overall sensitivity for cancer was 35%. In subjects with stage I or IIA cancer, the sensitivity for the combined tests was 27%, and for stage III or IV cancer it was 75%. A review of clinical data for subjects free of colorectal cancer but with a TIMP-1 result of more than 200 ng/mL showed associations with older age and hypertension.

Although the number of subjects with colorectal cancer in our study was modest, the sensitivity of TIMP-1 for colorectal cancer appears to be higher than that of CEA. By combining TIMP-1 and CEA testing, the sensitivity for the diagnosis of colorectal cancer increases and is comparable to that for fecal occult blood testing. Cardiovascular disease and advanced age are associated with elevated TIMP-1 plasma concentrations. Further studies on the use of TIMP-1 combined with CEA for colorectal cancer screening are warranted.

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Age- and Gender-Specific Pediatric Reference Intervals Are Necessary for Serum Aldolase and Uric Acid.

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Determination of appropriate pediatric reference intervals is difficult. Samples were collected from 873 healthy children with unremarkable history and physical examination findings (438 boys and 435 girls) between 7 and 17 years of age. Serum samples were then assayed on the Roche Modular P for 28 analytes: α_1-antitrypsin, angiotensin converting enzyme, α-fetoprotein, albumin, alanine aminotransferase, aldolase, alkaline phosphatase (ALP), amylase, aspartate aminotransferase (AST), β_2-microglobulin, bile acid, blood urea nitrogen (BUN), C3, C4, calcium, ceruloplasmin, creatinine kinase (CK), creatinine, γ-glutamyl transferase (GGT), IgE, lactate dehydrogenase (LD), lipase, pancreatic amylase, phosphorus, prealbumin, salivary amylase, transferrin, and uric acid. A total of 24,259 results were obtained with 185 expected results not available owing largely to insufficient sample volume. Results were entered into EP Evaluator 5 for data analysis. A total of 27 results (0.11%) were excluded as outliers.

Statistically significant gender differences were demonstrated by use of EP Evaluator's partition analysis function based on the criteria of Maximum Z more than Critical Z and/or SD ratio more than 1.5. Ten analytes were identified as having statistically significant gender differences: aldolase, ALP, AST, BUN, CK, GGT, IgE, LD, lipase, and uric acid. 90% confidence intervals (CIs) about the upper and lower reference limits obtained by application of the EP Evaluator full analysis function showed significant overlap at the reference limits in the majority of these analytes. Only aldolase (U/L; male, 10.7 [CI, 9.6-13.6]; female, 8.2 [CI, 7.8-8.8]) and uric acid (mg/dL; male, 8.7 [CI, 8.1-9.3]; female, 6.4 [CI, 6.2-6.8]) showed clear gender separation at the clinically significant upper reference limit. Nonparametric age partition analysis performed separately for males and females showed statistically significant differences for both analytes. The 90% CIs were generated as described previously for all significant age groups. Complete separation of upper limit CIs was found for males but not females for aldolase and uric acid. The results were as follows: aldolase (U/L): male, 7 to 11 years, 3.5 to 10.7; 12 to 17 years, 3.5 to 13.6; female, 7 to 17 years, 3.2 to 8.2; uric acid (mg/dL): male, 7 to 9 years, 2.0 to 6.0; 10 to 17 years, 2.7-8.9; female, 7 to 17 years, 2.5 to 6.4. Although 10 of 28 analytes demonstrated statistically significant differences for gender and age, clear separation of limits at the clinically significant upper end of the reference interval was demonstrated only for aldolase and uric acid.

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False-Positive Clonal Restriction of Lymphocytes in a Patient With Refractory T-Cell Prolymphocytic Leukemia Receiving Anti-CD52 Therapy: A Case Study.

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Our objective was to measure the interference, if any, of the drug alemtuzumab on fluorescence-activated cell sorting (FACS) analysis in the setting of a clinical immunohematology laboratory. Alemtuzumab, a monoclonal antibody that binds to surface CD52 on lymphocytes, is used to treat several forms of non-Hodgkin lymphoma, including refractory T-cell prolymphocytic leukemia (T-PLL). Based on the biologic properties of alemtuzumab, we speculated that this drug may influence FACS analysis of peripheral blood lymphocytes, particularly in the assessment of lymphocyte clonality and the uptake of anti-CD52 antibodies.

Peripheral blood specimens were obtained from 2 subjects, 1 receiving alemtuzumab for refractory T-PLL (of known immunophenotype) and 1 from a healthy volunteer. T and B lymphocytes from the specimens were analyzed by standard FACS protocol for the expression of several surface markers, including CD3, CD4, CD5, CD52, and κ and λ light chains. In addition, an aliquot of blood from the healthy donor was treated with alemtuzumab ex vivo and interrogated by FACS techniques.

In the patient with refractory T-PLL, a subset of malignant T cells expressing CD52 was identified. This malignant subset of T cells also demonstrated bright surface expression of κ light chain. In the healthy volunteer, no malignant cells were identified, and there was no expression of surface κ light chain within the untreated aliquot; CD52 was demonstrable. However, T cells from the volunteer sample treated with alemtuzumab demonstrated bright expression of surface κ light chain; expression of CD52 was similarly unaffected.

The administration of the anti-CD52 agent alemtuzumab causes binding of anti-κ to T cells, resulting in apparent κ restriction by FACS analysis. Therefore, treatment with this drug should be recognized as a potential interference for FACS-based diagnosis of a monoclonal lymphocyte population in peripheral blood. However, alemtuzumab does not diminish recognition of the CD52 receptor, nor does it hinder the ability to detect residual neoplastic T-cell disease by FACS analysis.

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Refining von Willebrand Disease Reference Ranges by Blood Type to Decrease Equivocal Results.

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Diagnosing von Willebrand disease (vWD) can be difficult and must include clinical history and laboratory tests. Routine testing should include von Willebrand factor antigen (vWF:Ag), von Willebrand factor activity (vWF:RCo), and factor VIII activity (FVIII) levels. Caution should be observed when interpreting results of these assays as vWD. It has been shown that normal subjects with type O blood have lower levels of these vWD factors than people with non–type O blood. The purpose of this investigation was to establish reference ranges for vWF:Ag, vWF:RCo, and FVIII for blood type O and non-O individuals to help better detect the presence of vWD.

We tested 137 normal control subjects with no history of bleeding and 27 subjects with known or suspected vWD for vWF:Ag, vWF:RCo, and FVIII. Of the normal subjects, 61 had type O blood and 76 had non–type O blood. Mean results and 2 SD ranges for non–type O, type O, and vWD subjects were as follows, respectively: vWF:Ag, 128 (67-189), 92 (31-152), and 40 (3-76); vWF:RCo, 112 (42-181), 86 (27-145), and 28 (0-65); FVIII, 142 (61-224), 112 (43-181), and 58 (12-103); and vWF:RCo/vWF:Ag ratios, 0.88 (0.53-1.22), 0.94 (0.67-1.21), and 0.65 (0-1.3). For vWD patients with historic blood types available, 4 were non–type O and 4 were type O. Results were as follows: vWF:Ag, 31 (0-81) and 47 (26-68); vWF:RCo, 18 (0-51) and 45 (15-75); mean FVIII, 42 (0-93) and 66 (24-107); and mean vWF:RCo/vWF:Ag ratio, 0.53 (0.13-0.93) and 0.94 (0.7-1.2), respectively.

By determining the vWD reference ranges for O and non-O blood types, we can reduce the number of patients in the equivocal range, which we define as the overlap between the upper limit of the 2SD range for patients with known vWD and the lower limit of the 2SD range for normal non–type O patients. When a patient's blood type is known to be non-O, the chance of an equivocal result is diminished because only 1 of the 76 non–type O normal subjects' vWF:Ag result was within the equivocal range.

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Effect of Hawthorn on Calcium Influx in Rat Cardiomyocytes: Differences Between Two Products.

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Hawthorn extracts (*Crataegus* species) are among the most popular herbal products in the United States and Europe (Pittler et al. *Am J Med.* 2003;114:665-674). These extracts are readily available and used by the general population to treat many cardiac diseases, including heart failure and angina. There are limited data on beneficial or detrimental effects of Hawthorn at the cellular level.

We studied potential cardiac effects of commercially available Hawthorn using rat cardiomyocytes. Two liquid extracts, a blend of Hawthorn flowers, leaves, and berries manufactured by Herb Pharm, Williams, OR (product 1), and an extract of Hawthorn berry manufactured by Gaia Herbs, Brevard, NC (product 2), were used to determine their effects on calcium influx in adult rat cardiomyocytes isolated using the Langerdorff procedure. Cells were incubated with Fluo4 (3 μmol/L), and calcium transients were recorded by real-time fluorescence spectrophotometry. Concurrent acquisitions of fluorescent images were also made after addition of the individual extracts, which mimicked in vivo serum concentrations after recommended dosages.

Addition of product 1 (1 μL/mL) resulted in initiation of robust calcium transients and eventual calcium overload. Addition of product 2 (1 μL/mL) resulted in increased sparking, eventual initiation of calcium transients, and, ultimately, an increased rate of beating, but no calcium overload was observed.

To further identify the mechanisms of increased calcium influx, adult rat cardiomyocytes were challenged with 10 μmol/L of ouabain, an Na+,K+-ATPase inhibitor, and, despite this addition, increased calcium transients were still observed, resulting in myocyte calcium overload. Adult myocytes were also challenged with nifedipine, a dihydropyridine calcium channel blocker, but the myocytes continued to proceed to increase Ca_i (ionized calcium) and eventual calcium overload.

Our findings reveal that 2 readily available Hawthorn preparations demonstrate markedly different effects on an isolated adult rat myocyte model, suggesting important implications for patients who are using these preparations to supplement or even replace their prescribed

cardiac medications. Our study indicates that the mechanism of cardiac activity of Hawthorn, at least to some extent, is independent of the Na+,K+-ATPase and the L-type calcium channel.

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Surrogate Markers of Epidermal Growth Factor Receptor Inhibition in Skin and Buccal Mucosa.

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Tyrosine kinase inhibitors targeting epidermal growth factor receptor (EGFR) have shown promise for treating some malignancies, but methods for selecting patients and determining optimal dosing are not yet defined. Demonstration of the desired effect on the targeted pathway during treatment could help guide therapy. Repeated sampling of the tumor is not always possible; therefore, surrogate markers are needed. Because EGFR has a role in renewal of the epidermis and oral mucosa, these are possible sites for measuring EGFR inhibition.

In a clinical trial of patients with advanced malignancies treated with the tyrosine kinase inhibitor gefitinib using 2 different dosing regimens, skin biopsy specimens were obtained before and on day 28 of therapy. These were stained immunohistochemically for the proliferation marker Ki-67 and for phospho-extracellular signal–related kinase (pERK), a molecule downstream from EGFR. Buccal swabs were collected before therapy and weekly during therapy, as were blood samples for measurement of serum gefitinib levels by immunoassay. Expression of c-fos mRNA, shown to be correlated with anti-EGFR response in preclinical experiments, was measured by RT-PCR in the buccal swabs.

Skin biopsies showed decreased nuclear staining for Ki-67 in the day 28 biopsy specimens relative to before therapy in 11 (79%) of 14 patients with available samples. Also, 11 (79%) of 14 cases showed the expected decrease in staining for pERK. However, neither the proportion of cells staining nor the magnitude of decrease correlated with steady-state serum gefitinib concentration measured on day 28. There was no correlation between buccal c-fos expression and serum drug levels. Patients displayed 2 distinct patterns of variation in buccal c-fos expression, with 13 (72%) of 18 showing initial suppression followed by rebound increase. The remaining 5 showed wide variation, some with more than 100-fold changes in c-fos expression, mostly above the pretreatment baseline.

Proliferation index and pERK expression in the epidermis decreased as expected during gefitinib therapy, but changes were not proportional to serum drug levels, suggesting a more complex relationship between dose and EGFR inhibition. Expression of c-fos in the buccal mucosa did not correlate with serum gefitinib levels. The finding of 2 distinct patterns of c-fos expression during treatment is intriguing and suggests distinct functional response to EGFR blockade by gefitinib.

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A 2-Year Study of Laboratory Safety Competency Assessment in 44 Clinical Laboratories.

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Under the Clinical Laboratory Improvement Amendments (CLIA), all laboratories are required to assess the competency of their personnel. Competency assessment (CA) can be accomplished by a variety of methods, including written testing. Laboratory safety is a fundamental aspect of all laboratory work and includes chemical safety, biosafety, electrical safety, phlebotomy safety, fire safety, and other topics. To comply with CLIA, we collaborated with a university-sponsored publishing company, Medical Training Solutions (Seattle, WA), to develop and implement an online CA tool for laboratory safety.

