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

The following abstracts were presented at Lab Medicine 2013, the 48th Annual Meeting of ACLPS, June 6 to June 8, 2013, in Atlanta, GA. Authors receiving a 2013 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 Computerized Physician Order Entry–Based Intervention to Improve Blood Utilization

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Blood transfusions can cause significant morbidity and mortality, and recent studies demonstrate that restrictive transfusion policies are safe for patients. At our institution, most inpatient transfusions for red blood cells (RBCs), platelets, and fresh frozen plasma (FFP) are ordered through a computerized physician order entry (CPOE) system. We developed a simple CPOE-based intervention to decrease inappropriate blood utilization (ie, transfusions that fall outside current best practice indicator thresholds). The data foundation for this intervention was detailed queries and analyses of transfusion records and relevant laboratory testing values in our electronic medical record (EMR). Baseline data of guideline adherence and transfusion rates allowed us to track changes in clinician ordering behavior following the CPOE intervention. To measure intervention outcome, the change in product transfused per inpatient admission was calculated. The intervention itself involved several simple changes to the CPOE ordering screen, including text guidance, convenient display of relevant laboratory values, and a mandatory field that required the ordering clinician to select from an approved list of indications for each transfusion order. After the intervention, the rate of transfusion per admission decreased for each blood component: from 1.004 units per admission to 0.822 (95% confidence interval [CI], 0.821-0.823) units per admission for RBCs, from 0.296 doses per admission to 0.267 (95% CI, 0.2667-0.268) doses per admission for platelets, and from 0.386 units per admission to 0.282 (95% CI, 0.281-0.283) units per admission for FFP. The postintervention data are from December 2012 and additional data are accumulating. The CPOE intervention had minimal impact on ordering clinician workflow and no clinical staff raised concerns about the minor change in process. By increasing adherence to institutionally accepted guidelines, we decreased utilization, anticipated cost savings, and improved patient care by avoiding unnecessary transfusions and their associated complications.

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Novel Spot Tests to Detect Zinc Sulfate in Urine: A Recently Introduced Urinary Adulterant For Invalidating Drugs of Abuse Testing

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Zinc sulfate has been promoted as an agent to beat urine drug tests. Vankatratam et al verified that zinc sulfate interferes with immunoassays for cocaine (measured as benzoylecgonine), methamphetamine, and THC; (measured as THC-COOH) and causes false-negative results. Currently, there is no suitable method to detect zinc sulfate in adulterated urine, including use of the specially designed dipstick such as AdultaCheck 10 (*J Anal Toxicol*. 2011;35:333-340). Therefore, we developed 2 rapid spot tests to detect the presence of zinc sulfate in adulterated urine. When drugs of abuse controls containing drugs at 25% higher concentration than cutoff were supplemented with 15 mg/mL of zinc sulfate (as per prior report), opiate, THC, cocaine metabolite, and phencyclidine (PCP) test results were negative using drugs of abuse testing panels on the Vista 1500 analyzer (Siemens Diagnostics). At higher zinc sulfate concentration (50 mg/mL), additional drugs such as barbiturates, benzodiazepines, and propoxyphene also tested negative. The amphetamine test was not affected as reported earlier. Drug-free urine was also supplemented with various drugs of abuse and zinc sulfate with similar effects observed. Zinc sulfate also falsely reduced the concentration of alcohol in urine (76 mg/dL in unadulterated urine vs none detected with 50 mg/mL zinc sulfate). The presence of zinc sulfate was not detected using Intect 7, another dipstick device effective in detecting adulterants in urine. Moreover, specific gravity of urine following

addition of zinc sulfate was not significantly altered. However, zinc sulfate at a lower concentration (10 mg/mL) than needed for producing false-negative results (15 mg/mL) could be detected by using novel spot tests we developed. Addition of 3 to 4 drops (approximately 100 μL) of 1N sodium hydroxide solution to approximately 1 mL of urine containing zinc sulfate led to the formation of a white precipitate. The precipitate could be dissolved upon addition of 10 to 15 drops of sodium hydroxide. The basis for this reaction is that zinc hydroxide is soluble in excess sodium hydroxide. In the second spot test, addition of 3 to 4 drops of 1% sodium chromate solution to 1 mL of urine containing zinc sulfate followed by addition of 4 to 5 drops of 1N sodium hydroxide led to the formation of a yellow precipitate (zinc chromate). Twenty drug-free urine specimens and urine specimens containing high amounts of sugar or reducing substances were tested with these spot tests and no false-positive result was observed. We conclude that either of these novel spot tests are useful tools for assessment of adulterated urine that contains zinc sulfate.

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Conundrums With Solid Phase HLA Antibody Testing

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The objective of this study was to investigate the conformational expression of HLA antigens on different solid phase HLA antibody detection platforms. Serum samples from 2 patients diagnosed with nonischemic cardiomyopathy and implanted with left ventricular assist devices (VAD) were tested for class I and II antibodies using the following platforms: (1) flow cytometric panel reactive antibody (PRA) beads; (2) a Luminex-based, single antigen bead (SAB) assay, (3) HLA phenotype beads by flow cytometry, and (4) cell-based testing (flow cytometric crossmatch). The antibody profiles observed for each patient depended on which detection platform was applied. Specifically, while no HLA class I antibodies from either patient were detected with the FlowPRA assay, each patient demonstrated antibodies to HLA-C*01, C*12, and C*15 when SAB testing was performed. To resolve the apparent discrepancy, the serum samples were tested with the HLA phenotype coated beads and in a flow cytometric crossmatch with cell lines expressing individual HLA-C locus antigens. Neither assay identified HLA-C locus antibodies, supporting the results of the FlowPRA screen. Finally, a serum sample from 1 patient was tested with the cell lines from which the recombinant HLA-C antigens were extracted. No antibody activity was detected, confirming the results of the FlowPRA, the flow specificity beads, and the flow crossmatch. The outlier was the SAB assay. Typically, antibodies detected on one platform are also observed on each of the other platforms. For the data reported here, we speculate that the adherence of recombinant HLA-C antigens to a plastic matrix results in a conformational change to these proteins and possible exposure of cryptic epitopes not apparent when native antigens are bound to plastic matrices (FlowPRA and phenotype beads). Thus, our data support the concept that serum samples from both patients possess antibodies reactive to the cryptic epitopes likely arising from denaturation or unfolding of proteins as they adhere to plastic microparticles. These antibodies may have developed as a consequence of VAD implantation in these patients. In summary, we observed that results from SAB assays do not always mimic the results of other solid phase antibody detection assays. As SAB assays are increasing in popularity and use, it is critical to determine whether the protein adhered to the matrix is expressed in its appropriate biological configuration. Failure to do so can lead to inappropriate identification of HLA antibody specificities and have a negative impact on patient access to an allograft.

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"In Control" in the HLA Laboratory: Using QC to Improve Consistency and Efficiency

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Monitoring of HLA antibodies is critical to minimizing the risk of antibody-mediated rejection (AMR) in pre- and post–solid organ transplant recipients. AMR is associated with the number and level of donor-specific HLA antibodies. The development of the Luminex-based solid-phase single antigen bead (SAB) assay has increased both the sensitivity and specificity to detect HLA antibodies. This approach has also provided a semiquantitative method to determine antibody levels (represented as normalized mean fluorescence intensity; MFI), and is considered a useful tool to assess risk of AMR and for management of patient care. However, these assays are predominantly manual, are not standardized, and have high variability. To improve consistency in both antibody identification and MFI values reported, we established quantitative quality control (QC) limits. SAB assays are typically performed in 96-well tray format batches. For every batch run over 6 months (n = 156), MFI values for class I SAB assays were identified for the internal negative (NC) and internal positive (PC) control beads for both the negative (NS) control serum and positive (PS) control serum. From these points, the mean and standard deviation (SD) for the NC and PC was determined for each control serum sample. Acceptability limits were defined by the mean ± 3 SD, which were 0-450 MFI for the NC and 16,500-27,000 MFI for the PC regardless of the serum; both NS and PS demonstrated essentially identical ranges. Technologist workflow mandates review of control serum samples (NS and PS) for each batch as well as internal control beads (NC and PC) for each patient serum sample preceding verification of the results. After implementation of more rigorous QC limits, assay performance was improved such that the coefficient of variation decreased by 20% for the NC and by 8% for the PC. Moreover, prior to the implementation of QC limits for SAB assays, patient results that did not match the patient history were not reported pending an investigation conducted to identify whether inconsistencies were due to preanalytic or analytic variables. The implementation of QC rules has improved the efficiency of the laboratory by giving the technologists the tools to identify problems with the analytic phase of the testing quickly, allowing more timely and consistent result reporting.

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Evaluation of Critical Laboratory Result Reporting Processes

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The timeliness of reporting critical values is vital to patient safety. Laboratories are required by regulatory agencies to communicate critical laboratory results to providers; however, the effectiveness and institutional variation of these notification practices is not well understood. The objective of this study was to evaluate the critical result reporting processes of 3 academic medical centers of similar size and patient complexity. Method: We first studied the time difference in the reporting of critical results and noncritical results by abstracting the data from each institution's laboratory information system for 3 tests that are run in different sections of the clinical laboratory (chemistry [potassium], immunoassay [troponin], hematology [platelets]). The time from when the result was ready on the instrument to its release into the electronic medical record was measured. Second, we benchmarked treatment intervention times based on the availability of a critical low potassium result by measuring the elapsed time from when the critical result was reported to when an order for potassium supplementation was placed. Results: Critical results were found to be released 3 to 20 times slower than noncritical results. Institutions that documented contact with a provider before releasing a critical result into the medical record were found to have results available in the medical record 2 to 3 times faster for potassium, 5 times faster for troponin, and 2 times faster for platelets than the institution that did not. In 1 institution, it was found that the median time to intervention for a critically low potassium level was 23 minutes for the emergency department, 31 minutes for the intensive care units, 14 minutes for the general medicine units, and 9.5 minutes for the surgical units. Conclusions: Our data revealed that in all 3 medical centers the critical results took longer to report in comparison to noncritical results. Furthermore, intervention orders were initiated shortly after the result was released, emphasizing that medical decisions/interventions are prompted by laboratory alerts. While calling critical results is intended to ensure that necessary interventions will not be delayed for the safety of the patient, our current reporting practices may actually be delaying their treatment. This is a first report to benchmark a relationship between laboratory critical value communications and treatment interventions.

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Appropriate Hb A1c Testing Frequency Is Not Associated With Proper Treatment Changes

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It is recommended that Hb A1c testing be performed at least twice per year on patients who meet treatment goals and demonstrate stable glycemic control. For those who have had treatment changes or are not meeting glycemic goals, quarterly testing is suggested. While Hb A1c utilization and adherence to testing frequency recommendations is important, so is the adjustment of treatment when changes in Hb A1c are considered significant. The objective of this study was to assess clinician practice by monitoring Hb A1c testing and medical chart review. Using retrospective data analysis, we determined whether recommendations are followed for Hb A1c testing frequency, and in those cases, whether appropriate treatment changes are made based on calculated Hb A1c reference change values. Hb A1c values (n = 32,112) over a 1-year period were extracted from the laboratory information system and the data filtered to include only patients who were tested at least twice within the time frame of our study. These data (n = 4,380) were partitioned into patients who were tested at the recommended frequency and those who were not. The testing frequency observed supports previous findings for Hb A1c overutilization and suggests only ~17% (n = 737) of patients were being tested at the recommended frequency. Patients tested at the recommended frequency were further partitioned into those who demonstrated glycemic control (Hb A1c <7.0%) (n = 410) and those who did not (Hb A1c ≥7.0%) (n = 327) based on their initial Hb A1c values. Chart review was conducted to assess treatment changes for patients whose initial Hb A1c demonstrated glycemic control but subsequent Hb A1c testing at the recommended frequency did not (n = 64). Based on the analytical performance of the instrument used for Hb A1c testing in this study, a calculated reference change value of ≥0.6 % Hb A1c must be met to be considered a significant change. Our data indicate that when Hb A1c testing frequency recommendations are met, treatment changes were made only 30% (n = 23) of the time even when the ΔHb A1c represented a significant increase. While the reasons behind the lack of treatment observed are not well understood, increasing communication between the laboratory and clinicians regarding these significant reference change values may help to improve awareness and interpretation of the results.