Laboratory safety competency of 591 laboratory staff from 44 subscribing laboratories in the United States was assessed via 10 multiple-choice questions administered semiannually during 2 years. Each of the 591 laboratory workers took all 4 examinations, which contained a total of 40 questions.

The overall mean score on the 40 questions was 92% (range, 62%-100%). The questions were moderately reliable (KR-20 coefficient, 0.65). Results for specific topics were as follows: chemical safety, n = 13 questions, mean score, 89%; biosafety, n = 12, 93%; electrical safety, n = 3, 97%; phlebotomy safety, n = 4, 83%; fire safety, n = 3, 95%; and miscellaneous (eg, ultraviolet safety, radioactivity safety), n = 5, 94%. Of the 5 questions that 100% of technologists answered correctly, 1 was about chemical safety, 2 were about biosafety, and 2 were in the miscellaneous category. These questions included first aid treatment (eg, eye rinses following a splash), use of personal protective equipment (eg, importance of buttoning a laboratory coat), and identifying physical hazards in the laboratory. Of the 5 lowest-scoring questions (mean scores, ≤79%), 1 was about chemical safety, 2 were about biosafety, 1 was about phlebotomy safety, and 1 was in the miscellaneous category. The lowest scoring question asked laboratory workers to identify the most common cause of needle-stick injuries among phlebotomists (62% correctly chose butterfly needles, whereas 34% incorrectly chose Vacutainer needles). Other low-scoring questions covered the proper use of a biologic safety cabinet (mean score, 67%) and proper cleaning of a biohazard spill (74%).

We successfully implemented a CA tool for laboratory safety across a large number of laboratories nationally. Examination scores for questions regarding phlebotomy safety and biosafety were in the lowest quartile, suggesting that additional education may be needed in this area. Overall, this online CA tool for laboratory safety is reliable. In addition, it allows laboratory supervisors to evaluate individual and group performance, compare performance with other subscribing laboratories, and set quantitative goals for improvement.

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Red Blood Cell Alloantibody Frequency, Specificity, and Properties in a Population of Male Military Veterans.

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The prevalence of RBC alloantibodies has been previously reported to range from 0.6% to 2.0% in general patient populations. The frequency and properties of RBC alloantibodies have never been studied in male military veterans in a hospital setting.

Transfusion records of 18,750 military veterans at a VA medical center were retrospectively reviewed. For patients with RBC alloantibodies, the following data were collected: sex, decade of birth, results of antibody screens, alloantibody specificity, reaction phase, and whether alloantibodies were detected at the time of initial testing or after at least 1 prior negative antibody screen.

RBC alloimmunization occurred in 2.36% (443 patients with 577 antibodies) of patients from a military veteran population that was 95% male. Alloimmunization rate varied with decade of birth, ranging from 1.23% (1941-1950) to 3.28% (1911-1920). The 10 most frequently identified alloantibodies in men, as a percentage of total antibodies, were as follows: K, 21.8%; E, 18.2%; D, 9.06%; Le^a, 7.39%; Fy^a, 5.36%; c, 4.81%; C, 4.62%; P_1, 3.88%; Jk^a, 3.70%; and Le^b, 3.51%. The stimulus for D alloimmunization in most men could not be identified because 80% developed this antibody before treatment at the VA. In the other 20% of cases, anti-D was most commonly associated with the infusion of Rh+ platelets. The percentage of patients making anti-D was highest in veterans born between 1911 and 1920 (0.44%). The majority of alloantibodies reacted at antiglobulin (AG) phase (93.9%). Even antibodies known typically to react at room temperature instead reacted mostly at AG and/or 37°C: anti-M, 18 of 18 (100%); anti-Lu^a, 10 of 11 (91%); and anti-P_1, 18 of 21 (86%). Of 405 alloantibodies for which at least 2 antibody screens were performed, 35.6% became undetectable over time. Anti-Jk^a had the highest rate of evanescence (11/14 [79%]) among alloantibodies occurring at least 10 times, whereas anti-D had the lowest rate (2/36 [6%]).

Overall alloantibody prevalence was as high as, or higher than, that reported in most mixed-gender populations. Anti-D was common despite the lack of pregnancy-related alloimmunization. Total and D alloimmunization varied by decade of birth and may reflect differences in medical and transfusion practice across combat eras. Compared with reports on other populations, alloantibodies made by male veterans tended to react more in AG phase and less at room temperature. Evanescence was found to vary with alloantibody specificity.

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A Rapid HPLC Method Used to Establish Pediatric Reference Intervals for Vitamins A and E.

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Retinol (vitamin A) and tocopherol (vitamin E) are fat-soluble vitamins that are measured in the clinical laboratory to assess deficiency or toxicity. Premature infants and children with diseases such as cystic fibrosis are often at risk of developing fat-soluble vitamin deficiencies; thus, establishing a pediatric reference interval is important. The purpose of this study was to evaluate reference intervals for vitamins A and E using an HPLC method that had been modified to decrease sample volume, run time, and mobile phase consumption.

More than 400 samples from healthy subjects were analyzed using this rapid HPLC method to establish pediatric reference intervals. Serum specimens were collected from healthy boys and girls, 7 to 17 years old. Samples were precipitated with ethanol, and retinol and α-tocopherol were extracted into hexane, evaporated under nitrogen, dissolved into ethanol, and injected onto an Agilent 1100 HPLC. The rapid HPLC method uses an Agilent Eclipse Plus C-18, 4.6 × 50 mm, 1.8-µm particle analytic column; 0.12-mm internal diameter tubing; and a diode array detector with a 10-mm path-length flow cell. Analytes were detected by their UV absorbance at 325 nm for retinol and 295 nm for α-tocopherol. The limit of quantification is 0.06 mg/L for retinol and is 0.6 mg/L for α-tocopherol.

Total imprecision values (% coefficient of variation) for retinol were 9.0%, 7.8%, and 8.0% at concentrations of 0.3, 0.59, and 2.04 mg/L, respectively. Total imprecision values for α-tocopherol were 6.3%, 5.0%, and 5.5% at concentrations of 5.6, 10.8, and 28.3 mg/L, respectively. For retinol, the Deming regression analysis gave a slope of 0.951 ± 0.023, a y-intercept of 0.004 ± 0.012, and an $S_{y/x}$ of 0.019. For α-tocopherol, the Deming regression analysis gave a slope of 1.030 ± 0.044, a y-intercept of –0.35 ± 0.69, and an $S_{y/x}$ of 1.17. The partitioning test for sex showed no statistical difference for either analyte. The nonparametric reference interval for retinol is 0.23 to 0.55 mg/L for ages 7 to 9; 0.28 to 0.68 mg/L for ages 10 to 12; 0.33 to 0.67 mg/L for ages 13 to 15; and 0.27 to 0.83 mg/L for ages 16 to 17. Partition testing for age showed no statistical difference for α-tocopherol; the nonparametric reference interval is 5.0 to 12.9 mg/L. The modified HPLC method reduced sample volume by 50%, decreased run time by 60%, and decreased mobile phase consumption by 38%. The method showed comparable results to the previous method and is employed for clinical use. Reference intervals determined using this rapid HPLC method are comparable to previously published intervals (Soldin et al, 2005).

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Pediatric Reference Intervals for Urine Calcium, Phosphorus, and Total Protein.

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Lack of pediatric reference intervals is an important issue in laboratory medicine. The aim of this study was to determine pediatric reference intervals for calcium, phosphorus, and total protein in urine. Determining pediatric reference intervals for these analytes in urine has important clinical implications. For example, urine total protein is helpful in monitoring children with renal disease. Urine phosphorus and calcium reference intervals can aid in predicting kidney stone formation and detecting disorders of calcium metabolism in children.

In this study, urine specimens were collected from 940 healthy children 7 to 17 years of age (469 boys and 471 girls). All analytes were measured using a MODULAR P analyzer (Roche Diagnostics, Indianapolis, IN). Results were normalized for creatinine and were reported as analyte/creatinine ratios. Reference intervals were partitioned by age and sex for each analyte. The 4 age groups were 7 to 9, 10 to 12, 13 to 15, and 16 to 17 years for boy and girls (8 groups total), with at least 83 samples in each group. Nonparametric analysis using EP Evaluator Release 5 software (David G. Rhoads Associates, Kennett Square, PA) was conducted to nonparametrically determine the central 95% reference intervals.

Results (in mg/g creatinine for all) were as follows: calcium: boys, 7 to 9 years, 7 to 434; 10 to 12 years, 7 to 300; 13 to 15 years, 7 to 304; and 16 to 17 years, 10 to 277; girls, 7 to 9 years, 17 to 589; 10 to 12 years, 11 to 355; 13 to 15 years, 5 to 334; and 16 to 17 years, 21 to 291; phosphorus: boys, 7 to 9 years, 164 to 1,707; 10 to 12 years, 121 to 1,309; 13 to 15 years, 102 to 1,269; and 16 to 17 years, 67 to 913; girls, 7 to 9 years, 118.5 to 1,347; 10 to 12 years, 152 to 1,365; 13 to 15 years, 80 to 996; and 16 to 17 years, 56 to 905; urine total protein: boys, 7 to 9 years, 63 to 233; 10 to 12 years, 60 to 243; 13 to 15 years, 40 to 510; and 16 to 17 years, 30 to 179; girls, 7 to 9 years, 71 to 545; 10 to 12 years, 60 to 429; 13 to 15 years, 33 to 287; and 16 to 17 years, 27 to 321.

In general, reference intervals decreased with age for all analytes, and boys had higher analyte/creatinine ratios than did girls in the comparable age group, although there were exceptions to both trends. The establishment of these pediatric reference intervals for urine will aid in the diagnosis and management of a variety of pediatric disorders.

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Thrombin Generation Alterations in Patients With Trauma and Disseminated Intravascular Coagulation.

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Severe trauma can induce coagulation factor consumption and disseminated intravascular coagulation (DIC), presumably due to release of tissue factor (TF) and phospholipids by the injury. We evaluated thrombin generation in 28 trauma patients, of whom 14 showed evidence of DIC. Diagnosis of DIC was based on the International Society on Thrombosis and Haemostasis (ISTH) DIC score, which incorporates changes in platelet count, fibrinogen, prothrombin time (PT), and D dimer.

In trauma patients without DIC, median values were as follows: platelets, 183,000/μL; fibrinogen, 199 mg/dL; PT, 18.8 seconds; and D dimer, 3.7 μg/mL; in trauma patients with DIC, median values were as follows: platelets, 60,000/μL; fibrinogen, 101 mg/dL; PT, 24 seconds; and D dimer, 18.7 μg/mL. Citrate-anticoagulated platelet-poor plasma was recalcified in the presence of corn trypsin inhibitor (which blocks contact activation) and a thrombin-sensitive fluorogenic substrate. Thrombin generation was initiated by in vivo TF present in the plasma. In normal plasma, the lag time (from recalcification to the onset of thrombin generation) was found to be inversely proportional to the TF concentration, whereas the peak thrombin generation was a complex function of the coagulation factor, phospholipid, and TF levels. To simulate hemodilution in severe trauma patients during resuscitation, we diluted normal plasma with physiologic buffer to 60% of the normal coagulation factor concentration, which had no effect on the lag time or peak thrombin generation. In 15 healthy subjects, the lag time was 38.3 ± 11.1 minutes, and peak thrombin generation was 101 ± 56 nmol/L. Trauma patients without DIC (ISTH DIC score, 0-4; mean, 2.7) had the shortest lag times (19.9 ± 7.4 minutes; $P < .0001$ vs normal subjects), and trauma patients with DIC (ISTH DIC score, 5-8; mean, 6.0) had lag times shorter than normal subjects but higher than trauma patients without DIC (28.6 ± 9.4; $P = .009$ vs normal subjects; $P < .02$ vs trauma patients without DIC; analysis of variance [ANOVA]). Peak thrombin generation had a similar distribution, with the most elevated levels in trauma patients without DIC (320 ± 117 nmol/L; $P < .0001$ vs normal subjects), whereas trauma patients with DIC had thrombin generation levels higher than normal subjects but lower than trauma patients without DIC (169 ± 70 nmol/L; $P < .04$ vs normal subjects; $P < .0001$ vs trauma patients without DIC, ANOVA).

These data suggest that moderate trauma and hemostatic insults but not overt DIC lead to increased thrombin generation, possibly due in part to TF in the plasma released during the trauma. In more severe trauma leading to overt DIC, thrombin generation is still elevated, even though coagulation factor levels are severely reduced.

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Measurement of the Active Leflunomide Metabolite A77 1726 in Human Plasma by High-Performance Liquid Chromatography.

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Leflunomide is a disease-modifying antirheumatic drug that not only alleviates symptoms of active rheumatoid arthritis, but also inhibits structural damage in affected joints. In vivo, essentially all leflunomide present is converted to its active metabolite, A77 1726. Therefore, most methodologies developed for pharmacokinetic studies monitor A77 1726 concentrations and not leflunomide concentrations. A simple method for the measurement of A77 1726 in human plasma by high-performance liquid chromatography analysis has been developed. The sample preparation protocol is short and straightforward, the main step consisting of protein precipitation with methanol. Chromatographic separation of A77 1726 and the internal standard, 4-aminopyridine, was achieved using a strong cation exchange polySULFOETHYL Aspartamide column with UV detection at 280 nm. Isocratic elution was performed using a pH 3 buffer containing 25% acetonitrile, 10 mmol/L of KH_2PO_4, and 150 mmol/L of KCl. Each run was 7 minutes long. The reproducibility (% coefficient of variation) for intraday and interday assays of spiked controls was less than 3%. The limit of quantification was 1 μg/mL. The average absolute recovery was approximately 100%. Based on these results, this assay is highly suited to the determination of A77 1726 concentrations in patients taking leflunomide owing to its simplicity, reproducibility, and rapid analysis time.

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Differentiation of Bone Marrow Mesenchymal Stem Cells Toward the Smooth Muscle Cell Lineage by Blocking the ERK-MAPK Pathway.

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Smooth muscle cells (SMCs) are major components of blood vessels, required for the engineering of large and medium caliber vessels. Adult bone marrow–derived mesenchymal stem cells (BMMSCs) are multipotent cells that may be used in vessel regeneration. This study aimed to evaluate whether adult BMMSCs can be converted into SMCs. We examined the ERK-MAP kinase pathway because in vitro dedifferentiation of SMCs is blocked by the MEK inhibitor, PD98059.

We used PD98059, an inhibitor of MEK to block ERK-MAPK pathway activation in BMMSCs. After 7-day treatment with PD98059, we evaluated SMC-specific marker gene expression in BMMSCs by RT-PCR and immunodetection. To elucidate the molecular target responsible for PD98059-induced BMMSC differentiation into SMC lineage, we used siRNA to knock down Elk-1, an antimyogenic transcription factor and a downstream target molecule of ERK-MAPK. We also evaluated ligand-induced cell contractility by measuring contraction of cell-embedded collagen gel (gel contraction).

Undifferentiated BMMSCs express most SMC marker genes, albeit mainly at low levels, except smooth muscle myosin heavy chain (SMMHC), the most definitive marker of differentiated SMCs. PD98059 treatment of BMMSCs up-regulated the expression of α-smooth muscle actin (ASMA), *h*-caldesmon, and SMMHC expression in BMMSCs in low serum conditions. PD98059-treated BMMSCs also acquired ligand-induced contractility to endothelin and carbachol. Interestingly, Elk-1 knockdown by siRNA induced SMMHC expression but decreased *h*-caldesmon expression and did not change ASMA expression.

ERK-MAPK pathway inhibition by PD98059 induces SMC marker genes, including SMMHC in BMMSC. These cells gain ligand-induced contractility to endothelin and carbachol. Elk-1 might be partially responsible for PD98059-induced SMMHC expression; however, signaling molecules for up-regulation of ASMA and *h*-caldesmon expression remain uncertain. These findings provide impetus for rationale bioengineering of blood vessels.

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Pediatric Reference Intervals for Three Markers of Bone Metabolism.

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The interpretation of pediatric bone marker results can be difficult because children have elevated bone marker concentrations compared with adults and bone marker values are known to vary with age. Presently, there is limited information on pediatric reference intervals for bone markers. The purpose of this study was to determine the reference intervals for 2 serum markers of bone formation, bone-specific alkaline phosphatase (BSAP) and osteocalcin (OSTEO) and 1 urine marker for bone resorption, cross-linked N-telopeptide (NTXC).

BSAP and OSTEO are molecules synthesized by osteoblasts that can be assayed in serum, and NTXC is generated by osteoclasts by cleaving the N-terminal domains of collagen and is present in serum and urine. Serum for BSAP and OSTEO and urine for NTXC was collected from 940 healthy children (470 boys and 470 girls) 7 to 17 years of age and assayed by chemiluminescent immunoassays according to manufacturer instructions. BSAP measurements were made using a Beckman Coulter DxI analyzer, OSTEO measurements were made using a Roche Elecsys E170 analyzer, and NTXC measurements were made using an Ortho Clinical Diagnostics Vitros ECi analyzer and reported in nM bone collagen equivalent (BCE)/mM of creatinine. In groups with 120 or more subjects, the reference interval was determined nonparametrically by the central 95% range, whereas in groups with fewer than 120 subjects, the reference interval was determined as a nonparametric index.

Results were as follows: BSAP (μg/L): boys, 7 to 9 years, 49 to 140; 10 to 12 years, 49 to 156; 13 to 15 years, 28 to 211; and 16 to 17 years, 15 to 127; girls, 7 to 9 years, 36 to 159; 10 to 12 years, 44 to 163; 13 to 15 years, 15 to 136; and 16 to 17 years, 11 to 45; NTXC (nM BCE): boys, 7 to 9 years, 167 to 578; 10 to 12 years, 152 to 505; 13 to 15 years, 103 to 776; and 16 to 17 years, 34 to 313; girls, 7 to 9 years, 201 to 626; 10 to 12 years, 173 to 728; 13 to 15 years, 38 to 515; and 16 to 17 years, 20 to 144; OSTEO (ng/mL): boys, 7 to 9 years, 66 to 182; 10 to 12 years, 85 to 232; 13 to 15 years, 70 to 336; and 16 to 17 years, 43 to 237; girls, 7 to 9 years, 73 to 206; 10 to 12 years, 77 to 262; 13 to 15 years, 33 to 222; and 16 to 17 years, 24 to 99.

The results of this study show that the reference intervals for each of these 3 analytes change rapidly with pubertal development and should be partitioned by age and sex. It is our hope that with these new reference intervals, clinicians will better be able to evaluate diseases and conditions that affect bone growth and to monitor the effects of therapy in pediatric patients.

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Assessing Renal Function Using a Novel Liquid Chromatography–Tandem Mass Spectrometry Method for Iothalamate Identification and Quantitation.

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Chronic kidney disease is a common and serious medical problem. The clearance of endogenous analytes, such as creatinine, is traditionally used to determine if a patient's kidneys are working effectively. However, this potentially fatal disease often goes undetected owing to the insensitivity of these methods. The clearance of exogenously introduced iothalamate, a commonly used radiopaque agent, has been proposed to be now the best assessment of renal function and serves as a necessary screening tool for kidney transplant donors.

Emory University Hospital (EUH; Atlanta, GA) currently uses iothalamate clearance to calculate glomerular filtration rate (GFR) as the method to screen individuals for potential kidney donations but does not perform iothalamate measurements in house. Methods currently available for the measurement of iothalamate require special equipment and up to a 5.5-minute run time. Therefore, we have developed a novel LC/MS/MS assay that can be used in house to specifically identify iothalamate in biologic samples (plasma and urine) and requires only a 3-minute run time per sample. Performing this method at EUH offers distinct practical and cost advantages.

After the addition of ascomycin, as the internal standard, iothalamate is isolated from urine and plasma by methanol extraction. Chromatographic separations were performed on an Atlantis reversed-phase dC18 column using an ammonium acetate/formic acid mobile phase. The compound was then quantified by electrospray ionization MS/MS in the multiple-reaction monitoring (MRM) mode (Waters Quattro Micro). Iothalamate demonstrated a reproducible elution time of 1.4 minutes, whereas ascomycin, the more nonpolar compound, consistently eluted from the column at 1.7 minutes. Iothalamate and ascomycin were monitored in the MRM mode using the hydrogen adduct mass transitions. Primary and secondary ion ratios were calculated as an additional check of compound specificity. The iothalamate standard curve for plasma displayed linearity up to 600 μg/mL with a limit of quantitation at 1.875 μg/mL. Precision was tested by within-run experiments using drug-free plasma and urine spiked with known low and high concentrations of iothalamate. Acceptable precision (coefficients of variation ≤6.6%) was demonstrated using the LC/MS/MS assay when measuring both of the control solutions. GFR is calculated using the patient's urine flow rate and plasma and urine iothalamate values. This assay holds promise for the fast, accurate assessment of GFR in patients with kidney disease and may serve to determine renal efficiency in potential kidney donors.

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Cytokine Production by Macrophage Cultures to Goldenseal and Astragalus Extract.

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Goldenseal is a native American medicinal plant used as an immune stimulant. Astragalus is a widely used herbal product in China, other Asian countries, and the United States as an immune stimulant for the prevention of colds and the flu. In this study, the innate effects of goldenseal and astragalus on cytokines and chemokines produced by cultured macrophages were examined using 2 brands of goldenseal (GS-1, Herb Pharm, Williams, OR; GS-2, Herbs etc, Santa Fe, NM) and astragalus (AS-1, Herbs etc, Santa Fe, NM; AS-2, Gaia Herbs, Brevard, NC) to test their ability to function as immune-modulating agents. Mouse macrophages (J774A.1; 5×10^5 per well) were incubated with goldenseal or astragalus liquid extracts at varying concentrations (1:50, 1:500, and 1:5,000 dilution vol/vol). Supernatants were collected at 24 and 72 hours postincubation and tested by enzyme-linked immunosorbent assay for the presence of secreted proinflammatory cytokines (interleukin [IL]-6, IL-10, IL-12, and tumor necrosis factor [TNF]-α) and chemokines (macrophage chemotactic protein [MCP]-1).

Both products of goldenseal demonstrated significant "fold" production of IL-6 at 24 hours at the 1:500 dilution (GS-1, 3.83-fold

± 1.0; GS-2, 15.50-fold ± 4.1). Increases in IL-6 production for goldenseal abated by 72 hours. In contrast, both brands of goldenseal demonstrated significant suppression of IL-10 and IL-12 at all 3 concentrations tested; production of TNF-α and MCP-1 was not significantly altered compared with controls. Both products of astragalus also demonstrated significant fold production of IL-6, with increases at the higher dose (1:50 dilution) at 24 hours (AS-1, 4.67-fold ± 0.7; AS-2, 33.86-fold ± 0.7) and significant production of TNF-α at 72 hours at the 1:50 dilution (AS-1, 4.21-fold ± 0.8; AS-2, 32.70-fold ± 3.2). In addition, both products of astragalus also demonstrated significant fold suppression of IL-10 at 24 and 72 hours for all 3 dilutions tested and significant fold suppression of IL-12 at 72 hours at the lowest dilution tested (1:50). AS-2 demonstrated MCP-1 production at 1:50 dilution, whereas AS-1 demonstrated no significant MCP-1 production compared with controls. Similar results were also observed after 72 hours of incubation.

Goldenseal and astragalus exhibit the ability to stimulate macrophage responses. However, both products differ in their immune stimulatory patterns, which may offer insight into differential use and the therapeutic potential of these products to regulate macrophage immune responses and activation events.

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Interaction of Grapefruit Juice With Acetaminophen in Mice.

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Grapefruit juice increases the bioavailability of a number of drugs due to inhibition of the P-glycoprotein pump by furanocoumarins contained within the juice. Moreover, intestinal CYP3A4 enzyme is also inhibited by these molecules. Acetaminophen is a popular over-the-counter pain medication that is toxic to the liver in high concentrations. The interaction of grapefruit juice with acetaminophen was examined in an in vivo mouse model. This drug-food interaction has never been previously reported.

Swiss Webster mice (Harlan, Houston, TX) were fed with 200 μL of white grapefruit juice (Ocean Spray, Lakeville, MA) or pink grapefruit juice (Texsun; Lake Wells, FL) by oral gavage (3 mice in each group) followed by orally delivered, 100 or 15 mg/kg, acetaminophen 1 hour later. Blood was withdrawn by retro-orbital bleeding 1 and 2 hours after feeding with acetaminophen. The concentrations of acetaminophen in serum samples of mice were determined by the fluorescence polarization immunoassay (FPIA) using an AxSYM analyzer (Abbott Laboratories, Abbott Park, IL). Our initial study with mice serum samples indicated that the FPIA designed for determination of acetaminophen in human serum samples can also accurately determine acetaminophen concentrations in serum samples of mice.