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A Novel Personalized Delta Check Approach

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Introduction: Inaccuracies in specimens of patients may lead to misdiagnosis and inappropriate therapy. The primary purpose of a delta check is to detect misidentified specimens. The delta check procedure compares the change in concentration of an analyte with a delta check limit (DCL) for that analyte. A change greater than the DCL sets a delta check flag for that analyte and will be further investigated by the technologist. This procedure is called univariate (multianalyte) delta check (UDC) and generates many false-positive flags. DCLs are typically taken from the literature independent from the patient population served by the laboratory. Here, we describe a novel software tool that customizes the DCL for historical patient data of that particular laboratory. Methods: The software accepts BMP or other laboratory results from an output file from the LIS, or from manual input. These results are assumed to be correctly identified and free of interferences and contamination. Next, it generates a set of misidentified samples by intentionally pairing results from 2 different patients. Additionally, for each analyte individually, the program determines which delta check type (absolute change, percent change, rate of absolute change, or rate of percent change) best differentiates correctly identified and misidentified samples for that analyte. The software uses the optimum delta check type for each analyte and calculates the sensitivity, specificity, and efficiency for each analyte alone and in all 255 possible combinations. Results: The output of the software is a table of the most efficient delta check combinations along with a comparison to the laboratory's current delta check system. Furthermore the software possesses a unique feature that allows the user to determine the maximum number of flags the laboratory can handle per day or per shift and adjusts the DCL accordingly. Conclusion: Here, for the first time we are able to allow the laboratory director to set the DCL according to the laboratory's patient population and staffing constraints. This novel software tool objectively optimizes the delta check procedure for a laboratory and can save time and money by reducing the number of false-positive delta check flags without sacrificing sensitivity.

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Urinary Galectin-3 Is a Potential Novel Biomarker of Renal Fibrosis

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Background: Nephrosclerosis is a common kidney biopsy finding and among those with renal disease is an important indicator of adverse prognosis. The invasive nature of the biopsy remains a barrier to obtaining consent for either initial or follow-up procedures. A biomarker indicative of ongoing/progressive renal fibrosis would be valuable for identifying individuals harboring the greatest risk for loss of future kidney function and may be useful to guide therapeutic response. Galectin-3 (gal-3) is a β-galactoside-binding lectin (molecular weight, ~30 kDa) upregulated during fibrotic reactions within diverse tissues, including the kidney. Objective: determine the potential utility of urinary gal-3 concentrations as a biomarker of renal fibrosis, compared with imaging and routine laboratory testing in controls (healthy kidney donor) and diseased (diabetic) cohorts. Methods: Gal-3 was quantitated in urine using a manual enzyme-linked immunosorbent assay (ELISA; BG Medicine). Intra- and interassay precision of urine pools was less than 6% and less than 11%, respectively. Healthy kidney donors were defined using stringent criteria for normal renal function assessed by iothalamate clearance (n = 455). Gal-3 was analyzed against kidney function tests, chronic kidney disease (CKD) risk factors, renal biopsy findings, kidney volumes, and other novel inflammatory markers. Urine specimens from 80 diabetic subjects comprised the diseased cohort. Gal-3 concentrations were normalized to urine creatinine (ng/mg). Results: Median concentrations of gal-3 were higher in healthy donor females compared to males (50 vs 37 ng/mg creatinine, $P < .0001$), and increased 0.41 ng/mg of creatinine per year of age ($P = .0016$). Gal-3 was significantly higher in the diabetic nephropathy group compared to healthy donors (102 vs 44 ng/mg creatinine, $P < .0001$). In kidney donors gal-3 associated with measured glomerular filtration rate (GFR; iothalamate clearance), microalbumin, albumin-creatinine ratio (UACR), systolic and diastolic blood pressure, serum uric acid, and creatinine (all $P < .05$). Independent predictors of gal-3 were older age, uric acid, and UACR ($P < .05$). Conclusions: Urinary gal-3 is significantly higher among individuals with traditional risk factors for CKD within the normal kidney donor cohort and in patients with established diabetic nephropathy. Gal-3 is a known mediator in the pathobiology of fibrosis, consistent with our results, which identify urinary gal-3 as a promising candidate biomarker for those with ongoing renal fibrosis and may assist in identifying those at greatest risk of CKD progression. Further long-term studies are warranted in cohorts where development of renal fibrosis occurs and outcomes are assessed.

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G6PD Deficiency in an HIV Clinic Setting in the Dominican Republic

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Glucose-6-phosphate dehydrogenase (G6PD) deficiency is the most common human enzymopathy, particularly in individuals of African descent. The epidemiology of this enzymopathy has not been studied in the Dominican Republic, where most individuals have African ancestry. Moreover, HIV–infected patients often receive prophylaxis with oxidative agents (eg, trimethoprim-sulfamethoxazole (TMP-SMX), which can induce hemolysis in G6PD-deficient patients. We aimed to determine the prevalence of G6PD deficiency and its significance in HIV-positive patients at Clínica de Familia La Romana, a free HIV clinic in the Dominican Republic. A medical history, chart review, and quantitative G6PD testing (Trinity Biotech) were performed for 238 consenting HIV-positive adults. The threshold for G6PD deficiency was 5.42 U/g hemoglobin (Hb; ie, 60% of the mean normal activity, by WHO criteria). Anemia was defined as Hb <12 g/dL for women and <14 g/dL for men. A history of hemolysis was defined as a report of dark urine or jaundice. An acute Hb decrease was defined as a documented decline in Hb of 2 g/dL or more within 30 days. Statistical analysis was performed using SAS 9.3. The overall prevalence of G6PD deficiency was 8.8% (21/238), similar in males (9.3% [9/97]) and females (8.5% [12/141]), but higher in Haitians (18% [9/50]), as compared to Dominicans (6.4% [12/187]) by maternal country of birth ($P = .011$). Approximately 60% of patients had received and approximately 15% were currently receiving TMP-SMX, regardless of G6PD status. Two patients, both G6PD-normal, received dapsone after TMP-SMX was stopped for allergy or anemia. A history of hemolysis was reported by 30% (65/217) of G6PD-normal and 57% (12/21) of G6PD-deficient patients. Patients reporting a history of hemolysis were 3.1 times more likely to be G6PD-deficient (95% confidence interval, 1.3-7.8; $P = .015$). However, G6PD-deficient patients were not more likely to be anemic or have acute decreases in Hb. There is a high prevalence of G6PD deficiency in this clinic population. Most patients, without knowing their G6PD status, are treated with agents that may cause hemolysis in patients with this enzymopathy. G6PD deficiency is significantly associated with a patient-reported history of hemolysis, but not with anemia or acute decreases in Hb. Developing patient history–based predictive criteria for identifying G6PD deficiency, prior to treatment with oxidative agents, may provide a cost-effective strategy for improving care in resource-poor settings.

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A Cost-Effective Approach to Improving Diagnostic Efficiency of *Trichomonas vaginalis*

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Trichomonas vaginalis (TV) is the most common nonviral sexually transmitted infection (STI) affecting approximately 8 million people in the United States each year with up to 50% of infections being asymptomatic. Untreated infections may lead to serious conditions such as preterm labor, low birth weight, pelvic inflammatory disease, and tubal infertility. Commonly used methods for detection of TV include culture and wet mount preparation, which suffer from poor sensitivity. The recently approved Gen-Probe APTIMA TV nucleic acid amplification assay demonstrates significantly higher sensitivity (95%-100%) but has increased cost and longer turnaround time compared to wet mount. With the goal of increasing the detection of TV while maintaining timely and cost-effective care, we introduced a testing option where TV-negative wet mounts are reflexed

to APTIMA TV. The practice change was evaluated to determine the impact of reflexed molecular TV testing on the TV positivity rate in adult emergency department (ED) patients. ED clinicians were educated on the reflex test choice and performance characteristics of the APTIMA TV assay. Patients with suspected STI requiring TV testing were either evaluated with wet mount alone (WM) or wet mount with reflex to APTIMA TV (WMreflex) per physician order selection. Over a period of 4 months (October 2012 to January 2013), 2,433 WM tests were ordered from different departments, which showed 233 (9.6%) positive TV results. Among these, 899 WM tests were from adult ED patients, which showed 98 (10.9%) positive TV results. Over the same period, 499 WMreflex orders were received, of which 376 (75%) were ordered from adult ED. Initial wet mount TV analysis of adult ED samples showed 49 (13.0%) positive results. Reflex APTIMA TV testing of adult ED samples with negative wet mount results (327 samples) revealed an additional 28 (8.6%) positive cases. Extrapolating this to the nonreflexed WM orders, approximately 69 cases of TV may be missed in the adult ED alone. Implementation of a reflex algorithm combining wet mount TV testing with APTIMA TV testing demonstrates increased detection of TV through sensitive molecular methods while maintaining the benefits of rapid TV diagnosis and treatment through wet mount examination.

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Diagnostic Accuracy of Point of Care Cardiac Troponin I Assays for Detection of Myocardial Infarction

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The objective of our study was to compare the diagnostic accuracy of a prototype point of care (POC) cardiac troponin I (cTnI) assay vs 3 commercially available POC assays and a sensitive contemporary central laboratory assay. Serial plasma (heparin) specimens were collected (presentation at 0, 3, and 6 hours) from 169 patients presenting with symptoms suggestive of acute coronary syndrome. cTnI was measured with the following assays: POC-Instrumentation Laboratory Gem Immuno 99th percentile 15 ng/L (prototype), Radiometer AQT90 99th percentile 23ng/L, Abbott POC iSTAT 99th percentile 40 ng/L, Mitsubishi Pathfast 99th percentile 29 ng/L and central lab–Ortho-Clinical Diagnostics Vitros ES 99th percentile 34 ng/L. 11.2% (n = 19) of patients had myocardial infarction (MI), classified along the universal definition of MI guidelines. At baseline, receiver operating characteristic (ROC) curve analysis revealed the highest diagnostic odds ratio for the Gem Immuno at 9.5 (area under the curve [AUC], 0.80; sensitivity, 63%; specificity, 85%), comparable to 9.0 for Vitros (AUC, 0.79; sensitivity, 68%; specificity 81%) and 6.8 for Pathfast (AUC, 0.76; sensitivity, 53%, specificity, 86%), but significantly ($P < .001$) greater than 5.3 for iSTAT (AUC, 0.77; sensitivity, 32%; specificity, 92%) and 4.5 for Radiometer (AUC, 0.68; sensitivity, 26%; specificity, 93%). In conclusion, we show that the prototype Gem Immuno POC cTnI assay has comparable diagnostic performance to both the Mitsubishi Pathfast POC and OCD contemporary assays in detecting acute MI at presentation in patients with clinical symptoms of acute coronary syndrome.

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Relative Accuracy of Automated Hematology Instrument and Laboratory-Developed Reticulated Platelet Assays for Categorization of Thrombocytopenia

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Background: Thrombocytopenia can be characterized as due to a destructive process, decreased production, or increased sequestration. The percentage of platelets that are most recently released from the marrow can help in this determination. Reticulated platelets (RPs) are young platelets recognized by their high RNA content, which can be identified by flow cytometry using nucleic acid specific fluorescent dyes. Several new automated hematology analyzers are able to calculate an RP percentage by using modified flow cytometry methods. The systems are more standardized and less laborious than manual laboratory-developed flow cytometry protocols and have been validated for clinical use. However, few studies have compared manual and automated RP fractions in routine clinical practice. Objectives: Evaluate the correlation between a manual flow cytometry–based RP assay and a corresponding automated hematology instrument RP measurement. Determine relative accuracy of the 2 methodologies in predicting categorization of thrombocytopenia based on follow-up clinical assessment. Methods: The percentages of RP were determined concurrently by a manual flow-based RP method and by the rP% parameter on a Cell-Dyn Sapphire (Abbott Diagnostics, Santa Clara, CA) on 100 consecutive patients with clinical requests for manual RP testing. Comparative evaluation included direct correlation of values and categorical separation into increased or decreased RP percentage based on laboratory- and literature-based cutoffs (increased production = 25% for manual RP and 10% for automated rP%). Final classification of thrombocytopenia was based on subsequent chart review. Results: The %rP as measured by manual and automated methods showed poor linear correlation ($R^2 = 0.07$). In terms of hypo- or hyperproduction, 25% of patients showed discrepant results, ie, would have been categorized differently by the automated rP%. Of these 25 discrepant results, 10 were able to be unambiguously characterized clinically. Of the 10 clearly characterizable discrepant results, 5 were production defects, 2 were sequestration, and 3 were destructive processes. In all production defect and sequestration cases, manual flow cytometry corresponded to the eventual clinical diagnosis, whereas destructive cases were split. Conclusions: The automated rP% parameter shows little numerical correlation with a manual laboratory-developed RP method. In this limited clinical set, the manual RP flow method showed greater relative accuracy in categorizing thrombocytopenia. Investigation of sources of discrepancy is warranted.

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A Next-Generation Sequencing Approach to Investigating Inherited Thrombophilias

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The risk of venous thromboembolism in protein C–, protein S–, and antithrombin-deficient individuals is approximately 5- to 10-fold higher compared with nondeficient individuals. Unlike the easily detected, recurrent mutations in the factor V and prothrombin genes, mutations in proteins C/S and antithrombin span the gene. In addition, phenotypic testing (ie, quantitative and functional assays) for deficiencies of these 3 proteins is affected by a myriad preanalytic variables such as age, vitamin K status, pregnancy,

hepatic function, consumptive coagulopathy, acute phase response, and anticoagulation therapy. To address these issues, our institution recently developed and validated clinical next-generation sequencing (NGS) testing for inherited genetic disorders. Among the 568 validated genes, we included 3 genes for investigating suspected thrombophilia, including: PROC (protein C), PROS (protein S), and SERPINC1 (antithrombin). A custom Agilent SureSelect sequence capture platform was utilized for target enrichment of exons and immediately adjacent intronic donor/acceptor splice site regions of the examined genes, prior to sequencing on an Illumina HiSeq 2000 instrument. NGS testing of 3 patients with clinical and laboratory evidence of hereditary thrombophilia identified single gene pathogenic mutations in all 3 patients, which included a missense mutation in SERPINC1, a splice site mutation in PROS, and a deletion in PROC. The SERPINC1 missense mutation affects the heparin-binding site of the enzyme and has been previously reported in individuals with type II antithrombin deficiency. Clinically, this patient has heparin resistance, requiring twice the dose of low-molecular-weight heparin to achieve therapeutic anticoagulation during her pregnancy. Due to the mutation leading to heparin resistance, she will be treated with antithrombin replacement at delivery. This case exemplifies the impact that identifying specific sequence variants can have on clinical decision making. Additionally, results can have a substantial impact on guiding family genetic counseling, and targeted Sanger sequencing of family members could be easily and cost-effectively performed if needed. As demonstrated by this limited study, clinical NGS testing is not only a powerful diagnostic tool for identifying pathogenic mutations in patients with inherited thrombophilia, but also important for making vital genotype-phenotype correlations.

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The Role of Complete Blood Count Parameters in the Diagnosis of Heparin-Induced Thrombocytopenia

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Heparin-induced thrombocytopenia (HIT), a platelet (Plt) destruction process, represents a small subset of thrombocytopenia, but it is always in the differential diagnosis due to its potential serious thrombotic sequelae. Enzyme immunoassay (EIA), a screening test for antibodies to the heparin-PF4 complex present in HIT, is over-ordered in many hospitals. The goal of this study is to investigate relationships between different parameters obtained from the complete blood count (CBC) and HIT EIA results. A prospective study was performed to identify all the samples sent for HIT EIA testing at Columbia University Medical Center between August 2012 and November 2012. Descriptive statistics and regression were performed to assess the relationship between HIT EIA results and various CBC parameters. During the study period, samples were received from 57 adult patients suspected to have HIT (median age, 65 years; 50.8% males; 35.1% had cardiac surgery; 29.8% had red blood cell transfusion within the past 7 days). 29.8% of the samples tested positive for HIT by EIA. In this study, patients with EIA optical density (OD) greater than 0.4 were classified as HIT EIA positive in the analysis. Of all the CBC parameters investigated, only the mean reticulocyte percentage (retic%) differed significantly between samples with positive and negative HIT EIA test results (3.97% vs 2.02%; $P = .01$). Similar results were observed for the mean immature reticulocyte fraction (32.4% vs 22.0%; $P = .02$). No difference in the immature platelet fraction (IPF) was observed between HIT EIA positive and negative samples (12.28% vs 12.29%; $P = .99$), although a weak correlation of –0.13 between EIA OD ratio and IPF was noted. In addition, neither hemoglobin (9.36 vs 9.95 g/dL; $P = .18$), nor Plt count (74.63 vs 95.71/μL; $P = .23$) differed significantly between HIT EIA positive and HIT EIA negative groups. Univariate logistic regression analysis showed that for every percent decrease in retic%, the odds of having a positive HIT EIA test result decreased by 51%. The sensitivity and specificity of the reticulocyte count for predicting a negative HIT EIA result were 65% and 65%, respectively, using a retic% cutoff value of 2.16%. If a retic% cutoff of 3.35% was used, the sensitivity and specificity for obtaining a negative HIT EIA were 95% and 40%, respectively. Further studies are required to confirm results of this limited study.

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Diagnostic and Social Work Yield of Newborn Drug Screening Using Meconium

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Objectives: Drug testing of newborns using meconium is commonly performed to determine fetal exposure to drugs. The objective of this study was to determine the diagnostic and social work yield of newborn drug screening over a 2-year period at an academic medical center. Methods: Retrospective analysis was performed on all meconium drug analysis performed on newborns in a 2-year period using an institutional review board–approved protocol. By institutional practice, the decision to perform newborn drug screening is based on assessment of maternal (eg, prior illicit use, poor prenatal care), delivery (eg, unexplained prematurity, precipitous labor), and newborn (eg, signs of drug withdrawal) risk factors. Detailed chart review examined clinical indications for newborn drug screening and whether drugs detected were illicit drugs or iatrogenic medications to mother or child. Results: Over a 2-year period, 976 newborn samples were analyzed by a meconium drug analysis panel (amphetamines, barbiturates, benzodiazepines, cannabis, cocaine, methadone, opiates, phencyclidine, propoxyphene), of which 344 were positive for at least 1 drug and/or metabolite. The most common illicit drugs detected (either parent compound or metabolite or both) were THC (n = 92), cocaine (n = 8), and methamphetamine (n = 3). Morphine was the most common drug detected overall (n = 195). In cases where morphine was detected in the absence of codeine (n = 106), 101 were in newborns where the morphine had been administered to the mother shortly before delivery or to infant after delivery but before meconium collection. Lorazepam was the most common benzodiazepine detected (n = 70), of which every case could be attributed to iatrogenic medications. For cases where an illicit drug was detected in meconium, 52.5% occurred with prior documented history of maternal illicit drug use and 77.8% occurred if one or more of the following factors were present: history of maternal illicit drug use, previous newborn with illicit drug detected, drug rehabilitation, and prior child protective custody involvement. Conclusions: Meconium drug screening picks up a significant amount of iatrogenic medications, especially morphine and lorazepam. Positive histories of maternal illicit drug use and/or prior child protective services involvement are strong risk factors for detection of illicit drugs in newborns.

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Analysis of Vitamin D Testing at 2 Medical Centers: Population Patterns and Chart Review

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Objectives: 25-Hydroxyvitamin D (25-OH) testing has increased in recent years. The objective of this study was to compare overall utilization and population results for 25-OH orders at 2 academic medical centers: one in New York (WCMC) and one in Iowa (UIHC). Methods: Retrospective analysis of all available 25-OH orders and results was conducted using laboratory information system queries according to IRB-approved protocols. Results were analyzed by age, gender, month, and location. Chart review was conducted for cases with very high or low 25-OH. Results: At WCMC, 20 months of data were available for analysis for 25-OH (n = 57,433 by immunoassay, n = 8,439 by LC-MS/MS; all sendouts). At UIHC, 144 months of data were available for analysis, representing 60,862 tests for 25-OH (all immunoassay; 78% sendouts). At both institutions, the majority of tests were ordered on females patients (WCMC, 69.2%; UI, 67.9%) and outpatients (WCMC: 95.4%; UIHC: 91.2%). 25-OH levels were higher on average in outpatients. At both institutions, 25-OH levels showed seasonal variation, with average levels higher in summer than winter. 25-OH levels were also higher in female vs male patients across age ranges at both institutions. Area plots (breaking down the percent of patients falling into specific reference intervals) demonstrated increased 25-OH insufficiency/deficiency in the adolescent females for whom 25-OH was ordered. Improved 25-OH status was observed in patients starting at approximately the fifth decade of life, possibly reflecting a supplementation effect. Chart review revealed over-supplementation (especially of 50,000 IU dose) in the majority of the rare cases of very high 25-OH levels. General nutritional deficiency and/or severe illness were found in most cases of severe 25-OH deficiency. Conclusions: Similar patterns of 25-OH orders and results were observed at 2 different academic medical centers. It appears that 25-OH status changes throughout age.

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Evaluation of PT or PTT Mixing Study With an Estimated Factor Correction Method

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Normal pooled plasma (NPP) mixing studies for prolonged PT or PTT are used to estimate whether the prolongation is due to an inhibitor vs factor deficiency. Current interpretation is based on empiric normal range (NR), percent correction (PC), or Rosner index (RI) method and assumes the same correction of single factor (SFD) vs multiple factor (vitamin K dependent or VKD) deficiency. We propose a new method of mixing study interpretation based on estimation of average factor level changes. VKD plasma (VKDP) was prepared by adsorption of the VKD factors in NPP to aluminum hydroxide. A standard curve (log clotting time vs log clotting factor concentration) was obtained by plotting PT or PTT results against diluted known factor-deficient plasma, including SFD plasma, VKDP, or all factor-deficient samples. Factor level was estimated based on PT or PTT. Similarly, a 1:1 mix PT or PTT could be predicted by estimated factor correction (EFC) and compared to actual mixing results to determine whether an inhibitor was present. We first applied this approach to a group of 24 patients who had known SFD, FVIII inhibitor, or lupus anticoagulant or who was receiving warfarin therapy. There was a 100% agreement between the interpretation based on our EFC method or NR method and patient's known conditions. However, a patient with FVIII inhibitor was misinterpreted as FVIII deficiency by PC or RI method. Next, we applied this EFC method to interpret all our mixing studies. A total of 82 mixing studies were performed in our coagulation laboratory in a large tertiary hospital in a 1.5-year period. The most likely type of deficiency was determined by patient's clinical history. VKDP curve (71%) or SFDP curve (29%) was chosen according to patient's clinical history and PT/PTT level. Using our EFC method, we found that 26 (32%) showed factor deficiency pattern, while 56 (68%) showed an inhibitor pattern, 1 with prolongation on incubation and factor VIII inhibitor, 55 with probable lupus inhibitor. In 40 patients additional studies were done, and lupus inhibitor was confirmed in 36 cases; of these, EFC detected 83%, NR 64%, PC 47%, and RI 58%. In conclusion, we developed a novel approach for interpretation of mixing studies based on estimated factor correction; this method was more sensitive at detecting lupus inhibitors, which represented approximately 70% of all samples sent for mixing studies.