White grapefruit juice increased the bioavailability of acetaminophen in mice and also significantly prolonged its half-life. In contrast, pink grapefruit juice only prolonged the half-life of acetaminophen without significantly increasing bioavailability compared with the controls. Specifically, in mice fed with white grapefruit juice followed by acetaminophen, the mean acetaminophen concentrations were 107.8 (SD, 18.3) and 40.27 μg/mL (SD, 16.1 μg/mL) 1 and 2 hours after feeding with acetaminophen, respectively. In contrast, the mean acetaminophen concentration in control mice was 62.2 μg/mL (19.3 μg/mL) 1 hour after administration of acetaminophen and 7.0 μg/mL (SD, 4.0 μg/mL) 2 hours after feeding. These differences were statistically significant by independent *t* test and Mann-Whitney *U* test ($P < .05$). When mice were fed with pink grapefruit juice followed by acetaminophen, the concentrations of acetaminophen were 76.9 (SD, 20.5) and 34.5 μg/mL (SD, 17.2 μg/mL) 1 and 2 hours after administration. Similar trends were observed when mice received a much lower dose of acetaminophen (15 mg/kg). In separate experiments, the duration of the effect of white grapefruit juice (up to 24 hours) was studied. The most significant effects lasted for 4 hours. We conclude that white grapefruit juice increased the bioavailability and prolonged the half-life of acetaminophen, whereas pink grapefruit juice only prolonged its half-life.

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HPC-A Collection Efficiency and Engraftment Between HIV and Non-HIV Patients With Lymphoma.

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This retrospective study evaluated hematopoietic progenitor cell-apheresis (HPC-A) collection efficiency and engraftment in HIV and non-HIV patients with lymphoma. We reviewed the files of patients who had HPC-A collection/transplant at Montefiore Medical Center between 2003 and 2007. We identified 8 HIV lymphoma patients (HIV lym pts) and 35 non-HIV lymphoma patients (non-HIV lym pts). The HPC-A collections were performed after mobilization with G-CSF and/or chemotherapy.

Mobilization of peripheral blood (PB) CD34+ cells was significantly lower in the HIV lym pts with an average number of 27.7/μL (range, 7.9-87.6 μL) compared with 85.1/μL (range, 8.9-746.7 μL) in non-HIV lym pts ($P < .01$). Also, the total CD34 cell collection average was significantly lower for the HIV lym pts, 1.71×10^6/kg (range, 0.26-4.2×10^6/kg) compared with 5.46×10^6/kg (range, 0.04-62.1×10^6/kg) in non-HIV lym pts ($P < .01$). Collection efficiency in both populations correlated with the PB CD34+ cell count. For HIV lym pts (n = 20 collections), the correlation coefficient was $r = 0.82$; for non-HIV lym pts (n = 78 collections), it was $r = 0.87$. The infused CD34+ HPC dose was lower in the HIV lym pts (4.22 vs 7.28 CD34+ cells × 10^6/kg in non-HIV lym pts; $P < .05$). Posttransplant engraftment data for the HIV lym pts were similar to those for non-HIV lym pts. The median times until engraftment for absolute neutrophil count (ANC) more than 500/μL, WBC count of 1,000/μL, and platelet count more than 20,000/μL were 11.80 days (range, 10-13 days), 11.80 days (range, 10-13 days), and 18.50 days (range, 12-35 days) in the HIV lym pts and 10.89 days (range, 8-33 days), 11.33 days (range, 8-25 days), and 19.50 days (range, 7-118 days) in the non-HIV lym pts, respectively.

HIV lym pts have significantly lower PB CD34+ HPC mobilization and HPC-A collection average than non-HIV lym pts, but there is a similar collection efficiency in both groups. Although the infused CD34+ cell dose was lower in the HIV lym pts, engraftment was similar in both groups.

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Blood Detection in Urine: Lower Threshold for the Clinitek Status Compared With the Yellow IRIS.

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Presence of even a trace of blood in urine can reflect a serious condition such as bladder cancer. However, a tiny amount of blood can also be normal, and commercial systems using peroxidase dipstick methodology may differ in their threshold of detection. Because

we recently adopted the Clinitek Status (Siemens Medical Systems) dipstick reader for use in a point-of-care (POC) setting, we compared its blood detection with our main laboratory system, the Yellow Iris (IRIS International), which provides a dipstick analysis and RBC count. We retrospectively examined results during two 7-day periods, 2 months apart. The Status reports urine blood in 1 of 6 categories: negative (NEG), trace intact (TI), trace lysed (TL), small (S), moderate (M), and large (L). The Iris dipstick reader does not distinguish intact and lysed, reporting only trace (T). The quantitative cell counts from the IRIS were categorized as POS if more than 2 RBCs per high-power field were reported, and otherwise as NEG. A total of 501 urine samples were examined in the POC laboratory during the study. The POC laboratory serves only the Emergency Department (ED), so the study population included patients who presented to the ED for any reason and had a stat urinalysis ordered. The patients were 48% male and ranged in age from 1 month to 94 years (median, 44 years).

The results of the Status blood dipstick test were as follows: 241 (48%) NEG, 76 (15%) TI, 15 (3%) TL, 42 (8.4%) S, 49 (9.8%) M, and 78 (16%) L. The Status NEG specimens were not, per our clinical protocol, retested on the Iris. However, 10 Status NEG specimens were NEG on the Iris by dipstick and cell count. Trace and negative results were reproducible on the Status. Of the 76 specimens that were TI on the Status, 73 (96%) were NEG on the Iris by dipstick and cell count. Of the 15 specimens that were TL, 14 (93%) were NEG on the Iris by dipstick and cell count. Of specimens that were S, M, or L on the Status, NEG results by dipstick and cell count on the Iris occurred, respectively, in 10 (24%) of 42, 1 (2%) of 49, and 3 (4%) of 78 cases. None of the 260 specimens in any positive category on the Status were in a higher "more positive" category on the Iris dipstick.

In a diverse population of patients presenting to an ED, 52% tested at least trace positive for urine blood using the Status, and 87 (96%) of 91 of the Status trace positives were not confirmed as positive by dipstick or cell count using the Iris. By contrast, only 14 (8%) of 169 Status results in a higher positive category (S, M, or L) were not confirmed as positive by dipstick or cell count using the Iris. Using the Clinitek Status method for urine screening could result in considerably more follow-up testing than using the Yellow Iris, but further studies will be needed to determine if such follow-up is clinically appropriate.

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The Role of Flow Cytometry in Routine CSF Specimens.

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In patients with central nervous system involvement of hematologic malignancies, conventional cytomorphologic analysis of cerebrospinal fluid (CSF) has limited sensitivity in detecting malignant cells (15%-25%). Flow cytometric immunophenotyping (FCI) has been found to have at least 25% higher sensitivity than conventional cytomorphologic analysis in detecting malignant cells. At our institution, the clinician directs CSF specimens to hematology for a cell count or to cytology for conventional morphologic analysis (cytopath). After cytopath review, the specimen can then be redirected for FCI, usually with a delay of up to 24 hours. We wanted to investigate whether this is the best use of a sample that is difficult to collect and easily degraded by time and improper collection media.

Cytopath results were examined on all CSF specimens collected in 2005 at a large academic medical center. Cytopath and FCI reports were correlated for all specimens available. Results were collected and catalogued according to the age, sex, submitting specialty, stated clinical history, cytopath diagnosis, and FCI diagnosis. Cytopath diagnoses were classified as negative or abnormal, which consisted of atypical, "suspicious," or positive. We reviewed 211 CSF cytopath specimens. Of the 211 specimens reviewed, 85 (40%) were classified as abnormal. Of the cytopath abnormal cases, 55 (65%) were sent for FCI, in which 38 (69%) were negative, 8 (15%) were insufficient, and 9 (16%) were positive. Of the 85 abnormal cytopath cases, 30 did not have FCI performed, although 9 were submitted with a suspicion of hematologic malignancy. Of 211 specimens, 126 (60%) were classified as negative by cytopath. Of the cytopath-negative cases, 56 (44%) were sent for FCI, in which 27 (48%) were negative, 27 (48%) were insufficient, and 2 (4%) were positive.

Based on the data, proper handling of CSF includes a laboratory capable of immediate triage of specimens to the appropriate diagnostic modality and proper preservation media. Use of FCI with its greater diagnostic sensitivity might help the diagnosis in the 48% of cytopath-negative/FCI-insufficient specimens and in the 15% of cytopath-abnormal/FCI-insufficient cases. Routing of specimens in this way would eliminate the 35% of cytopath-abnormal specimens with no FCI analyses performed, especially when 11% of these cytopath-abnormal cases had a diagnosis suspicious for a hematologic malignancy. Proper routing of limited specimens such as CSF is paramount to institutional quality assurance.

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M Proteins as Potential Early Markers of Posttransplant Lymphoproliferative Disorder.

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Posttransplant lymphoproliferative disorder (PTLD) is a potentially life-threatening complication occurring in up to 30% of graft recipients. Early diagnosis and intervention are important prognostic factors. Currently available screening assays have significant limitations; Epstein Barr viral (EBV) load, EBV-specific T-cell response, and serum interleukin-10 concentrations have been studied with varying results but are not routinely available. An increased incidence of monoclonal gammopathy associated with an elevated risk of developing PTLD was reported in liver and kidney allograft recipients. We investigated the prevalence of monoclonal gammopathy in serial serum samples from heart transplant recipients by 2 widely available tests: serum protein electrophoresis (SPE) and immunofixation electrophoresis (IFE).

For the study, 61 samples from 20 heart transplant recipients with PTLD were analyzed by SPE and IFE and interpreted by 3 blinded interpreters. Samples were interpreted as positive or negative for monoclonal gammopathy based on unanimous analysis. Discordant results were reviewed, and resolution was attempted in a blinded manner. Tests were scored as indeterminate if no unanimous interpretation was achieved.

The median time from transplantation to the diagnosis of PTLD was 5.6 years (SD, 4.4 years), and the median time from the first sample to diagnosis of PTLD was 5.1 months (SD, 0.4 months). The interobserver variability was 14.8%. All 5 available pretransplant samples were negative. M proteins were detected in 11 of the remaining 56 samples; 2 were interpreted as indeterminate. These 13 samples came from 7 PTLD patients (35%). It is important to note that IFE was positive in all 7 patients, whereas SPE detected the M protein in only 3 patients. Of the 7 patients, 6 (86%) had an abnormal SPE/IFE before the diagnosis of PTLD (4 positive, 2 indeterminate). The M proteins were IgGκ (n = 3), IgGλ (n = 1), IgMκ (n = 1), IgAκ (n = 1), and λ light chain (n = 1). It is interesting that 1 EBV-negative patient and 1 patient with T-cell PTLD had monoclonal gammopathy.

SPE and IFE are widely available, noninvasive, and affordable tests that could complement current investigational screening assays for PTLD. With 1 exception, M proteins were always detected before diagnosis of PTLD in our series. IFE detected more than twice as many cases of M protein–positive PTLD than did SPE. This emphasizes the need for clinical laboratories to consider using both tests rather than using reflex IFE testing only for positive SPE samples

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Comparison of Hemagglutination and a Solid Phase ELISA for Determination of ABO Blood Group Antibody Titers.

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One solution to the increased demand for solid-organ transplants is the use of ABO-incompatible (ABOI) organs. The ABO blood group anti-A/B titer, as measured by classical hemagglutination, is widely used to make clinical decisions on the timing of transplants and on the diagnosis of acute humoral rejection in ABOI cases. However, studies have shown poor interinstitutional reproducibility of this assay, and we sought to develop and validate an enzyme-linked immunosorbent assay (ELISA) using red cell ghosts as the solid phase antigen to produce a more precise and accurate quantitative measure of anti-A/B antibody concentrations in this growing patient population.

As proof-of-principle, we used 3 anonymized plasma samples from group B individuals representing high, medium, and low anti-A titers, 2 serial dilutions of each of these 3 samples, each in replicates of 5; and 3 undiluted, anonymized samples from an AB individual; to prepare a total of 48 blinded samples for testing. We then compared results obtained by a single trained blood bank technologist performing standard hemagglutination IgM titers in saline with group A red cells with the optical density results detecting IgM anti-A obtained by ELISA. These results showed a good correlation between ELISA and standard hemagglutination ($R^2 = 0.912$). The ELISA showed an interassay coefficient of variation ranging from 1.3% to 11.5% for low and high antibody concentrations, respectively. In contrast, the difference between maximum and minimum titer obtained by hemagglutination for replicates of the same sample was at least 2-fold, resulting in serial 2-fold dilutions that differed from each other by 0- to 4-fold.

We developed a solid-phase ELISA method for quantifying IgM anti-A concentrations that is more precise and accurate than the standard hemagglutination titer. In addition, this approach has the capability of quantifying IgG anti-A concentrations and those for individual IgG subclasses (ie, IgG1, IgG2, IgG3, and IgG4), which may prove to be more clinically relevant in this setting.