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Analytical Performance of QMS Everolimus Assay on Ortho Vitros 5,1 FS Chemistry Analyzer: Measuring Everolimus Trough Levels for Liver Transplant Recipients

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Objective: We evaluated the analytical performance of the Thermo Fisher Scientific Quantitative Microsphere System (QMS) Everolimus immunoassay adapted to the Ortho Vitros 5,1 FS chemistry analyzer to support the immunosuppressant management of post solid organ transplant patients, including liver recipients. Background: Everolimus was approved by the Food and Drug Administration (FDA) in 2010 for graft maintenance in kidney transplant recipients. This drug has a narrow therapeutic index (3.0-8.0 ng/mL) and benefits from close blood level monitoring. Currently, QMS Everolimus immunoassay is the only commercial immunoassay approved by the FDA for monitoring everolimus levels specifically in kidney transplant patients. Additionally, several studies have shown liver recipients also benefit from everolimus therapy. The application of the QMS assay to monitoring drug levels in this particular patient population is warranted. Methods: The QMS Everolimus immunoassay is a homogeneous, competitive particle-enhanced turbidimetric immunoassay. The assay was adapted to the Ortho Vitros 5,1 FS chemistry analyzer, and patient samples were treated according to manufacturer instructions prior to analysis. The immunoassay was compared to a liquid chromatography tandem mass spectrometry (LC-MS/MS) assay. Results: The assay was linear between 0.8-20.0 ng/mL. Limit of detection (LOD) was 0.7 ng/mL and lower limit of quantitation (LLOQ) was 0.8 ng/mL. Over the period of 20 consecutive days, between-day coefficients of variation (CV) were 16.5%, 8.3%, and 3.1% at mean levels of 5.3, 12.0, and 17.2 ng/mL, respectively. Method comparison was carried out using 32 samples from treated transplant recipients, including 1

double lung and liver, 9 liver, and 2 kidney recipients. By the time the patients were tested for everolimus trough levels, the patients were co-administered with other calcineurin inhibitors: tacrolimus (3.5-20.2 ng/mL) or cyclosporine (97-175 ng/mL). The patients were not previously administered or transferred from sirolimus, another mTOR inhibitor. Everolimus levels in the patients ranged from 2.6 to 15.1 ng/mL, and we obtained a Deming regression of y = 1.276 – 0.697 (r = 0.930). The drug levels determined to be within the therapeutic index were in agreement between the 2 assays in comparison. Conclusion: The performance of the QMS Everolimus immunoassay by the Ortho Vitros analyzer was satisfactory for monitoring drug levels of liver transplant recipients.

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A Wiki-Based Decision Support Tool for Clinical Next Generation Cancer Sequencing

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The goal of this work was to develop a decision support tool to aid in the interpretation of a multiplexed 194 cancer gene sequencing panel in clinical use at the University of Washington. The panel, UW-OncoPlex, uses next generation deep sequencing to detect mutations in tumor tissue that may have relevance for treatment, prognosis, and diagnosis. The proper interpretation of the resulting sequencing data presents a significant challenge. Currently available resources (such as My Cancer Genome and COSMIC) did not offer sufficient annotation to meet our needs, so we developed our own wiki-based decision support tool we call the OncoWiki. This web-based support tool was built using the wiki engine MoinMoin. Each gene for which clinical information is available has a page which contains fully referenced information on the known, clinically relevant mutations that can occur in that gene broken down by prognostic, diagnostic, and therapeutic relevance. The site can be made accessible to the general public and is also password protected with the option of defining user permissions. Multiple users can upload and edit content on the site, all of which can be reviewed and signed off by the laboratory director. The rapid expansion of clinical information on cancer mutations is addressed with 2 main functionalities. The first is a system of automated PubMed searching that allows users to rapidly update pages with the most current research. Second, all pages contain a revision history, which allows users to see how current the page is and allows the laboratory director to rapidly identify out-of-date sections. To date, the OncoWiki has been utilized in the sign-out of over 125 clinical UW-OncoPlex cases and has served as both a resource for clinical sign-out and as a genomic training tool for residents and fellows. The OncoWiki is an easy to implement and update, low-cost decision support tool, which has facilitated institutional memory of rapidly evolving clinical information of cancer mutations.

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Age-Related Vitamin D Concentration Distribution and Vitamin D Ordering Patterns in a VA Population

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Objective: It is unknown if frequent 25-hydroxy vitamin D (OHD) testing improves patient outcome, therefore we investigated the clinical efficacy of OHD ordering in a VA population. Methods: Serum 25(OH) vitamin-D2 and -D3 (OHD2, OHD3, respectively) were measured by an in-house LC-MS/MS assay, calibrated to the NIST NRM 2972 standard. Total OHD was reported as the sum of OHD2 and OHD3. Assay AMR were 6-200 and 4-200 ng/mL for OHD2 and OHD3, respectively. Assay CVs were <10%. Patients' OHD results of 32 months' duration were obtained from the laboratory information system. Vitamin D status was defined as "deficient" (<15 ng/mL), "insufficient" (16-31 ng/mL), "normal" (32-99 ng/mL) and "toxic" (>100 ng/mL). Results: 43,661 results from 25,305 patients, mean age = 60 years, were collected. 14,894 patients had 1 and 10,411 patients (41%) had multiple OHD tests over the study period. 4,623 results (10.6%) were from female veterans. The number of OHD orders per patient ranged from 1.42 to 1.87 depending on patients' age. Mean OHD concentration was 32 ng/mL (SD = 13.4). 6,212 results (14%) had detectable OHD2. Eight percent of all results were deficient, 45.6% were insufficient and 0.1% were in the toxic range. Older patients had higher mean OHD. Mean OHD concentrations varied with seasons, and the lowest and highest values were observed in January and August, (mean OHD 28.2 and 34.4 ng/mL, respectively). We separately analyzed patients' OHD results if they had multiple orders during the study period. 70% of the follow-up tests were done between 90-365 days from the initial order. Trend analysis of results by linear regression did not show statically significant change in vitamin D status. Comparing results of the same months from consecutive years showed a statistically significant increase of average OHD concentration during the summer months only for patients with single and multiple orders. Patients' results were analyzed depending on their OHD status at the time of their first test. Mean OHD and vitamin D status of deficient patients improved >30 days after the initial measurement, but patients with insufficient OHD results have also shown improvement. Approximately 50% of all deficient and insufficient patients returned to normal vitamin D status. Conclusion: Our patients had higher OHD concentration than non-veteran populations, and their mean OHD concentration increased with increasing age, contrary to other studies, and an increased proportion of them had low OHD concentration during winter. 14% had detectable OHD2. Of all patients with multiple OHD tests, those with pathologically low OHD benefited from repeat testing but the overall mean OHD concentration did not change, suggesting that frequent testing should be done only in patients with clinically low OHD.

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A Case of False-Positive HIV Test in a Pregnant Woman at Delivery

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Introduction: Human immunodeficiency virus (HIV) is a leading cause of illness and death in the United States. Annually about 500,000 children become infected with HIV, mostly acquired perinatally from their mother. However, prevention of mother-to-child transmission is achieved by (1) maternal HIV screening, and (2) HIV treatment and counseling during pregnancy. Indeed, prenatal screening for HIV infection is currently recommended by the Centers for Disease Control and Prevention. With this increased effort for prenatal screening, more low-risk women are being tested. Since pregnancy itself has been recognized as a risk factor for false-positive results in HIV immunoassays, the positive predictive value of HIV testing is reduced when screening a lower prevalence population such as

ours. Accordingly, many laboratories are currently using fourth generation immunoassays which simultaneously detect p24 antigen and HIV antibodies. However, fourth-generation HIV immunoassays have lower specificity than earlier generation immunoassays, resulting in higher false-positive rates. Case report: We report a case of a false-positive HIV test in a 20-year-old primigravida woman, 39 weeks' gestation, who presented with spontaneous rupture of membranes. Since no prenatal HIV testing was done, an HIV serology test was ordered to determine the mother's HIV status. Results on the Architect HIV Ag/Ab Combo assay (fourth generation immunoassay) were repeatedly reactive on a single serum sample (Gold BD clot tube) but nonreactive on a plasma sample (Lavender BD EDTA tube). However the same serum and plasma samples were nonreactive on the Advia Centaur HIV 1/O/2 Enhanced assay (third generation immunoassay) and Oraquick Advance Rapid HIV-1/2 Antibody test. Because of these discrepant results and for further confirmation, an HIV-1 Western blot and HIV-1 viral load were performed with the blot results found to be intermediate while the viral load was undetectable. Therefore, the repeatedly reactive Gold BD serum sample results, analyzed on the Architect, were considered false positive. Conclusion: We describe a case of a false-positive HIV serum test performed on the Architect in an apparently healthy pregnant woman. While the cause of this false reactivity is not clear, most probably fibrin microclots in the serum sample interfered with the assay and thus accounted for the false positive results. Plasma may provide a more appropriate sample when using the Architect HIV Ag/Ab Combo assay. Proper and close follow-up and the use of supplementary tests are highly recommended.

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The Use of Thawed Type A Plasma in Emergency Situations

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Patients with massive hemorrhage may benefit from having thawed plasma immediately available for transfusion prior to blood type determination. Due to the limited availability of type AB donors and the rapid expiration of thawed plasma, routinely providing thawed AB plasma is an impractical solution for many hospitals. Based on institutional platelet transfusion practices, a decision was made to change our clinical practice to allow the storage and use of thawed type A emergency plasma in the trauma bays. In response to this change, a quality review of 130 consecutive plasma units issued to the trauma bays was conducted. Segments from each plasma unit were titrated for anti-B using tube serial dilution methodology. In 11 of the 130 units, the titer results were recorded as less than 1:128 without an exact titer result. Of the remaining 119 units, titers were as follows: [1:2] 1 unit (1%), [1:4] 12 units (10%), [1:8] 31 units (26%), [1:16] 39 units (33%), [1:32] 24 units (20%), [1:64] 11 units (9%), and [1:128] 1 unit (1%). No units had a titer greater than 1:128. Of the 130 units issued to patients, 50 plasma units were transfused to 37 adults before a patient blood type was available. Of these patients, 21 received a single unit and 15 received 2 units of plasma. In assessing whether any recipients were of incompatible blood groups, 2 of the 37 patients typed as AB and none typed as B. Among the 2 group AB patients, 1 received a single unit of plasma (titer < 1:128) and the other received 2 units (titers 1:4, 1:32). There were no reported clinical signs of hemolysis and the direct antiglobulin test (DAT) remained negative in each patient. The literature suggests that an anti-B titer of less than 1:256 may be of little clinical significance. In our donor population, the likelihood of an anti-B titer being 1:256 or greater appears to be low. Based on these preliminary data, transfusion of 1 to 2 units of plasma containing anti-B alloantibodies to patients expressing the B antigen is unlikely to result in acute hemolysis and is not associated with a positive DAT. These results suggest that small volumes of type A plasma with low titer anti-B may be considered acceptable for emergency transfusion of adults.

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Vitek2 Under-calls Cefepime Compared to Etest for Extended Spectrum Beta-Lactamase (ESBL)– and Carbapenemase-producing Enterobacteriaceae (CREs)

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There are increasing reports of false susceptibility with some automated susceptibility testing platforms for certain gram-negative (GN) pathogens against some antimicrobial agents. We previously demonstrated on the GN28 card that Vitek2 exhibited a high percentage of very major errors for cefepime (CP) compared to reference broth microdilution when testing *Klebsiella pneumoniae* carbapenemase-producing isolates. In the current study, we compared 2 recent Vitek2 cards (GN59 and GN74) to Etest to determine the degree of categorical agreement between the 2 methods for CP with ESBLs and CREs. Over 2 months in 2011, all consecutive ESBLs and CREs from any site were tested on the GN59 card (122 isolates). Similarly, over a 6-week period in 2012, all consecutively recovered ESBLs and CREs (59 isolates) were tested on the GN74 card. The species tested was similar each year with 65% *Escherichia coli* (98% ESBLs) and 31% *K pneumoniae* (60% CREs). The ESBL/CRE ratio was also similar each year at 78%/22%. For both Vitek cards, 32% of all isolates demonstrated categorical disagreement between Vitek and Etest. For the GN59 card, 62% of discrepancies were Vitek susceptible [S]/Etest resistant [R]); the remainder were Vitek intermediate [I]/Etest R or Vitek S/Etest I. Of the CREs and ESBLs in 2011, 56% and 25%, respectively, were discrepant by the testing method. An equal proportion (approximately 60%) of Vitek S/Etest R was seen in ESBLs and in CREs in 2011. For the GN74 card, 90% of all discrepancies were seen when testing ESBLs, and the majority of discrepancies (84%) were Vitek I/Etest R or Vitek S/Etest I. Only 2/19 discrepant isolates (10.5%, both ESBLs) were Vitek S/Etest R. Vitek under-called resistance for CP as compared to Etest on both cards, with categorical disagreement for 1/3 of isolates. There was a higher proportion of Vitek S/Etest R for the GN59 card as compared to the more recent GN74. In general, CREs showed the pattern of Vitek S/Etest R more frequently than ESBLs. Improved formulation of cefepime on Vitek2 is needed in order to direct more appropriate therapy for resistant gram-negative organisms, as CP remains a potential treatment option for some ESBLs and CREs.