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Determination of 8-Isoprostanes in Human Serum Using LC/MS/MS.

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The isoprostanes, which are prostaglandins produced via free radical–induced peroxidation of arachidonic acid, are gaining acceptance as reliable indicators of lipid peroxidation in vivo. However, owing to their labile nature and complicated formation pathway (different products and isomers are formed), the methods available for their measurement remain a challenge. Current methods for measuring isoprostanes in serum and urine include immunoassays or mass spectrometry. However, most of these methods suffer from poor reproducibility or long sample preparation times. The aim of this project was to develop a method to measure 8-iso-prostaglandin F2α (8-iso-PGF2α) in human serum using liquid chromatography–tandem mass spectrometry (LC/MS/MS).

To accomplish this, hormone-free human serum was spiked with 8-iso-PGF2α and its deuterated internal standard and extracted using a solid-phase extraction technique. We injected 50 μL of the sample into a Waters 2795 HPLC system using a Waters XTerra MS C18 3.5-μm column. The initial gradient consisted of 5% acetonitrile in ddH_2O with a flow rate of 0.3 mL/min, ending at 33% acetonitrile in ddH_2O at 20 minutes. 8-iso-PGF2α and 8-iso-PGF2α-d4 were detected using a Waters Micromass Quattro Micro tandem MS operated in the negative ion, multiple reaction mode with the following transitions: 8-iso-PGF2α, m/z 353.20>193.25; 8-iso-PGF2α-d4, m/z 357.20>197.25 (retention time [Rt], 16.16 minutes for both). Our results showed clear separation of the 8-iso-15(R) PGF2α (Rt, 15.71 minutes), 15(R)-PGF2α (Rt, 17.22 minutes), PGF2α (Rt, 17.8 minutes), and 11β-PGF2α (Rt, 17.82 minutes) isomers. The assay was linear from 20 pg/mL to 25 ng/mL (correlation coefficient = 0.9999). Recovery of 8-iso-PGF2α ranged from 81% to 106%. Precision studies showed an intra-assay coefficient of variation (CV) of 5.3% at 1.4 ng/mL.

We introduced a simple, sensitive, and reproducible LC/MS/MS assay to quantify 8-iso-PGF2α in human serum. Determination of the interassay CV and reference range is underway.

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The Utility of Fetal Fibronectin in Preterm Labor Decision Making.

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Approximately 2% of pregnancies are complicated by threatened preterm labor (tPTL), treated with ultrasound (US), steroids, and tocolysis although 80% of these pregnancies will carry to term. Fetal fibronectin (fFN) has been used to predict outcome for these pregnancies. We evaluated fFN testing, with turnaround time (TAT) of 24 to 96 hours, to determine if fFN results predicted PTL or affected treatment and if fFN testing through a reference laboratory provided adequate care.

The fFN test was provided to 240 women with tPTL during 12 months. Retrospective chart reviews determined symptoms, treatment, fFN result, and gestational age (GA) at presentation, testing, and delivery. The 2-tailed, unpaired *t* test and the Fisher exact test (significance $P < .05$) were used for analysis. Demographics were similar in the test positive (fFN+) and negative (fFN–) groups. During the study, 236 of 240 women met GA requirements. Forty-nine had not delivered by the conclusion of the study or were lost to follow-up. An fFN+ result correlated with a relative risk of 4.3 (95% confidence interval, 2.2-8.6; $P = .001$), sensitivity was 43.8%; specificity, 89.9%; positive predictive value, 25.9%; and negative predictive value, 95.2% for delivery within 7 days of testing. The fFN result was available before delivery in 94.7% of patients (177/187). The median times from testing to delivery were 23 and 47.2 days for fFN+ and fFN– results, respectively. One patient delivered before

fFN+ and 9 before fFN– results were available. Twenty-six patients had fFN+ results before delivery, and 17 (65.4%) received treatment. In the fFN– group, 44.0% (71/151) received treatment. Tocolytic use was similar between fFN+ and fFN– groups (P = .0718). Steroids (P = .0008), US (P = .018), and antibiotics (P = .02) were associated with fFN+ results and increased length of hospital stay (LOS; P = .0078). Seven patients with an fFN+ result and no tPTL-related treatment had urinary tract infection, closed cervix, and no signs or symptoms of tPTL. In the fFN– group, 85.4% (129/151) were discharged before a result was issued. The fastest TAT of our results (0.73 days) was used for comparing patients with an fFN– result and hospital stay longer than 0.73 days (n = 34). Treatment was different in patients discharged before or after a result (P = .03), but treatment was similar whether the patient was discharged the day of or multiple days after a negative result (P = .0). The LOS was the same if the patient was discharged before or after a negative result (P = .4).

Although a +fFN result correlates with a higher likelihood of delivering within 7 days and additional therapy with tocolytics, our data suggest that clinical decisions may be independent of a negative result.

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The Functional Polymorphism of the Hemoglobin-Binding Protein Haptoglobin Influences Susceptibility to Idiopathic Parkinson Disease.

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Oxidative stress and iron have been widely implicated in the etiology of Parkinson disease (PD). Hemoglobin is the richest source of iron in the body and is capable of inducing oxidative stress damage. Human haptoglobin (Hp) is a plasma α_2-glycoprotein that removes free hemoglobin from circulation and is important to protect kidneys and other tissues from hemoglobin-induced damage. Hp also functions in immune system regulation and angiogenesis; thus, it is potentially involved in protection from the development of PD. A common genetic polymorphism of Hp exists in the population, in which the different Hp forms, Hp 1-1, Hp 2-1, and Hp 2-2, exhibit profound functional differences. In this study, the Hp genotype corresponding to phenotypes Hp 1-1, Hp 2-1, and Hp 2-2 was determined in a population of 312 idiopathic PD patients and 420 normal control subjects.

Genotyping was performed by polymerase chain reaction (PCR) on the subjects' genomic DNA using Hp allele–specific primers, followed by agarose gel electrophoresis of the PCR products. A significant increase in the number of subjects carrying the Hp 2-1 genotype was present among the population of PD patients. Our results showed that the distribution of Hp genotypes among PD cases (Hp 1-1, 16.0%; Hp 2-1, 56.4%; and Hp 2-2, 27.6%) was significantly different from the distribution in normal control subjects (Hp 1-1, 15.2%; Hp2-1, 48.1%; and Hp 2-2, 36.7%; $\chi^2 = 6.99$; P = .030). The odds ratios for PD risk for Hp 2-1 and Hp 1-1 vs Hp 2-2 genotype were 1.51 (1.07-2.12) and 1.36 (0.86-2.15), respectively. Differences in Hp genotype distribution between cases and controls were stronger in subjects who were never smokers compared with ever smokers.

Our results show that subjects with different Hp phenotypes have significantly different risks for idiopathic PD. To our knowledge, this is the first study that investigated the association of Hp phenotypes with idiopathic PD risk.

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Characterization and Optimization of a Real-Time PCR Assay for SRY Determination.

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Sex determining region (SRY) contains a gene that encodes a transcription factor that is a member of high mobility group (HMG-box) family of DNA binding proteins. SRY is also called testis determining factor and testis determining factor on Y. Its main function is to initiate male sex determination during the embryonic developmental stage. The detection of SRY is useful clinically in cases of infertility, ambiguous genitalia, and gonadal dysgenesis, as well as other disorders. The presence of SRY in maternal circulating free DNA has also been used for fetal sex determination. The aim of the present study was to develop, characterize, and optimize a real-time polymerase chain reaction (PCR) assay for SRY for application in clinical laboratory.

DNA was extracted from the whole blood of 20 normal males and females each, using the MagNA Pure LC instrument for nucleic acid extraction. DNA extracted from 20 cell pellets sent for SRY detection by fluorescence in situ hybridization analysis was also used. As a control for extraction and amplification steps, a small amount of mouse DNA (Jackson Laboratories, Miami) was added to each patient's sample. Amplification was carried out in a Light Cycler 2.0 instrument (Roche Diagnostics, Germany). The amplification of 115 base pairs of the *SRY* gene was performed. Two hybridization probes, one labeled at 5' with LCRed640 and the other labeled with fluorescein isothiocyanate at 3', were used. PCR reactions were set up in a final volume of 20 µL using the Fast DNA Master Hybridization Probes Kit (Roche Biochemicals) with 0.5 µmol/L of each primer, 0.25 µmol/L of each probe, 1.25 U of uracil N-glycosylase (Applied Biosystems), 5 mmol/L of magnesium chloride, and 1 µL of extracted DNA. Cycling conditions were as follows: denaturation at 95°C for 10 seconds, annealing at 56°C for 10 seconds, and extension at 72°C for 15 seconds. The sensitivity of the SRY assay was evaluated by using serial dilutions of male DNA with water. The reproducibility of the assay was determined by amplification of DNA from a normal male and a normal female in replicates of 3 per run and over 5 runs on different days.

All serum samples from normal male individuals gave positive results for *SRY* gene detection, whereas all serum samples from females were negative for SRY. There was no amplification seen with mouse DNA, which was used as the external control. *SRY* amplification by closed-tube, real-time PCR is a highly sensitive and specific, rapid, and cost-effective assay.

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Effects of Hemoglobin C and S Traits on Two Commercial Glycated Hemoglobin Assays.

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Glycated hemoglobin (HbA_{1c}) is formed by a posttranslational, non-enzymatic condensation of the aldehyde group of glucose with amino terminus of the β chain of hemoglobin. The measurement of HbA_{1c} can be used to assess long-term glycemic control in patients with diabetes mellitus. Hemoglobin C (HbC) and S (HbS) traits have

a prevalence of 10% in African Americans, and it is estimated that 200,000 African Americans with diabetes in United States have HbC or HbS trait. Hemoglobin variants are known to produce clinically significant effects on HbA_{1c} measurements. We evaluated the effects of HbC and HbS on 2 commercial HbA_{1c} methods, using the Primus CLC 330 boronate affinity method as the comparison method.

Whole blood samples from people with diabetes mellitus who were homozygous for HbA (HbAA), heterozygous for HbC (HbAC), and heterozygous for HbS (HbAS) were analyzed by the following methods/instruments: Afinion AS 100 and Microgenics DRI glycated hemoglobin. For each test method, results of each type of sample were compared with those by the CLC 330 method, which is not affected by the presence of HbC or HbS trait. For the Afinion method, the numbers of samples in the HbAA, HbAC, and HbAS groups were 23, 22, and 24 respectively. For the Microgenics method, the numbers of samples in the HbAA, HbAC, and HbAS groups were 40, 28, and 43, respectively. Deming regression analysis was performed. To determine whether the presence of HbC or HbS traits could have a clinically significant effect on HbA_{1c} measurements, evaluation limits of 6% and 9% were chosen. After correcting for calibration bias with the HbAA sample group, we evaluated net method bias attributable to the presence of HbC or HbS traits. A clinically significant difference was more than 10% (ie, 0.6% at 6% HbA_{1c} and 0.9% at 9% HbA_{1c}).

For the HbC trait, net bias for the Afinion method was 0.06% at 6% and 0.2% at 9%. For the Microgenics method, net bias values were 0.3% and 0.06% at 6% and 9%, respectively. For the HbS trait, net bias values for the Afinion method were –0.07% and –0.2% at 6% and 9%, respectively. For the Microgenics method, net bias values were 0.1% and –0.4% at of 6% and 9%, respectively.

Although small differences were noted from patients who had HbC and HbS traits, we do not consider these differences clinically significant for the Afinion or the Microgenics method.

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Competency-Based Training in Clinical Chemistry for Pathology Residents.

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The Accreditation Council for Graduate Medical Education (ACGME) has endorsed The Outcome Project, which mandates that training programs in all medical specialties must address development of skills in 6 core competencies. Training programs in clinical chemistry have found it difficult to involve residents in the full spectrum of laboratory activities and to strike a balance between didactic teaching and involvement with development activities and research. We developed a training program in clinical chemistry for residents in pathology that conforms to ACGME guidelines, addresses all 6 core competencies, and incorporates didactic and experiential elements.

At our institution, chemistry testing is performed in 14 laboratories, including high-volume (routine) and specialized laboratories. To provide universal exposure to important core tests and to allow for participation in 1 or more laboratory projects, we divided the 12-week rotation into a 5-week mandatory segment and a 7-week elective. In the mandatory segment, residents rotate for 1 week each in the following laboratories: central clinical, hospital clinical, protein, renal, and cardiovascular. This experience includes daily contact with laboratory directors for personalized seminars with the resident and test sign outs. This portion of the rotation emphasizes the following 4 core competencies: medical knowledge, patient care, interpersonal and communication skills, and professionalism.

Before beginning the elective portion of the rotation, residents review online descriptions of the research and development activities in each chemistry laboratory and select a staff mentor to aid in choosing their elective(s). Residents meet with their mentors, discuss possible projects, and meet with individual laboratory directors to design their projects and establish specific learning objectives. Each project is designed with the resident's career goals in mind and focuses on the remaining core competencies of practice-based learning and improvement and systems-based practice. Each resident is expected to make a presentation to the laboratory staff, develop an abstract or manuscript, and participate in practice-based activities such as introducing a test for clinical service.

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Clinical Chemistry Education of Pathology Residents.

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The Academy of Clinical Laboratory Physicians and Scientists recently published a detailed proposed curriculum for training of pathology residents in clinical pathology. Our main objective was to focus on the current state of clinical chemistry education and identify areas of success and difficulty in teaching this subject to pathology residents.

We first attempted to identify the primary person responsible for clinical chemistry education at all accredited pathology residency programs in the United States. An anonymous, open-ended survey was sent to these faculty that inquired about the following: (1) aspects and methods of the clinical chemistry rotation that work particularly well, (2) major challenges in teaching residents clinical chemistry, and (3) impact of the shortening in length of pathology residency on chemistry education. Faculty members from 49 institutions returned surveys or agreed to telephone interviews.

Many respondents described in detail their efforts in engaging residents, including the challenges of having residents serve effectively on call. The major challenge in teaching clinical chemistry to pathology residents was identified by 59% of respondents as a lack of interest by residents. This was especially evident for residents seeking combined training in anatomic and clinical pathology, most of whom plan a future career with little or no responsibility for clinical chemistry. Nearly 40% of respondents indicated that the shortening of pathology residencies in 2002 negatively impacted clinical chemistry by shortening rotation time and/or forcing more anatomic pathology responsibilities during the chemistry rotation. The respondents also highlighted poor background knowledge and skills exhibited by many residents, especially quantitative, statistical, and abstract problem-solving skills. This hampers understanding of chemistry instrumentation and quality control methods. Last, some faculty members were positive about the future of clinical chemistry education in that areas such as genomics, proteomics, and molecular techniques are developing rapidly and are likely to be of more interest to pathology residents than "classical" clinical chemistry.

Clinical chemistry education of pathology residents faces a number of challenges, including lack of resident interest in the subject, compressed rotation schedules, and deficiencies in background knowledge and skills by residents. Opportunities to address these challenges include ongoing initiatives to reform medical school curricula and the incorporation of genomic and proteomic applications into clinical chemistry.

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Molecular-Based Identification of *Nocardia* Species in the Clinical Microbiology Laboratory.

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Nocardiae are ubiquitous, saprophytic bacteria that can also cause human disease (>30 species are known to infect humans). Correct identification of *Nocardia* to the species level is important because mortality rates and antimicrobial resistance patterns differ among species. However, the microbiologic culture and chemotaxonomic methods for species assignment are time-consuming, inaccurate, and not commonly practiced in many clinical laboratories. Molecular methods, such as *16S rRNA* gene sequencing, have the potential to improve species identification. We evaluated a multigene sequencing approach to study the relative prevalence of *Nocardia* species at our institution.

We subcultured 34 clinical isolates, initially identified as *Nocardia* species by routine microbiologic methods, from frozen stocks or collected prospectively. Direct polymerase chain reaction and sequencing analysis of the *16S rRNA* (1,512 base pairs [bp]), *hsp65* (441 bp), and *rpoB* (559 bp) genes were performed from cultures of single colonies. The resulting sequences were compared with entries in GenBank by BLAST analysis. The *16S rRNA* gene was considered the "gold standard" for species assignment. Selected chemotaxonomic tests were used to validate identity.

Since 2002, 34 clinical strains have been isolated at our New York City institution. By the *16S rRNA*, 13 (38%) were identified as *Nocardia nova*, 9 (26%) as *Nocardia cyriacigeorgica*, 6 (18%) as *Nocardia farcinica*, 2 (6%) as *Nocardia niigatensis*, and 1 (3%) each as *Nocardia abscessus*, *Nocardia otitidiscaviarum,* and *Nocardia transvalensis*. The sequence of 1 isolate did not significantly match any GenBank entry and is most likely a novel species. Species identities based on *hsp65* gene sequences were identical to those obtained by *16S rRNA* analysis for 33 of 34 isolates; the *hsp65* sequence of the *N abscessus* isolate was most similar to a *Nocardia arthritidis* GenBank sequence entry. Owing to the current paucity of entries, reliable identification based on *rpoB* sequences was not possible by BLAST analysis in GenBank. However, *hsp65* and *rpoB* consistently showed higher intraspecies variability than the *16S rRNA* gene.

Overall, 82% of our New York City clinical isolates belonged to 3 distinct species: *N nova*, *N cyriacigeorgica*, and *N farcinica*. This distribution differs from reports in the southwestern United States and central Europe. Identification of *Nocardia* species can guide treatment, and *16S rRNA* sequencing provides rapid and accurate identification that can reduce time to diagnosis. However, the higher ratio of polymorphisms to length in *hsp65* and *rpoB* potentially makes these individual targets more suitable for *Nocardia* strain discrimination than *16S rRNA*.

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Histologic and Microbiologic Diagnosis of Infective Endocarditis.

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Treatment of infective endocarditis (IE) often involves replacement of the damaged valve. Histologic examination and culture of the excised valve confirm the diagnosis and can provide information on the adequacy of previous antibiotic treatment and/or guide future treatment. The objective of this study was to correlate histologic and bacteriologic results in patients with acute endocarditis.

We identified patients at our institution who had a histologic diagnosis of IE based on visualization of microorganism and/or changes consistent with IE during the 2002-2006 period and correlated this with valve tissue Gram stain (GS), culture (vcult), and blood culture results. The most frequent etiologic agents were viridans streptococci (n = 9), *Staphylococcus aureus* (n = 8), and *Enterococcus faecalis* (n = 5). In 29 specimens, organisms were seen in the histologic section; 15 were GS–/vcult–, 5 were GS+/vcult–, 4 were GS–/vcult+, and 5 were GS+/vcult+. In 3 specimens, organisms were not seen in the histologic section but were seen in the tissue GS (1 was also vcult+). The low incidence of positive valve cultures (10/32) was probably due to antibiotic treatment before valve replacement. Of the 10 positive valve cultures, 5 grew *S aureus* (3 were methicillin resistant). This probably reflects the difficulty of treating and/or the rapidly progressive nature of *S aureus* endocarditis. Of 29 specimens, 19 had a negative GS despite the observation of organisms in the paraffin section. This discrepancy is not readily explained by previous antibiotic treatment. Examination of the histologic slides revealed that only small numbers of microorganisms were present in many of the sections, even when there were large vegetations. In addition, organisms were often localized in microcolonies rather than uniformly distributed.

At our institution, the surgeon sends part of the surgical specimen to the anatomic pathology laboratory and part to the microbiology laboratory for GS/culture. We suspect that the low yield of the tissue GS in the microbiology laboratory is due to inadequate sampling. Having the surgical pathologist assess the entire specimen and decide which part of the specimen should be sent to the microbiology laboratory may improve the yield of valve GS/culture.

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Sepsis-Induced T- and B-Lymphocyte Apoptosis Is MyD88-Dependent.

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Sepsis induces global lymphocyte apoptosis, resulting in impaired immune defenses and subsequent increased morbidity and mortality. There are multiple potential triggers or signaling molecules in mediating death signals. Elucidating the specific signaling pathways in sepsis-induced lymphocyte apoptosis may lead to improved therapies of this highly lethal disorder.

Presently, our studies were performed using the cecal ligation and puncture (CLP) model of clinical sepsis using specific gene-targeted deletions of the following receptors and signaling molecules: toll-like receptors 2 and 4 (TLR2/4), interleukin 1 receptor (IL-1R), myeloid differentiation factor 88 (MyD88), toll/IL-1 receptor domain–containing adapter-inducing interferon-β (TRIF), signal transducer and activator of transcription 1 and 4 (STAT1 and STAT4), and c-Jun N-terminal kinase (JNK). CLP-induced lymphocyte apoptosis was evaluated 24 hours postoperation by active caspase-3 and TUNEL staining.

Surprisingly, the only genetic construct that ameliorated sepsis-induced apoptosis occurred in the MyD88–/– mice. T- and B-lymphocyte apoptosis was significantly decreased in MyD88–/– mice by 80% and 85%, respectively, as evidenced by decreased active caspase-3

and TUNEL staining. MyD88 is a common adapter signaling protein of the TLRs and IL-1R. As expected, there was a significant decrease in serum proinflammatory and anti-inflammatory cytokines in the MyD88–/– mice compared with controls. In contrast with MyD88–/– mice, TLR2, TLR4, IL-1R, TRIF, STAT1, STAT4, and JNK knockout mice demonstrated no significant changes in CLP-induced lymphocyte apoptosis compared with controls. Our data suggest that the post–receptor signaling protein, MyD88, has a pivotal role in CLP-induced lymphocyte apoptosis and mediator production.

Lymphocyte death in sepsis likely involves multiple pathogen-sensing receptors and redundant signaling pathways. Ultimately, deletion of MyD88, which has an essential role in mediating numerous pathogen recognition pathways, was effective in blocking apoptosis.

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Comparison of Roche's New Prolactin II Assay With Roche's Initial Method Based on Three Months of Patient Samples Before and After the Change.

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Our objective was to determine if the new, reformulated Roche Elecsys Prolactin II assay has less cross-reactivity with macroprolactin than the initial Roche Elecsys Prolactin assay.

We reviewed data from the last 3 months of use of the initial Roche method (August 2006-October 2006; total number of patients, 356; number of hyperprolactinemic patients, 77 [females, 47; males, 30]) and compared it with data from the first 3 months of use of the reformulated method (November 2006-January 2007; total number of patients, 330; number of hyperprolactinemic patients, 88 [females, 53; males, 35]). The presence of macroprolactin was reported when the prolactin level fell to within the normal range after treatment with polyethylene glycol.

The data revealed an increase in the number of patients reported to have hyperprolactinemia with the reformulated method (88 of 330 patients tested [26.7%]) compared with the initial method (77 of 356 patients tested [21.6%]). This may reflect the fall in the reference range between methods. The percentage of patients reported to have macroprolactinemia, however, was not significantly different (initial method, 30 of 356 patients tested [8.4%]; reformulated method, 27 of 330 patients tested [8.2%]). By using a negative control sample (Biorad Lyphochek 3), we found the precision to be improved in the new method (SD, new method, 3.1 ng/mL; initial method, 4.4 ng/mL). Finally, we compared the average prolactin level in hyperprolactinemic women vs men between the initial and reformulated methods. The average prolactin level in women fell by 13% with the reformulated method (81.2 to 70.6 ng/mL) compared with the percentage decrease of 19.0% in the reference range (old method, 0.4-29.0 ng/mL; new method, 0.1-23.5 ng/mL). In contrast, the average prolactin level in men fell by 49.3% with the new method (90.8 to 46.1 ng/mL), which is significantly greater than the corresponding fall of the reference range of 26.8% (old method, 0.4-20.5 ng/mL; new method, 0.1-15.0 ng/mL)

Changing to the new prolactin assay appeared to have no significant impact on the number of patients reported to have macroprolactinemia. However, we identified a significant drop in the prolactin level in men with the new method. The explanation for this fall in prolactin is presumed to be related to the change in assay and not to clinical differences in the patient population. The exact nature of this discrepancy remains unclear.

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Aberrant T-Cell Antigen Expression in B-Cell Lymphoma.

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B-cell lymphomas often aberrantly coexpress T-cell antigens other than CD5 and CD43. We studied the incidence and spectrum of aberrant T-cell antigen expression in a large series of B-cell non-Hodgkin lymphomas analyzed in our laboratory between 2003 and 2006 using 4- or 8-color flow cytometry.

We searched our database for B-cell lymphomas in blood, body fluid, lymph node, tissue, or bone marrow specimens that were analyzed for expression of B- and T-cell antigens. We identified 1,042 cases of B-cell lymphoma in which neoplastic B cells represented more than 10% of the sampled WBCs, and we analyzed these cases for expression of CD2, CD3, CD4, CD7, and CD8. A T-cell antigen was considered aberrantly expressed if more than 25% of the abnormal B cells expressed this antigen and if the staining was confirmed not to represent nonspecific antibody binding, autofluorescence, or other artifact.