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A Case of Biotin Interference in the Vitros 5600 Thyroid-Stimulating Hormone (TSH) Assay

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Objective: We determined that the biotinylated molecules present in specimens who take high doses of biotin may interfere with immunoassays that use biotin streptavidin mechanisms in their

assay designs. Background: A 44-year-old woman with Grave's disease post radioiodine therapy was evaluated for further management. Her low free T4 (reference range, 0.70-1.85 ng/dL) and low TSH (reference range, 0.4-5.0 mU/L) of 0.65 ng/dL and 0.75 mU/L, respectively, did not clinically match. Free T4 by equilibrium dialysis confirmed the low free T4, which prompted investigation into the low TSH result reported by the Vitros 5600 TSH assay at our institution. Simultaneous samples were analyzed for TSH on the Vitros 5600 analyzer and the Roche Elecsys 2010 analyzer. Discrepant values of 1.8 mU/L and 17.1 mU/L were obtained, respectively. Dilution studies confirmed the presence of a negative interference in the Vitros assay since TSH was higher in the diluted samples compared to the undiluted sample (undiluted = 1.8 mU/L; diluted 1:10 = 26.4 mU/L). After heterophilic-blocking reagent revealed no effects, we reviewed the patient's medical history, which suggested that biotin may be the cause of the interference since the Vitros 5600 assay uses biotin-streptavidin mechanisms. Methods and Results: To confirm the interfering role of biotin, we treated the specimen using streptavidin-coated microparticles, which removed biotin, and compared the TSH results before and after the treatment. The TSH concentration in the patient's specimen increased from 1.8 mU/L before to 18.2 mU/L after treatment, which identified biotin as the potential interference in the Vitros TSH assay. Interestingly, our investigation showed that although the TSH assay on the Roche Elecsys 2010 analyzer also uses biotin-streptavidin mechanisms, the Roche TSH assay was not affected by the biotin interference present in the specimen. Conclusions: Using the streptavidin microparticle treatment, we determined the interfering role of biotinylated molecules present in specimens who take high doses of biotin with immunoassays that use biotin streptavidin interactions. In the future, we will determine the biotin concentration in the specimen, and study the effects of free biotin to the Roche Elecsys 2010 and Vitros 5600 TSH assays, which may explain the difference in their tolerance to the biotin interference. We will conduct an in vitro interference study to determine whether large dose intake of biotin (10 mg/day) would interfere at the normal TSH levels.

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Simulated Annealing Optimizer for Moving Averages Parameters

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Background: The application of moving averages as a laboratory QC measure was proposed in 1965 and is designed to detect short- and long-term analytical shifts by smoothing data points thought to reflect a pseudo-normal range. Previous publications have focused on a priori knowledge of the characteristics of normal data including assumptions on normality, means, and statistical measures of dispersion, which in many laboratory tests are not necessarily true. Objectives: We propose a method of developing moving averages parameters without a priori knowledge of the distribution of patient values using a simulated annealing search algorithm to minimize false error detection (FPrate) as well as the average number of patient samples affected until error detection (ANPed). Methods: Using MATLAB to create and run simulations, we injected artificial incremental positive and negative errors into 96 days of patient values obtained from our laboratory information system. From this we determined the ANPed and FPrate for a fixed number of patient samples averaged (N), while control and truncation limits were adapted from methods described by Cembrowski et al in 1984. These initial protocols were then compared to a second set generated via a simulated annealing search algorithm with asymmetric variation of truncation limits and N, while control limits were set at the calculated reference change value or CLIA limits for each assay. The N and truncation limits were optimized by minimizing a cost function consisting of a linear combination of ANPed and FPrate with coefficients empirically chosen. Results: The optimized protocols showed superior ANPed performance compared to the original protocols. For example, the original plasma phosphorus protocol detected a ±0.9mg/dL shift in 939 and 657 specimens, respectively, while the optimized protocol detected the same magnitude shift in 39 and 37 samples. Likewise, while the original plasma creatinine protocol was able to detect a ±0.3mg/dL shift in 139 and 348 specimens, our optimized protocol was able to detect the same shift in 35 and 45 samples, respectively. Interestingly, optimized plasma potassium protocol showed similar performance with minimal improvement of the ANPed at 73 vs 76 samples originally. In all assays modeled the FPrate was zero. Conclusions: This is a robust method for optimizing moving average parameters independent of a priori assumptions of normality. For assays in which normality and variance assumptions can be made, the optimized protocol closely matches the theoretical performance of the Cembrowski model, while in other assays we have shown 10-fold performance increases in ANPed without increasing FPrate.

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Prevalence of Paraprotein Inference in Beckman Coulter Synchron Measurements of HDL

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Complete suppression of high-density lipoprotein (HDL) measurements has been reported to occur with absorbance-based determinations in patients with paraproteinemia. However, the prevalence of this interference and relationship to M-protein concentrations and clonal types have not been fully explored. Additionally, there are reports of decreasing HDL concentrations with increasing amounts of clonal protein that may be related to incremental assay interference. A systemic evaluation of the correlation between monoclonal (M) protein concentration and HDL assay interference with varying M-protein concentrations and clonal types was initiated. One hundred three patients were selected from a population undergoing workup and follow-up for multiple myeloma that had undergone M-protein concentration determination by serum protein electrophoresis (SPEP) and capillary zone electrophoresis (CAPYLLARYS Sebia) M-protein determination. Samples were selected to represent a range of M-protein concentrations for each immunoglobulin clonal type. These included samples without detectable M-protein (n = 10), as well as IgA (n = 32), IgG (n = 43), IgM (n = 10), free kappa (n = 5), and free lambda (n = 3). One patient was excluded because of nondetectable HDL. Mean M-protein concentration was 1.85 g/dL (0.0-10.0 g/dL). Two forms of HDL measurements were then performed on split serum samples: Synchron LX, a common absorbance-based technique, and Atherotech Vertical Auto Profile (VAP), an ultracentrifugation method not subject to interference by paraproteins. Mean HDL concentration of the Beckman technique was 45.9 mg/dL (16-108 mg/dL) and of the Atherotech technique was 46.0 mg/dl (12-111 mg/dL). In this population of 103 patients, a single patient with 6.1 g/dL IgG kappa M-protein exhibited suppressed HDL measurement (<5 mg/dL with a corresponding VAP level of 33 mg/dL). For the other patient samples both Synchron LX HDL and VAP HDL (mg/dL) plotted vs increasing M-protein concentration (g/dL) revealed similar

negative correlations (m [slope])= –3.87 and –3.85, respectively), demonstrating that as M-protein increases, HDL levels decrease. Overall, there was no significant difference between the 2 methods (P = .40). However, for IgM monoclonal protein samples, HDL values were significantly less with the Synchron LX method compared with the VAP method (P = .03), with observed differences between methods increasing with immunoglobulin concentration. For other immunoglobulins, the differences between HDL determinations were not significant. As serum M-protein concentration increases, there is an observed inverse relationship with HDL. This does not seem to be related to paraprotein interference in HDL assays. Approximately, 1% (1/93) of patients with measurable M-protein exhibited strong suppression in HDL concentration determination.

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Revisiting the Dynamic Relationship Between Erythropoietin and Hematocrit

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Erythropoietin (EPO) is a hormone produced by the kidney that acts on bone marrow to stimulate erythropoiesis. An inverse log-linear relationship between EPO and hematocrit was first described in the 1980s, in which a marked increase in EPO was observed as hematocrit decreased (Erslev et al. *J Lab Clin Med.* 1987;109[4]:429-433). The 95% confidence limits of this relationship are still used today in the interpretation of EPO. However, these early studies utilized EPO bioassays and radioimmunoassays which are no longer used clinically and differ significantly from modern assays. The objective of this study was to reevaluate the relationship between EPO and hematocrit using modern clinical laboratory methods. We conducted a retrospective review of EPO and hematocrit results analyzed at our facility on samples collected within 2 days of each other for patients ≥18 years of age (n = 208). Additional sample data included age, gender, hemoglobin, and a general marker of kidney function, when available. The semilog plot of EPO vs hematocrit was evaluated using nonlinear regression as well as the Erslev et al confidence limits. The correlation (R^2 value) of a nonlinear regression of the data was 0.085. Dividing the data based on hemoglobin (Hb) concentration resulted in R^2 values of 0.066 (Hb <12 g/dL), -0.060 (Hb 12-18 g/dL), and 7.8e-5 (Hb >18 g/dL), indicating no correlation between EPO and hematocrit regardless of the hemoglobin concentration. Additionally, regression analysis of the data subdivided into age groups of approximately 10-year ranges indicated no correlation between EPO and hematocrit based on age, with the exception of the 61-70 year old group (R^2 = 0.977, n = 94). Interpretation in the context of the Erslev et al confidence limits indicated a population that fell within the expected range, but also distinct populations (approximately 30%) that fell outside the Erslev et al limits. These populations had EPO values predominantly within the reference interval (4-27 mU/mL) with corresponding hematocrit values that were either increased or decreased (reference interval, 35%-52%). This indicated a lack of dynamic response by EPO to changes in hematocrit in these subpopulations. The current analysis revealed that a log-linear relationship between EPO and hematocrit was not maintained when using current analytic methods. Since many factors can influence EPO and hematocrit, investigation of the clinical status of subsets of these patients may help identify the expected response of EPO to changes in hematocrit in different clinical situations. Updating our understanding of these relationships will aid in further result interpretation.

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Validation of the Bruker MALDI BioTyper for the Identification of Rapid-Growing Mycobacteria

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Rapid-growing mycobacteria (RGM) are ubiquitous environmental organisms that cause a spectrum of clinical ailments ranging from localized pulmonary and soft tissue infections to disseminated disease. Despite their defining characteristic of growth on subculture within 7 days, traditional identification methods can take weeks to finalize and susceptibility testing is not widely available. The majority of infections can be attributed to 1 of 3 species (*M abscessus*, *M chelonae*, and the *M fortuitum* group); antibiotic susceptibility profiles are predictable but dramatically different among species. Narrow-spectrum empiric treatment could therefore be guided by rapid identification of RGM to species level. To achieve this goal, we are validating identification of RGM isolates from liquid and solid media on the Bruker MALDI Biotyper system (version 3.1, with *Mycobacteria* Database 1.0; Bruker Daltonics, Billerica, MA). A diverse set of RGM isolates previously identified to the species level by reference laboratories were subcultured, subjected to standard N-acetyl cysteine and sodium hydroxide decontamination, and cultured in Mycobacterial Growth Indicator Tubes (MGIT; Beckton Dickinson), Lowenstein-Jensen (LJ), 7H10 and 7H11 slants and plates at 30°C. Analysis of MGIT samples (24-48 hrs after initial positivity) and solid media (at 7 days) was performed in triplicate following a bead lysis/heat inactivation protocol developed by the manufacturer. A species level identification was assigned for a BioTyper score of ≥ 2.0 per Bruker's guidelines and compared to the gold-standard identification. Preliminary data demonstrated that the rate of identification to species level from MGIT tubes, LJ, 7H10 and 7H11 solid media was 80% (n = 26, mean score = 2.18), 62% (n = 26, mean score 2.05), 50% (n = 29, mean score = 1.99) and 37% (n = 27, mean score = 2.00), respectively. With the exception of 1 MGIT identification (*M smegmatis* called *M wolinsky*, score 1.85), no misidentifications were made, even at scores down to 1.48. Upon completion of the validation, cutoff scores will therefore be determined for each media type using ROC analysis. Rates of identification were not affected by subculture, freezing, or temperature variation (35°C vs. 30°C). Decreased identification rate from solid media may reflect variability in inoculum size and the effect of media type on protein expression. These data provide preliminary evidence that MALDI-TOF technology may be a robust and clinically useful method to reliably identify RGM from broth and solid media to species level, allowing for early initiation of appropriate antimicrobial therapy.