Overall, 3.5% (36/1,042) of the B-cell lymphomas aberrantly expressed 1 or more of these T-cell antigens. The most commonly expressed antigen was CD7 (17 cases) followed by CD8 (14 cases), CD2 (9 cases), and CD4 (1 case). No cases of CD3+ B-cell lymphoma were identified. Aberrant T-cell antigen expression was identified in 2.6% of cases having a CD5+ small cell immunophenotype (7/273), 1.6% of cases having a CD5–/CD10– small cell immunophenotype (3/186), 20% of hairy cell leukemias (3/15), 8.0% of cases having a CD10– large cell immunophenotype (9/113), and 3.1% of cases having a CD10+ immunophenotype (14/455). Of the 14 CD10+ cases with T-cell antigen expression, 8 had a strikingly similar immunophenotype characterized by coexpression of CD7, CD5 (5 of 8 cases), CD8 (5 of 8 cases), and λ light chain restriction (all 8 cases). These represent the only cases in our series with convincing aberrant coexpression of multiple T-cell antigens. Histologic review revealed that 6 of these 8 cases were diffuse large B-cell lymphomas and that 2 were low-grade follicular lymphomas. Further characterization of these cases will determine whether this unique immunophenotype represents a novel subtype of follicle center lymphoma.

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Impact of Critical Value Cutoffs on Laboratory Operations.

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Immediate reporting of critical laboratory values is an integral part of quality patient care; however, no clear consensus exists in the literature as to what values should be considered critical for each laboratory test. As a result, defining critical values varies widely among hospitals. Our objectives were to determine the distribution of critical value results in our institution, compare our cutoffs for critical values with those for a peer group of 8 academic medical centers, and assess the impact of altering those values on call volume and allocation of laboratory personnel time.

We assessed our weekly critical result reporting data and determined the most frequent laboratory tests for which critical result calls were necessary. We then compared our critical value cutoffs for these tests with those used at 8 peer institutions. By using the average critical value for each test, we determined the potential impact on the number of critical result calls per week. In addition, we assessed the average time necessary to document notification of the critical result. By using

this information, we calculated the potential laboratory personnel time that could be saved by using the average critical result cutoff values.

Our institution reports an average of 2,753 critical results per week (excluding microbiology and blood bank sections). Of the 1,577 called results, the following 8 tests constitute the majority, or 83% (1,309), of these calls: hematocrit, 318 (24%); hemoglobin, 201 (15%); potassium, 230 (18%); troponin, 167 (13%); platelet count, 134 (10%); WBC count, 117 (9%); glucose, 78 (6%); and calcium, 64 (5%). Wide variability was found in the critical value cutoffs used among peer institutions. By using the average critical values for our 8 most common critical results, we estimate a 59% reduction in our critical result call volume, eliminating 933 total calls per week. This significant decrease in total calls, with an average time to documentation of 1 minute, would save an estimated 16 hours per week of laboratory personnel time, or the equivalent of 0.4 full-time equivalents per year.

In this study, we showed that critical value cutoffs vary widely among peer institutions. Although critical values must be chosen to accurately represent the severity of the clinical situation, appropriate choice can significantly impact call volume and, therefore, utilization of laboratory personnel time. Recently, the Joint Commission on Accreditation of Healthcare Organizations established a requirement that clinicians document the action taken on receiving a critical result. Review of this documentation could lead to the establishment of institution-appropriate critical values that maintain the highest degree of patient safety while improving laboratory personnel time management.

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Use of CD38 to Differentiate Follicular Lymphoma From Follicular Hyperplasia by Flow Cytometry.

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Follicular lymphoma can be difficult to differentiate from follicular hyperplasia in some cases by flow cytometry. In addition, low-level involvement of bone marrow by follicular lymphoma can be difficult to detect by flow cytometry given the normal CD10+ immature B-cell population typically present. It has been our observation that follicular lymphoma demonstrates CD38 expression at decreased levels compared with that present in follicular hyperplasia. This study was aimed at documenting this observation in an effort to determine if the level of CD38 expression by flow cytometry can help differentiate follicular lymphoma from follicular hyperplasia.

CD38 expression was assessed by flow cytometry in 11 lymph nodes with immunophenotypic changes characteristic of follicular hyperplasia and 18 lymph nodes selected based on the presence of a mature B-cell neoplasm with CD10 coexpression. Each of the 18 lymph nodes demonstrating neoplastic immunophenotypic changes by flow cytometry had a confirmatory morphologic diagnosis of follicular lymphoma. The level of CD38 was measured as the median fluorescence intensity of the atypical CD10+ cells in cases of follicular lymphoma and compared with the median fluorescent intensities of the CD10+ polyclonal germinal center population in cases of follicular hyperplasia.

The median fluorescent intensity in the neoplastic population was significantly different compared with normal germinal center B-cell cases of follicular hyperplasia ($P < .005$). Levels of CD38 did not differ significantly between the nonneoplastic B cells in cases of follicular lymphoma and non–germinal center B cells in follicular hyperplasia ($P < .2$), between T cells and nonneoplastic B cells in cases of follicular lymphoma ($P < .3$), between T cells and non–germinal center B cells in cases of follicular hyperplasia ($P < .3$), or between T cells in cases of follicular lymphoma and follicular hyperplasia ($P < .3$).

Based on this review, the median fluorescent intensity of CD38 is significantly decreased in follicular lymphoma compared with the germinal center B cells in follicular hyperplasia as measured by flow cytometry and does not vary significantly between background bystander B- and T-cell populations (serving as internal controls). These findings suggest CD38 is a useful marker for differentiating follicular lymphoma from follicular hyperplasia and may be helpful in identifying low-level involvement by follicular lymphoma in bone marrow aspirates.

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Use of a Resident On-Call Database to Characterize Communication Failures in Critical Value Reporting.

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The Clinical Laboratory Improvement Amendments require that laboratories have a procedure for reporting "imminent life-threatening results" to caregivers. Although this system has improved timely intervention, communication failures undermine its impact. When laboratory personnel at our institution are unable to reach care providers with critical laboratory results (CLRs), the laboratory medicine resident on-call ensures that the value is communicated and/or that appropriate action is taken on the patient's behalf. We evaluated all resident-involved CLR calls to determine reasons for the communication failure and to define potential interventions.

The University of Washington Clinical Pathology program maintains an online database of all calls received by residents and fellows on call. From August 2004 through January 2006, the database contained 6,700 calls to residents and fellows on call. For the study, 592 calls regarding critical values were accrued by searching the "Call Classification," "Specific Request," and "Relevant Information" fields for the word critical.

In 318 (61%) of the 524 proven CLR calls, there was a documented reason for a communication failure. Of these 318 cases, 117 calls (37%) were because the ordering or covering providers did not return pages or calls; 37 calls (12%) were for patients from closed outpatient clinics; 26 calls (8%) were for discharged inpatients; 35 calls (11%) were related to confusing requisitions; 16 calls (5%) were related to confusing and/or unusual results; 7 calls (2%) were because the provider refused to accept the CLR; and in 6 calls (2%), there was an insufficient attempt to contact the provider. Of interest, 43% of the unreturned pages or calls were calls to outpatient providers, which was in keeping with the fraction of the *total number* of calls to outpatient providers (38%). Microbiology and chemistry initiated 44% and 36% of all calls, respectively.

Unreturned pages and inability to reach care providers for outpatients or discharged patients constitute the largest sources of communication failures. We are devising interventions to address the causes of communication failures identified in this analysis.

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Prognostic Potentials of Haptoglobin Phenotypes in Identifying Diabetics at Risk for Developing Diabetic Retinopathy.

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Diabetic retinopathy (DR), a retinal microangiopathy, is the leading cause of blindness in persons aged 20 to 74 years in the United States. Retinopathy has 2 stages: nonproliferative (NPDR), which consists of intraretinal microaneurysms, hemorrhages, and soft and hard exudates, and proliferative (PDR), a more serious condition characterized by neovascularization and fibrovascular growth from the retina. Retinal neovascularization has a tendency to be fragile and prone to hemorrhage and leakage. It has been suggested that hemorrhagic events, inflammation, and angiogenesis can have a major role in the etiology of DR. Free hemoglobin (Hb) released from RBCs following a hemorrhagic event can cause oxidative damage in different tissues. Haptoglobin (Hp), an α_2-glycoprotein, can bind free Hb and remove it from the circulation to prevent Hb and/or iron-mediated tissue damage. Also, Hp can modulate immune response and affect angiogenesis. Hp has 3 major phenotypes, Hp 1-1, Hp 2-1, and Hp 2-2. The overall biologic activity of Hp 2-2 is considerably lower than that of Hp 1-1. The focus of this project was to study the possible role and frequency of Hp phenotypes and oxidative stress (OS) in diabetics with various stages of DR.

So far, 20 diabetics with at least 20 years of type 1 diabetes have been involved in this project. Hp phenotyping was performed by gel electrophoresis. OS was assessed by measuring 8-isoprostane (LC/MS/MS), conjugated dienes, serum free iron, and total antioxidant capacity (photometry).

Our results showed that 63.2% of diabetics with DR had Hp 2-2, 26.3% had Hp 2-1, and 10.5% had Hp 1-1 phenotypes compared with 36%, 48%, and 16% in the general population, respectively. Also, the Hp concentration was lowest in Hp 2-2 (85 ± 36 mg/dL) and highest in Hp 1-1 (190 mg/dL), with Hp 2-1 (95 ± 26 mg/dL) in between. At this point, we have not seen significant differences in levels of serum markers of OS among our subjects. This study will be continued in our laboratory for the next few years.

Our preliminary results suggest that Hp 2-2 is highly associated with the presence of DR in type 1 diabetics and may be used to identify diabetics at higher risk for developing DR.

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Effect of Asian, Indian, and Siberian Ginseng on Human Erythrocytes Membrane-Bound ATPases.

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Asian and Siberian ginseng, although prepared from different plant species and containing different alkaloids, cross-react with the polyclonal immunoassay of digoxin owing to structural similarities between these alkaloids and digoxin. Moreover, the FAB fragment of antidigoxin antibody (DIGIBIND) recognizes these alkaloids and neutralizes them in vitro. Earlier studies also demonstrated that ginseng can inhibit microsomal Na+,K+-ATPase isolated from rat brain. Our earlier work also indicates that Asian and Siberian ginseng can increase calcium influx in cardiomyocytes isolated from rats, and the mechanism of such influx involves more than Na+,K+-ATPase inhibition (Poindexter et al. *Life Sci.* 2006;79:2337-2344). The aim of this project was to study the effect of both ginsengs on the Na/K pump ATPase and Ca pump ATPase from human erythrocytes.

Hemoglobin-free membrane was prepared from fresh human erythrocytes and was incubated with different concentrations of ginseng at 37°C for 60 minutes. At the end of incubation, membranes were washed and the ATPase activity was determined by measuring inorganic phosphate generated from ATP used by the membrane-bound ATPases.

Our results showed that Asian ginseng enhanced Na/K pump ATPase and Ca pump ATPase in a dose-dependent manner (Na/K = 6.7 ± 2.0 and 11 ± 0.1; Ca = 13 ± 5.7 and 31 ± 1.4 nmol/Pi/mg/min, with or without ginseng, respectively). Indian ginseng enhanced Ca pump but not Na/K pump ATPase, also in a dose-dependent manner (Ca = 11.4 ± 5.2 and 33 ± 13, nmol/Pi/mg/min, with or without ginseng, respectively. However, at higher concentrations, both ginsengs completely inhibited the pumps.

Our results suggest that depending on the dose, ginseng can act as an aminoglycoside, increasing the peripheral vascular pressure by inhibiting Na/K pump ATPase and Ca pump ATPase.

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Development of a Functional ELISA for von Willebrand Factor Activity Using the Extracellular Domain of Human Platelet Glycoprotein Ibα Secreted From Chinese Hamster Ovary Cells.

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The classic ristocetin cofactor assay for von Willebrand factor (vWF) functional activity is not only very demanding technically, but also suffers from poor reproducibility. Functional enzyme-linked immunosorbent assays (ELISAs) have recently been developed, using recombinant (r) glycoprotein (GP)Ibα fragments captured by monoclonal antibodies (mabs) and mabs to vWF whose binding is thought to mimic that of GPIbα.

In the present studies, virtually the entire extracellular portion of human GPIbα (residues 1-483) was secreted in culture by stably transfected Chinese hamster ovary (CHO) cells, including wild-type (WT) and increase-of-function (PT-vWD) mutants containing $233_{gly\rightarrow val}$ and $239_{met\rightarrow val}$. Provision of a his-tag at the carboxyl terminus permitted oriented coupling to nickel chelate–coated wells, eliminating mabs and any associated nonspecific binding. Immobilized rGPIbα was subsequently incubated with plasma in the presence of ristocetin or botrocetin and bound vWF detected with an HRP-conjugated polyclonal anti-vWF antibody.

By using assayed normal plasma, standard curves could reproducibly be constructed with WT or mutant receptors with sensitivities well exceeding the RCo assay. Despite this, however, when this method was used to assay the level of functional vWF in the plasma of certain patients with von Willebrand disease, it tended to seriously overestimate vWF activity compared with the classic ristocetin cofactor assay. Correction of overestimation of the patient samples was successfully achieved by marked reduction of receptor coating density, together with the use of relatively low modulator concentrations. Whereas adoption of these conditions was possible with the mutant rGPIbα, a comparable combination of receptor density and modulator concentration was insufficient to support vWF binding above background levels in the case of the WT receptor.

Excellent agreement between ristocetin cofactor assays and the ELISA using the mutant GPIbα under these conditions was achieved for a broad array of normal and patient plasma samples. This new assay retains the classic attribute of dependency on modulator-induced binding of vWF to the principal platelet receptor for vWF in a simpler and more reproducible testing format.

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Evaluation of Reference Intervals for Methylmalonic Acid in Serum and Urine.

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Methylmalonic acid (MMA), an intermediate in the metabolic transformation of propionic acid to succinic acid, is used as an indicator of vitamin B_{12} deficiency. Vitamin B_{12} (cobalamin) is an essential cofactor for the enzymatic carbon rearrangement of MMA to succinic acid. Vitamin B_{12} deficiency leads to increased concentrations of MMA. MMA reference intervals found in the literature were developed from studies of relatively small samples using gas chromatography–mass spectrometry, and they vary considerably. The objective of our study was to evaluate reference intervals for MMA in serum and urine, measured by a liquid chromatography–tandem mass spectrometry (LC-MS/MS) method used in our laboratory, to provide interpretive guidance to clinicians.

MMA in serum and urine specimens was identified and quantified by LC-MS/MS. Nonfasting serum samples (n = 40) collected from self-reported healthy subjects (24 women and 16 men) and random urine samples (n = 130) collected from self-reported healthy subjects (53 women and 77 men) were analyzed. Results were evaluated using a nonparametric method for reference interval determination. We also performed a database study. Results from 4,944 consecutive MMA tests in serum performed between November 1 and November 30, 2006, and results from 5,012 consecutive MMA tests in urine performed between September 2, 2002, and December 30, 2006, were evaluated using the nonparametric method following exclusion of the highest 10% of results. The reference intervals from healthy subjects for MMA in serum and urine are 0.09 to 0.35 μmol/L and 0.43 to 1.88 mmol/mol creatinine, respectively.

Representative ranges obtained from the database study were 0.05 to 0.45 μmol/L for MMA in serum and 0.20 to 3.00 mmol/mol creatinine for MMA in urine. Representative ranges determined by age were less than 0.51 μmol/L for the first decade, less than 0.26 μmol/L for the second decade, less than 0.33 μmol/L for the third decade, less than 0.40 μmol/L for the fourth and fifth decades, less than 0.42 μmol/L for the sixth decade, less than 0.44 μmol/L for the seventh decade, and less than 0.48 μmol/L for older than 70 years. Urine MMA representative ranges determined by age were less than 3.4 mmol/mol creatinine for the first decade and less than 3.0 mmol/mol creatinine for all others. These ranges are consistent with incidence of congenital methylmalonic aciduria in young children and prevalence of vitamin B_{12} deficiency in elderly people.

We have validated the reference intervals for MMA in serum and urine using an LC-MS/MS method. These results are comparable to those reported in the literature.

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Comparison of Serum Free Light Chain Quantitation With Urine Electrophoresis Techniques in Testing Specimens With Monoclonal Urine Light Chain Proteins.

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Serum free light chain measurement by immunonephelometry has been proposed to replace the need for quantitation of urine monoclonal free light chains (Bence-Jones protein). To test this hypothesis, we performed serum and urine free light measurement on a sample of clinical specimens received in our laboratory for monoclonal protein detection by electrophoresis. During the study period, we found 28 urine samples that had a monoclonal protein detected by electrophoresis or by immunofixation and concurrent serum samples on which serum free light chain detection was performed. Only 21 of the 28 samples had an abnormal serum free light chain ratio, yielding a sensitivity of 75% for serum free light chain quantitation. Of the patients, 7 had normal serum light chain ratios, and 2 of these had normal serum electrophoresis and immunofixation results. In the group of samples with normal serum free light chain results and monoclonal free light chain proteins in the urine, 4 were from patients with a diagnosis of light chain amyloidosis. The remaining patients had treated multiple myeloma or monoclonal gammopathy of undetermined significance.

We also investigated if urine free light chain measurement by nephelometry can substitute for urine monoclonal protein quantitation by electrophoresis. A total of 133 clinical urine samples were tested. Compared with urine protein electrophoresis and immunofixation, the urine free light chain test had good sensitivity (90%) but poor specificity (55%) in this set. Of note, no samples with monoclonal urine free light chain protein of more than 0.1 g/24 hours had a normal urine light chain ratio. Of the 3 patients with positive immunofixation results but negative urine nephelometric light chain results, 1 had κ and λ monoclonal components. Two others had very faint monoclonal bands. Results of urine free light chain analysis correlated well with Bence-Jones protein quantitation ($r = 0.98$); however, the slope of the line was 5.9, and at low Bence-Jones protein concentrations, many outliers were encountered.

Urine electrophoresis and immunofixation may still have a role in diagnosis and follow-up of select patients with monoclonal gammopathies, particularly when primary amyloidosis is suspected. Moreover, quantitative urine free light chain analysis may be a reasonable substitute for monoclonal protein urine quantification by electrophoresis.

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An Analysis of the Association Between Cytochrome P-450 2D6 Polymorphism and Incidence of Adverse Effects Caused by Tamoxifen in Women with Breast Cancer.

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Tamoxifen is an important drug for treatment and prevention of estrogen receptor–positive breast cancer. However, not all patients with estrogen-positive tumors can benefit from tamoxifen. No well-established markers are available to predict the efficacy and side effects of tamoxifen. Tamoxifen acts by blocking estrogen receptors and is metabolized in the body by cytochrome P-450 2D6 (CYP2D6) to endoxifen, which is 10-fold more potent in blocking estrogen receptors. Polymorphisms in CYP2D6, such as the *4 allele, can significantly decrease the enzyme activity. The Food and Drug Administration Clinical Pharmacology Subcommittee has recommended that drug manufacturers include information about CYP2D6 genotype and tamoxifen on the package insert label. However, clinical studies that examined the effect of the CYP2D6 *4 polymorphism on efficacy and side effects of tamoxifen have shown contradicting results. Some suggested poor metabolizers had a higher risk of disease relapse and lower incidence of hot flashes; others found no correlation between CYP2D6 *4 allele status and clinical outcomes; 1 study indicated patients with *4 alleles had better outcomes.

We analyzed the effect of CYP2D6 *4 polymorphism on side effects of tamoxifen in an ethnically diverse breast cancer patient population. Sixty patients who receive tamoxifen for breast cancer treatment were genotyped for CYP2D6. DNA extracted from whole blood was amplified, followed by allele-specific extension and

hybridization to a microarray. Of the 60 patients, 36 (60%) were homozygote wild type, 21 (35%) were heterozygote, and 3 (5%) were homozygote mutant for the *4 allele. Side effect information was available for 58 patients.

In univariate analysis, Caucasians had a significantly higher incidence of *4 polymorphisms compared with Asians (P = .036; 95% confidence interval, 0.01-0.31). No significant association was found between the incidence of adverse effects of tamoxifen (hot flashes, vaginal dryness, and sleeping problems) and *4 allele status (odds ratio, 1.9-8; P = .1-.6). However, the number of patients who were homozygote mutant (*4/*4) was very low, making the statistical comparison difficult. Because intake of comedications that are CYP2D6 inhibitors also decreases the enzyme activity, we plan to increase the statistical power of the study by stratifying patients according to their status of CYP2D6 inhibitor intake and also including more study subjects. We will also analyze the association between CYP2D6 polymorphisms and incidence of cancer relapse, as well as serum endoxifen levels, in the same population.

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Development of a Seizure Panel to Detect Common Drugs Involved in Drug-Induced Seizures Using LCMS/MS.

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Many seizures in patients presenting to emergency departments have unexplained causes, and there is currently no method to quickly or reliably determine if a seizure is drug induced. Therefore, our objective was to develop a seizure panel to detect drugs in patient blood samples that may be the cause of a seizure.

A retrospective study of California Poison Control Center cases in 2003 revealed 386 seizures to be drug related. From this study, we chose 12 drugs that accounted for about 75% of all the drugs implicated in seizures to use in a seizure panel. The drugs are bupropion, citalopram, cocaine, diphenhydramine, isoniazid, lamotrigine, methamphetamine, MDMA, quetiapine, tiagabine, tramadol, and venlafaxine. Liquid chromatography–tandem mass spectrometry (LCMS/MS) was chosen as a technique for the seizure panel owing to its sensitivity and ability to detect a diverse range of compounds. Serum and plasma samples were subjected to solid-phase extraction before the 10-minute LCMS/MS run. The assay is semiquantitative, with a cutoff of 50 ng/mL for all of the drugs except isoniazid and lamotrigine, which have a cutoff of 500 ng/mL owing to their higher therapeutic blood levels. Drugs were also qualitatively identified, requiring a 60% or higher purity match between unknown and library product ion spectra. In a preliminary analysis of 33 samples from patients who had seizures, 2 were positive for lamotrigine.

A test that can detect the presence of the common drugs implicated in drug-induced seizures has the potential to help physicians rule in or rule out drug toxicity as the cause of an unexplained seizure. This knowledge can help in triaging patients and improve patient care.

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Evaluation of the Use and Utility of the Coagulation Screen.

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Coagulation tests are typically ordered to evaluate ongoing bleeding episodes, establish a preprocedure baseline, and/or monitor therapy. The number of monthly coagulation screens (prothrombin time [PT], partial thromboplastin time [PTT], thrombin time [TT], and fibrinogen) ordered at our institution almost tripled from just more than 1,200 in May 2000 to more than 3,000 in May 2006, out of proportion to the number of patients seen or admitted. We evaluated coagulation screens for a 6-month period in 2005 to evaluate use and improve utilization of coagulation testing.

We used laboratory criteria to classify the coagulation screens into diagnostic groups and did chart reviews to determine if the designated laboratory criteria were sufficient to accurately identify patients in each group.

A total of 28,737 screens were evaluated; 9,184 were initial screens and 19,553 were repeated screens. Of the repeated screens, 98% had normal initial and subsequent fibrinogen levels. All patients whose charts were reviewed had histories consistent with their assigned cohorts. Of all the coagulation screens, 40% were normal (PT, <15.6; PTT, <35), 36% had mild vitamin K–dependent factor deficiency (international normalized ratio [INR], 1.2-1.9; TT, <30), and another 12% had a probable lupus inhibitor. None of these screens had critical fibrinogen levels (<100 mg/dL). TT and fibrinogen levels are not useful in patients with normal PTs/PTTs, mild vitamin K–dependent factor deficiency, or a probable mild lupus inhibitor. Of the coagulation screens, 4% indicated vitamin K–dependent factor deficiency, most patients were taking warfarin (INR >2), and another 4% of coagulation screens indicated heparin therapy (PT, <20; PTT, >60). TT and fibrinogen levels are not useful in patients being monitored for anticoagulant therapy. Of the screens, 99.5% had fibrinogen levels higher than the treatment threshold. In the 0.5% (160 panels, 70 patients) with critical fibrinogen levels (<100 mg/dL), we recommended the faster emergency hemorrhage panel (PT, fibrinogen, platelets, hematocrit; turnaround time [TAT], 15 minutes) and not a coagulation screen (PT, PTT, TT, and fibrinogen; TAT, 45-90 minutes) to help decide what components (fresh frozen plasma, cryoprecipitate, platelets, RBCs) are needed. These patients all had ongoing bleeding or hemostatic abnormalities, and 73% of them died during or shortly after their hospitalization. Each patient with a critical fibrinogen level had an average of 15.2 panels done (range, 1-55 per patient).

TTs and fibrinogen levels are not routinely needed in patients with normal PT and PTT, mild vitamin K deficiency without bleeding, or lupus inhibitors without bleeding or for monitoring warfarin or heparin therapy. We are now changing the current coagulation screen (PT, PTT, fibrinogen, TT) to one consisting of PT and PTT only with the option to order TT and fibrinogen and recommending an emergency hemorrhage panel for patients who are actively bleeding or in unstable condition. A similar strategy applied to D dimer testing reduced utilization by more than 80%.