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Utility of Immature Granulocytes for the Prediction of Septic Shock

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Septic shock represents the most severe form of sepsis with an almost 70% in-hospital mortality rate. Biomarkers are needed to identify at first presentation, which patients may benefit from early and aggressive therapies. This study investigated

if immature granulocytes counts (IG and IG%) could add to the conventional complete blood count (CBC) to predict which patients would develop septic shock in a medical intensive care unit (ICU). Methods: White blood cell count (WBC), neutrophil count (NEUT), IG and IG% were obtained using the Sysmex XT-2000i (Sysmex America, Mundelein, IL) hematology analyzer. Central 95% reference intervals were established using nonparametric analysis (n = 120 normal subjects). Intra- (n = 20) and interassay (n = 20 days) coefficients of variation (CV) were determined using residual whole blood pools and quality control material, respectively. Analytical measurable range (AMR) was validated by linear regression analyses of mixed high and low IG-containing residual whole blood pools. Retrospectively, CBC results were matched with 94 adult ICU patients at high risk for septic shock who underwent case review by a single senior critical care physician. Only CBCs collected within 48 hours of initial sepsis panel blood cultures were included. Patients who met international consensus criteria for septic shock (n = 78) were compared with nonseptic patients (n = 16) in this ICU population. Results: Reference intervals for IG (0-0.01 × 10^3/μL) and IG% (0%-0.2%) were established. Intraassay precision increased (58%, 30%, <10% CV) with increasing IG (0.01, 0.05, 0.4 × 10^3/μL) and IG% (0.1, 0.4, and 3.6%), respectively. Interassay precision was less than 27% CV at 3 levels of IG (0.10, 0.15, and 0.24 × 10^3/μL) and IG% (1.5%, 2.2%, and 3.3%). The AMR for IG (0.004-1.02 × 10^3/μL) and IG% (0.06%-15.6%) yielded slopes of 1.02 and R^2 less than 0.99. Using the upper reference limit, CBC parameters had modest clinical sensitivities with poor specificities for predicting septic shock: WBC (61%, 44%), NEUT (72%, 31%), IG (62%, 31%), and IG% (51%, 56%). Receiver operating characteristic curve analysis was used to optimize cutoffs for each CBC parameter. Used in combination, the parameters yielded a specificity of 94%, but a poor sensitivity of 11% for prediction of septic shock. Conclusions: Among the CBC parameters, NEUT was the most sensitive and IG% was the most specific biomarker for septic shock. Based on these results, there may be added benefit of reporting IG% along with CBC results for the ability to predict, at first presentation, which patients will develop septic shock in the adult ICU population.

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Predicting Succinylcholine Recovery Time: A Study of Butyrylcholinesterase Genotype and Phenotype in Normal Individuals

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Objective: Butyrylcholinesterase (BChE) is encoded by the *BCHE* gene and metabolizes ester-containing drugs including the neuromuscular blocking agent succinylcholine. Individuals with wild-type BChE activity experience neuromuscular blockade (NMB) of short duration (≤10 min) while extended NMB (0.5-8 h) may be observed in those with inherited BChE variants. The BCHE genotype could lead to a personalized prediction of NMB duration from succinylcholine. As a first step, we investigated if a BCHE genotype could predict a biochemical phenotype and the duration of NMB. Methods: The BChE activity, dibucaine number (DN), and BCHE genotype was determined in 50 adults (18-65 y) undergoing elective surgery under general anesthesia at the University of Utah Hospital and requiring NMB with succinylcholine (1 mg/kg). Duration of paralysis (recovery time) was measured by acceleromyography and a recovery time of ≤10 minutes was considered normal. BChE activity in the presence and absence of the inhibitor dibucaine (0.4 mmol/L) was determined using acetylthiocholine as a substrate and was used to calculate the DN. An uninhibited BChE activity ≥3,300 U/L with a DN ≥83% was considered normal. BCHE genotype was determined by sequencing MagNAPure-extracted patient DNA using BigDye Terminator chemistry. Results: The BCHE genotype was U/U in 54%, U/K in 30%, U/AK or A/K in 8%, K/K in 6%, and indeterminate in 2%. 90% had a genotype (U/U, U/K, and K/K) that would be expected to produce an NMB of ≤10 minutes. The mean ± SD NMB recovery time of these individuals was 8.2 ± 2.2 min (range, 4.0-14.3 min) and was not significantly different (P = .25) across genotypes. The mean ± SD BChE activity and DN of individuals with these genotypes was 4,584 ± 1,262 U/L (range, 1,457-6,987 U/L) and 84% ± 2.3% (range, 72%-85%), respectively, both of which were significantly different across genotypes (P = .04). Seventy-eight percent had a BChE activity ≥3,300 U/L with a DN ≥83% and 89% had an NMB ≤10 minutes. NMB was negatively correlated with BChE activity (r = –0.45; P = .002). Conclusions: The NMB duration showed little variation among individuals with U/U, U/K, and K/K BCHE genotypes but BChE phenotype was highly variable. As a group, these BCHE genotypes were fair predictors of a normal BChE biochemical phenotype and good predictors of succinylcholine NMB duration.

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Use of Cystatin C–Based Estimated Glomerular Filtration Rate to Improve Diagnostic Accuracy for Chronic Kidney Disease (CKD)

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Background: The accuracy of creatinine-based equations for estimated glomerular filtration rate (eGFRCr) is known to depend on patient characteristics and can lead to overdiagnosis of CKD in healthier populations. Recently, equations have been developed to estimate GFR using serum cystatin C (eGFRCys). Our objectives were to (1) compare eGFRCr and eGFRCys with measured GFR in clinically mixed populations; and (2) determine the impact of KDIGO guidelines suggesting eGFRCys for patients with eGFRCr 45-59 mL/min/1.73m^2. Methods: Physician-ordered measured GFR (iothalamate clearance) was obtained (n = 502). Group A included transplant recipients (229 kidney; 123 other organ) and group B included all other presentations (CKD [n = 117], postnephrectomy donors [n = 12], and potential kidney donors [n = 21]). Serum creatinine (enzymatic) and cystatin C were assayed using Roche Cobas platforms (Roche Diagnostics). eGFRCr and eGFRCys were calculated using CKD-EPI 2009 and CKD-EPI 2012 cystatin C equations, respectively. Cystatin C was also measured in 93 nontransplant outpatients with physician-ordered creatinine and eGFR (MDRD) between 45-59 mL/min/1.73m^2. Results: The mean measured GFR was 66 (group A) and 59 mL/min/1.73m^2 (group B). In Group A, bias (median difference between measured and eGFR) was 1.5 (eGFRCr) and 6.9 mL/min/1.73m^2 (eGFRCys; P < .001). In group B, bias was 5.2 (eGFRCr) and 2.4 mL/min/1.73m^2 (eGFRCys; P < .001). Precision (interquartile range of the difference) was similar for both groups (15.8, eGFRCr vs 15.0, eGFRCys). Accuracy (percent of eGFR results within 30% of measured GFR) was similar for eGFRCr and eGFRCys in group A (20% vs 18%), but significantly different in group B (30% vs 16%; P = .003). The sensitivity and specificity for detecting a measured

GFR <60 mL/min/1.73m^2 were: group A: eGFRCr (86%, 69%); eGFRCys (94%, 57%), and group B: eGFRCr (96%, 72%); eGFRCys (99%, 88%). Across both groups, 50 patients with measured GFR >60 mL/min/1.73m^2 had eGFRCr 45-59 mL/min/1.73m^2; of these, 24 (48%) patients were correctly classified using eGFRCys. In the second cohort of 93 outpatients with eGFR (MDRD) between 45-59 mL/min/1.73m^2, eGFRCys reclassified 37 (38%) as GFR >60 mL/min/1.73m^2. Conclusions: eGFRCys demonstrates superior accuracy and sensitivity compared to eGFRCr among the nontransplant outpatient populations, while eGFRCr is better for the transplant recipient group. These data suggest that cystatin C testing could reduce unnecessary nephrology referrals when used to verify reduced GFR in nontransplant patients with eGFRCr of 45 to 59 mL/min/1.73m^2.

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Why GCMS Remains an Invaluable Tool in a Toxicology Lab: A Novel Approach to Clinical Validation of a High Sensitive GCMS Platform for Urine Drug Screening

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We have validated a new gas chromatography mass spectrometry (GCMS) platform that facilitates routine screening and automated reporting of up to 212 drugs, in any shift, by laboratory technologists without the need for sign-out by an onsite mass spectrometry-trained toxicologist. The platform uses a programmable temperature vaporizer (PTV) injector for large sample volume injection and free data purification and spectral matching software (AMDIS) for analyte identification. Validation data from 118 patient samples demonstrate that this platform provides multifold improvement in sensitivity as well as detection of an increased number of drugs per patient compared with an established assay. Further examination of the role of the data processing tools and the in-house databases used in the 2 platforms demonstrates that the improved analytical sensitivity of the new platform is attributed in large measure to the superiority of the new GCMS platform, while the use of AMDIS for data processing irrespective of the type of in-house library used provides additional improvement in performance and specificity. Clinical validation of the use of AMDIS (or any other freeware) for routine automated screening and unsupervised reporting of a large number of drugs in a clinical environment has not been previously reported. Newer mass spectrometry techniques such as liquid-chromatography mass-spectrometry (LCMS) and high-resolution mass spectrometry (HRMS) offer better analytical sensitivity, broader molecular range, and mass resolution compared with GCMS and is currently being adopted in the toxicology laboratories. With the advent of such high performance instruments, an emerging question facing the clinical laboratory community is whether the use of GCMS is appropriate in the current toxicology setting. GCMS instruments are a mature and reliable technology, the platforms are substantially cheaper than LCMS platforms, and they are easier to use by technologists with no mass spectrometry expertise. We present a case study that demonstrates the capability of our GCMS analysis in a toxicology laboratory to provide real-time identification of drugs with high confidence and improved clinical outcome. In this case, we were able to report in under an hour that the altered mental status of a 71-year-old nursing home resident admitted to ER was caused by the mix-up of her prescription drugs with those of her roommate.

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Prevalence of Hemoglobin Variants in Stored Blood

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Simple and exchange transfusions are used to treat sickle cell anemia. To prevent alloimmunization in these patients, antigen matching is used in the selection of RBC units. Recent data suggest an increased frequency of glucose-6-phosphate dehydrogenase (G6PD) deficiency in antigen-matched RBC units that are transfused into sickle cell patients. Similarly, there is a higher incidence of hemoglobin (Hb) variants in individuals of African descent than in Caucasians. The majority of the blood donor population in the United States is Caucasian, which suggests a low incidence of Hb variants in the general inventory of the blood bank. We hypothesize that sickle cell anemia patients who receive antigen-matched pRBCs are more likely to receive blood from donors of African descent and thus may potentially receive a unit containing an Hb variant. Although there are published case reports describing transfusion-acquired Hb variants, the frequency and clinical significance of this are unclear. Donated blood is not routinely screened for Hb variants, and there are no current guidelines for blood donors with Hb variants. The current study investigates the frequency of Hb variants in the general donor inventory and antigen-matched units. Using alkaline Hb electrophoresis, we compared the prevalence of Hb variants in Rh-positive (D+) RBC units that were negative for the C, E, and K antigens and that were used for exchange transfusion to the prevalence of Hb variants in D+ RBC units in the transfusion service general inventory. We evaluated 157 total pRBC units. Of these, 105 were D+ pRBC units from the general inventory of the blood bank, 40 were D+ units lacking the C, E, and K antigens used for RBC exchange procedures, and 12 were D+ units used for RBC exchange that were not matched for the C, E, and K antigens. No hemoglobin variants were detected, either in pRBC units in the general inventory or in those used for RBC exchange. The results of this study suggest that the risk of acquiring an Hb variant through transfusion is low. The most common variant in the African-American population is hemoglobin S, but it is our standard practice to provide Hb S–negative pRBCs for transfusing sickle cell patients. The prevalence of Hb C trait, 2% in African-Americans, suggests that additional units from the D+, C–, E–, and K– inventory would need to be tested to find a unit from a donor with Hb C trait. Examination of additional units is needed to better assess the risk of transfusion-acquired Hb variants to help determine if screening of the donor population for Hb variants is warranted.

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How Clinical Pathologists Can Guide the Evaluation and Management of Patients With an Underrecognized, Potentially Fatal Cause of Anemia and Thrombocytopenia

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Bone marrow necrosis (BMN) and the subsequent embolism of fat and necrotic marrow into systemic circulation is a rare but potentially fatal complication of compound heterozygous sickle cell disorders. Delayed diagnosis may bring catastrophic results. This report demonstrates the critical role pathologists play in the diagnosis and treatment of BMN, a cause of anemia and thrombocytopenia in patients with sickle cell-β+-thalassemia (HgbSβ+) or hemoglobin SC. In the past 3 years, 8 patients, including 2 diagnosed postmortem, were transferred to our institution with unrecognized BMN.

Their presumed diagnoses included thrombotic thrombocytopenic purpura (TTP) and acute leukemia. Careful review of their laboratory data by our Clinical Pathology consultants excluded those diagnoses in the 6 patients who survived. Unfortunately, the first 2 surviving patients already had permanent neurologic deficits with multiorgan failure due to fat/BM emboli. In contrast, the third patient, who had SC disease, was profoundly anemic (Hgb 4.5 g/dL) upon admission and received 4 PRBC transfusions. She recovered quickly and was discharged 2 weeks later. Based on her favorable response to transfusion, we elected to perform emergent RBC exchanges on subsequent patients with clinical features consistent with BMN, even in the setting of unknown hemoglobinopathy. The next 3 patients were also transferred with presumed TTP. They had neurologic deficits, renal insufficiency, increased LDH, anemia, thrombocytopenia, and leukoerythroblastic changes on peripheral smear. One patient had known HgbSβ+, and the other 2 were diagnosed with HgbSβ+ during hospitalization. Signs and symptoms supported the clinical diagnosis of BMN. After treatment with emergent RBC exchanges (70% of blood volume replaced by donor HgbS-negative PRBCs), all 3 patients had immediate mental and respiratory status improvement while undergoing the procedure. Two patients were discharged within 1 week of treatment; the other was discharged 2 weeks later. Remarkably, the 3 patients treated with RBC exchanges experienced full recovery. We report the successful diagnosis and treatment of 3 HgbSβ+ patients who presented with BMN and multiorgan failure. Due to a lack of awareness of this rare clinical entity, BMN may be initially misdiagnosed, delaying life-saving therapy. As pathologists, we have access to key clinical and laboratory data and play a pivotal role in guiding our clinical colleagues in the evaluation and management of such patients.

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Evaluation of Deuterium-Labeled Internal Standards for the Multiplex Measurement of Cyclosporin A, Sirolimus, and Tacrolimus by HPLC Electrospray Ionization Tandem Mass Spectrometry (LC-MS/MS)

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Deuterium-labeled analogs are valuable as internal standards (IS) for quantitative analysis of drugs by liquid chromatography tandem mass spectrometry (LC-MS/MS). Compared with the analyte, they have identical extraction characteristics and similar chromatographic and ionization properties. For the past 10 years, our laboratory has utilized the analog IS for therapeutic drug monitoring of the immunosuppressant drugs: cyclosporin D IS for cyclosporin A measurement, and ascomycin IS for a measurement of sirolimus and tacrolimus. The objective of this study is to evaluate the newly acquired deuterium-labeled IS for the multiplex measurement of cyclosporin A, sirolimus, and tacrolimus. The deuterium-labeled IS were obtained from the following companies: Cyclosporin A-D12 from Analytical Services International Ltd, Sirolimus-D3 (ie, Rapamycin-16-methoxy-D3) from IsoSciences, and Tacrolimus-13C,D2 (ie, FK-506-13C,D2) from Toronto Research Chemicals Inc. Tuning for IS was performed on Waters Xevo LC-MS/MS equipped with Masslink software. Three deuterium-labeled IS were combined in acetonitrile to a concentration that yielded similar area under the peaks when compared to analog IS under the same injection conditions. Cyclosporin A, sirolimus, and tacrolimus were enriched from patients' whole blood (EDTA) by protein precipitation with $ZnSO_4$ and acetonitrile, containing either the deuterium-labeled IS or analog IS. Extracted samples were injected on to the HPLC, passed through a short C-18 reverse phase column, and analyzed on Waters Xevo LC-MS/MS. Analysis of the data was performed on the Masslink, Excell and EP Evaluator software. We have validated the new assay by assessing precision and linearity of a new method comparing to the method that utilized the analog IS. Precision and accuracy of quality control samples at 3 concentrations (×5 for each concentration on each of the 5 days of the experiment) were similar for deuterium-labeled IS and analog IS both intrarun and interday: CVCyclosporin A-D12 = 10.1%, CVTacrolimus-13C,D2 = 7.6%, CVSirolimus-D3 = 6.5%, compared with CVCyclosporin D = 12.7%, CVAscomycin = 10%. For linearity studies, 30 patient samples were analyzed for each of the 3 immunosuppressant drugs with either new deuterium-labeled IS or analog IS on the same instrument following the same extraction method on the same day. For each of the 3 analytes, patient samples were carefully selected to span the whole range of clinically important values. Patients' results were not significantly different on average with new deuterium-labeled IS vs analog IS. In summary, we have evaluated 3 deuterium-labeled IS for therapeutic drug monitoring of cyclosporin A, sirolimus, and tacrolimus immunosuppressant drugs. To our knowledge, these deuterium-labeled IS were assessed for the first time for the LC-MS/MS multiplex assay.

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An Approach to Optimize FISH Test Utilization for Multiple Myeloma

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Detection of recurring cytogenetic abnormalities in multiple myeloma (MM) by fluorescence in situ hybridization (FISH) provides critical prognostic information. An 11-probe FISH panel is ordered on all MM patient bone marrows at our institution, regardless of disease status. However, reagent and labor costs are high, highlighting the need to ensure appropriate test ordering. We hypothesized that low MM cell content in samples by morphology and flow cytometry (FC) would predict negative/noninformative FISH results. To test this hypothesis, we retrospectively analyzed FISH data to better understand patterns of utilization and the role of CD138 antibody enrichment of MM cells in increasing detection of abnormalities. We retrieved demographics and results of marrow morphology, FC, FISH, and concurrent serum paraprotein data and marrow CD138 enrichment use for all 794 cases (630 patients) with MM and related histories for which FISH was performed from May 2011 to June 2012. Results were stratified into morphology and/or FC-positive cases (M/F+, n = 390), and, dual morphology and FC negative (M–/F–, n = 404). CD138 enrichment was performed for 505 of 794 cases. Of the nonenriched M/F+ cases, FISH yielded a positive result in 51 (43%) of 118 cases. With CD138 enrichment, positive results jumped to 258 (95%) of 272 cases. Importantly, of 171 nonenriched M–/F– cases, none yielded a positive FISH result (0%). Even in M–/F– cases in which enrichment was performed, only 37 (16%) of 233 cases were FISH positive. These FISH-positive cases showed a higher percentage of abnormal serum protein immunofixation (72%) compared with FISH-negative cases (40%). However, positive paraprotein data were a suboptimal predictor of FISH positivity among M–/F– cases since 28% (10/36) of informative results would have been missed. Our results suggest a means to optimize FISH utilization using same-sample morphology and FC results and CD138 enrichment status to predict whether FISH results

will be informative. For M/F+ cases, high rates of FISH positivity argue for proceeding with FISH testing and consistent use of CD138 enrichment. By contrast, cancellation of FISH testing should be considered for M–/F– cases for which CD138 enrichment cannot be performed since 100% of 171 cases in our series displayed negative/noninformative FISH results. Such an approach, if verified, could result in significant cost-containment for MM patients for whom serial bone marrow studies over many years are being performed more frequently.

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UMN Experience with Next Generation Sequencing (NGS) of Previously Test-Negative Ataxia Patients

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The hereditary ataxias (HAs) are a heterogeneous group of more than 50 genetic disorders characterized by progressive incoordination of gait, hand movement, speech production, swallowing, and extraocular muscle control. The most common HAs (Friedreich ataxia, SCA1-3, SCA6 &7, and fragile X tremor ataxia syndrome) are caused by trinucleotide repeat expansions and can be easily diagnosed using well-validated polymerase chain reaction (PCR) strategies. In patients with other forms of ataxia it is often difficult to arrive at a precise diagnosis due to the rarity of these individual diseases, the large number of ataxia genes, and large size of the genes. NGS allows comprehensive and cost-effective testing of the large number of genes involved in ataxia. We report our experience utilizing NGS to identify gene mutations in 10 patients evaluated in the University of Minnesota Ataxia Clinic in whom prior testing excluded the common trinucleotide repeat–mediated ataxias. Our panel included 20 well-characterized HA genes and a total of 238 neurologic disease genes, including those associated with spastic paraparesis, Parkinsonism, dementia, motor neuron disease, and mitochondrial disorders. We hypothesized that this broad-based approach would allow us to establish specific diagnoses in patients with previously negative testing. We utilized sequence capture (Sure Select Capture reagents, Agilent) to enrich for targeted genes on a HiSeq 2000 (Illumina). The data were analyzed using a modified Genome Analysis Toolkit (GATK). Synonymous variants and intronic variants that did not affect the canonical splice sites were filtered from the initial dataset. An average of 4 candidate variants was identified in each subject. These candidate variants showed very low allelic frequencies, were listed as damaging using in-silico analysis modeling, and/or were listed as pathogenic in mutational databases. NGS analysis identified causative mutations in approximately 25% of screen-negative ataxia patients. Our expanded NGS panel also identified multiple concurrent abnormal variants involving other genes known to be involved in other forms of neurodegeneration. Analysis to determine the significance of this latter finding in the setting of ataxia is ongoing. Our results strongly support the utility of NGS in identifying disease causing mutations in HA patients.

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Can Markers of Coagulation Identify Types of Hepatic Disease?

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Introduction: Different mechanisms of liver disease produce different patterns of liver enzyme alterations. As the majority of coagulation factors are produced or cleared by the liver, it is reasonable to hypothesize that coagulation parameters could also be useful to define the type of liver disease. Methods: We performed a retrospective study of 214 patients who had liver disease and had a cadre of coagulation markers performed between January and December of 2011. After review of patients' charts to identify the primary liver disease, we calculated the frequency of the normal and abnormal results for each type of disease. Results: We identified 76 cases with hepatitis C, 29 with alcoholic liver disease, 24 with cryptogenic cirrhosis, 22 with nonalcoholic steatohepatitis (NASH), 11 with autoimmune hepatitis, 19 with diseases that targeted bile ducts such as primary sclerosing cholangitis, 17 with acute liver injury mostly due to drug overdose, and 16 cases with other liver diseases. Platelets were most frequently abnormal in patients with hepatitis C and most frequently normal in those with acute liver injury. Prothrombin time (PT), partial thromboplastin time (PTT), fibrin monomers, prothrombin fragment 1.2 (PF1.2), and thrombin-antithrombin complexes (TAT) were most frequently abnormal in patients with acute liver injury. Fibrinogen was most frequently abnormal in patients with hepatitis C and most frequently normal in those with NASH. Antithrombin was most frequently abnormal in alcoholic liver disease and most frequently normal in bile duct obstruction. D-dimer was most frequently abnormal in bile duct obstruction and most frequently normal in autoimmune hepatitis. Fibrin monomers, PF1.2, and TAT were most likely to be normal in those with autoimmune hepatitis. AST and ALT were most frequently abnormal in patients with acute liver injury while alkaline phosphatase and GGT was most frequently abnormal in those with autoimmune hepatitis. Discussion: Two interesting trends were seen in this cohort of patients: Those with autoimmune hepatitis had frequent abnormal liver enzyme results while their coagulation markers (D-dimer, fibrin monomer, PF1.2, and TAT) were normal. In contrast, those patients with acute liver injury tended to have frequently abnormal liver enzymes and coagulation markers and the levels of these markers were usually higher than those seen in the other patients. These changes are most likely due to liver failure.

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Prothrombin Fragment 1.2 and Other Coagulation Markers in Patients With Hepatitis C

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Introduction: Liver diseases result in alterations in production or impaired clearance of a variety of analytes and coagulation markers. The aspartate aminotransferase (AST) to platelet ratio index (APRI) has been used as a hepatic fibrosis marker. The number of platelets is certainly altered in hepatic diseases as portal hypertension ensues; however, there are many other coagulation markers that could be potentially used to identify hepatic fibrosis and other consequences of hepatitis C such as hepatocarcinoma. Methods: We identified 77 patients with hepatitis C undergoing evaluation for a liver transplant from January-December of 2011. A variety of liver enzymes and hepatic derived analytes, along with a variety of coagulation markers, were collected for each patient. A chart review identified comorbidities such as cancer, thrombosis, bleeding, ascites, and varices. A Fisher exact test was used to identify significance of different correlations. Results: There were 23 patients with APRI <1.5 (2 of

these had an APRI <0.5) and 54 with an APRI >1.5. Six patients had cancer, 2 had thrombi, 4 had bleeding, and 5 had varices. In addition to the AST and platelets that constitute the basis for the APRI, we found that alterations of alanine aminotransferase, total bilirubin, mean corpuscular volume, prothrombin time, fibrinogen, and antithrombin were significantly associated with hepatic fibrosis score. Cancer was significantly associated with elevations in prothrombin fragment 1.2 levels. Discussion: Our data indicate that prothrombin fragment 1.2 can be used as a marker for carcinoma in patients with hepatitis C. The degree of abnormal prothrombin time, fibrin monomers, and antithrombin appears to correlate with the degree of fibrosis as calculated in the APRI using the AST and platelet levels; however, further studies correlating these coagulation markers with amount of fibrosis in biopsy specimens need to be conducted.

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Reducing the Rate of Blood Culture Contamination: The Success Experience from a Large Urban Hospital

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Objectives: Blood culture is one of the most important laboratory tests in detecting pathogens causing systemic infections and sepsis, and in directing subsequent management in patient care such as antibiotic usage. False-positive results due to contamination during blood culture collection can have far-reaching consequences including inappropriate antibiotic therapy, longer hospital stay, additional or unnecessary cultures and testing, an overall increase in workload on laboratory or hospital staff, and increase in health care costs to both the patient and the hospital. Previous College of American Pathologists (CAP) Q-probe studies have defined the baseline rate of blood culture contamination of 2.5% to 3.0%, which has become the standard threshold. The objective of this study is to see if using a specialized team, namely a phlebotomy team, for collection of blood culture samples can reduce contamination rates, especially in in-patient, intensive care, and emergency room settings. Methods: After implementing phlebotomy service in the inpatient setting, Grady Memorial Hospital instituted maximum if not full coverage of emergency room (ER) blood culture collection by phlebotomy teams in 2011 and maximum coverage of intensive care units (ICU) in 2012. The blood culture contamination rates are analyzed during the period from 2009 to 2012. Results: Prior to 2011, the overall blood culture contamination rate, calculated monthly, ranged from 3.1% to 4.6% during the period from 2009 to 2010. With maximum ER phlebotomy team coverage in 2011, the blood culture contamination rates decreased, from as high as 3.8% in March to as low as 1.9% in December, and were between 2.5% and 3.5% for most months in 2011. With maximum ICU phlebotomy team coverage in 2012, the blood culture contamination rate decreased further, from as high as 2.9% in January to as low as 1.7% in December, and was between 2.0% and 2.5% for most months in 2012, well below 3% of the threshold. Conclusions: These data support findings that utilization of specialized teams, such as phlebotomists, for blood culture collection in hospital areas such as the ER and ICU can help significantly reducing the blood culture contamination rates.

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Can the hCG Discriminatory Zone be Utilized Without Regard to a Specific hCG Assay?

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Background: Ectopic pregnancy is the leading cause of maternal death in the first trimester. Serum concentrations of human chorionic gonadotropin (hCG) in conjunction with transvaginal ultrasonography are used to evaluate a potential ectopic pregnancy. The hCG discriminatory zone is the hCG concentration at which an intrauterine pregnancy should be visualized by ultrasound and is frequently defined as 1,500 IU/L but may be as high as 3,500 IU/L. The objective of this study was to evaluate the lack of hCG assay harmonization and the effect it has on the interpretation of the hCG discriminatory zone. Methods: Deidentified, residual serum samples sent to ARUP Laboratories for hCG testing were selected based on hCG concentrations. Samples were prepared by pooling, if necessary, to create a set of 80 samples. By design, 25%, 50%, and 25% of samples were created to target hCG concentrations of <1,000, 1,000-4,000, and >4,000 IU/L, respectively. The hCG concentration of each sample was determined using the Abbott ARCHITECT Total β-hCG, Beckman DxI Total hCG, Roche E170 hCG+β, Siemens Centaur Total hCG, Siemens Dimension hCG, Siemens IMMULITE hCG, and Ortho 5600 β-hCG assays. The Roche method was selected as the comparative method. Results: Measured hCG concentrations across all assays ranged from 74 to 6,660 IU/L. Results were significantly different for 90% (19/21) of unique assay pairs ($P < .02$). When samples were selected based on Roche hCG concentrations between 1,500 and 3,500 IU/L (n = 43), results were significantly different for 81% (17/21) of pairs ($P < .05$). Deming regression analyses produced slopes of more than 1.0 (range 1.006-1.431) for all methods except for Siemens IMMULITE (0.911). Compared with Roche, the calculated hCG discriminatory zone for each method was within 10% of 1,500 and 3,500 IU/L for all methods except for the Siemens Centaur for which a range of 2,020 to 4,881 IU/L would be required for equivalence. Conclusions: Significant differences were observed across most hCG assays within the hCG discriminatory zone but were within 10% of the frequently used cutoffs. The use of an hCG discriminatory zone between 1,500 and 3,500 IU/L may be used without regard to a specific hCG assay with the exception of the Siemens Centaur method for which a higher cutoff may be required.

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Comparison of Glutamate Dehydrogenase (GDH), Toxin, and Real-Time PCR for *Clostridium difficile* Screening: Algorithm for Testing

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Objective: Infection control is an important part of patient care and hospital management especially when it comes to *Clostridium difficile* infection (CDI). Major contributor for the prevention and control of CDI is accurate and timely laboratory diagnosis of CDI. Many assays available for detection of CDI have variable sensitivities and specificities, assay times as well as the costs. The objective of this study is to find a stepwise diagnostic algorithm for diagnosis of CDI. Methods: Stool samples from 68 patients at Grady Memorial Hospital were used at the same time by 3 different methods: (1) the

C difficile Quik Chek Complete test by Techlab for rapid detection of both glutamate dehydrogenase (GDH) antigen (GDH-Complete) and *C difficile* toxin (toxin-Complete) at the same time; (2) the *C difficile* Tox A/B II enzyme immunoassay test by Wampole for detection of *C difficile* toxin by enzyme immunoassay (toxin-EIA); and (3) the GeneXpert real-time PCR by Cepheid (Xpert PCR). In addition, cultures for *C difficile* were performed. Results: The consensus for determining the diagnosis of CDI was reached by comparing results from all methods. Culture results were used to solve the discrepancy among 3 methods. Of 68 samples, 47 (69%) were confirmed negative by all the methods, and 21 (31%) were positive by at least one method. Of 21 positive samples, 17 (81%) were confirmed positive for CDI. Of 17 confirmed cases, 17 were positive by GDH-Complete, and 16 were positive by Xpert PCR; however, only 5 were positive by toxin-Complete and 9 positive by toxin-EIA. Conclusion: We found that toxin test by EIA or by Complete had low sensitivity, and the GDH-Complete and Xpert PCR had the high sensitivity. Due to high unconfirmed toxin-Complete results, GDH antigen detection with real-time PCR for confirmation could be a good algorithm for accurate and cost-effective testing for diagnosis of *C difficile* infection.

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Evaluation of Alternate Hemoglobin A1c Assays for Use in a Population with a High Prevalence of Hemoglobin Variants

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OBJECTIVES: The role of hemoglobin A1c (Hb A1c) in monitoring glucose control in patients with diabetes is well established. A variety of analytical methodologies are available for determining Hb A1c, including several immunoassays, boronate affinity chromatography, cation exchange liquid chromatography, and capillary electrophoresis. Grady Memorial Hospital (GMH) in Atlanta, GA, is one of the largest public hospitals in the United States, serving a predominantly inner-city, African American population known to possess a high prevalence of hemoglobin variants, especially S and C. The clinical laboratory at GMH transitioned to a Beckman Coulter AU chemistry analyzer in July 2011 and soon discovered several falsely elevated Hb A1c values (up to 40%) in patients with Hb S and Hb C traits, which appear to be due to an alteration in the epitope targeted by the antibody in that assay. To correct this problem, 3 alternate Hb A1c methods were evaluated: Trinity Biotech Premier Hb9210 (boronate affinity); BioRad D-10 Analyzer (cation exchange); and Sebia Capillarys 2 Flex system (capillary electrophoresis). METHODS: The precision of each instrument was evaluated using 2 levels of control material, tested daily on weekdays for 3 to 4 weeks. The comparison of methods study was performed between the AU immunoassay and each respective platform using samples from patients known not to harbor hemoglobin variants (n = 117). Additional samples from patients with hemoglobin variants were tested on each platform (n = 33). RESULTS: Based on the performance goals set by the authors, all 3 instruments were sufficiently precise. The comparison of methods study showed that in patients without hemoglobin variants, the BioRad D-10, displayed a relatively consistent high bias of 0.60% A1c compared with the AU immunoassay. In patients with hemoglobin variants, there was high concordance in values between the 3 test instruments, while the expected bias was observed in the immunoassay. CONCLUSIONS: At GMH, it is critical to have an A1c assay which is not affected by common Hb variants. Among the 3 instruments evaluated in this study, all appeared to provide nonbiased results in patients with hemoglobin variants. In patients without hemoglobin variants, the BioRad D-10 showed a positive bias. On the basis of the accuracy data and subjective ease-of-use as determined by the medical technologists, the Premier Hb9210 was selected for Hb A1c testing at GMH.

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Decision Support for Sign-out of a Multiplexed LC/MS Urine Opioid Assay Using a Machine Learning Approach

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Laboratory testing for commonly prescribed opioids is key to monitoring therapeutic compliance in patients who are on chronic pain therapy. Because the number of patients receiving chronic pain therapy is growing and immunoassays for these drugs lack specificity, examining and signing out mass spectrometric data is becoming an increasing burden. While determining whether a patient has tested positive for a drug most often requires a simple comparison of their sample concentration with a fixed threshold, some ambiguities such as the intersection of metabolic pathways of related drugs and the presence of process impurities make some cases challenging. The aim of this study is to explore whether applying machine learning techniques to urine opioid liquid chromatography mass spectrometry data could make the sign-out process more efficient. We analyze data from our LC/MS chronic pain screening panel of 20 opioids and metabolites plus their sign-out interpretations for over 400 patients using 2 supervised learning techniques: recursive partitioning and random forests. Recursive partitioning divides the total feature space of possible drug concentrations into defined areas such that samples that test positive for a drug are grouped together. Random forests uses a similar principle but randomly generates decision trees based on sampling a subset of the drugs tested and then chooses the mode of these decision trees to classify the data. Using freely available packages in the R software environment, we generate recursive partitioning algorithms using a training set of 80% of the data to classify samples in the remaining 20% of the data. After aggregating the data from 5 trials of this procedure, we demonstrate that recursive partitioning achieves classification accuracies of 99.3%, 99.8%, 99.5%, and 96.5% for oxycodone, methadone, morphine, and hydrocodone, respectively. Since most cases require only a simple comparison to determine positivity or negativity, recursive partitioning is well suited to classify a majority of the data. We repeat this procedure using random forests and demonstrate accuracies of 99.5%, 99.5%, 98.7%, and 96.7% for oxycodone, methadone, morphine, and hydrocodone, respectively. However, by using random forests, a probability of positivity can be provided rather than a simple classification. If thresholds of 5% to 95% probability of positivity of a drug are applied, only 25% of cases would require review, with perfect accuracy in classifying the remaining cases. These supervised learning techniques therefore represent promising techniques to select only subsets of drug testing data for manual review based on analysis of historical cases.

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Hyperfibrinolysis in a Patient With Amyloidosis

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Approximately one third of patients with amyloidosis experience hemorrhage. Due to the progressive nature of the disease, prompt recognition and therapeutic action are essential. While adsorption of

factor X is known to account for amyloidosis-associated hemorrhage, we describe a patient with a complex presentation that could not be explained by a singular factor deficiency. He was a 57-year-old man with no history of coagulopathy who presented with refractory bleeding from a dialysis fistula despite ligation and multiple transfusions of PRBCs and platelets. He had a diagnosis of nephrotic syndrome due to amyloidosis 18 months prior to presenting to our institution and had undergone an autologous stem cell transplant 8 months later. Unfortunately, his renal function continued to deteriorate and dialysis was initiated 4 months later. He also complained of fatigue, but had no bruises or ecchymoses. His initial laboratory workup showed anemia (hemoglobin, 8.9 g/dL), thrombocytopenia (77,000/mL), prothrombin time (PT) of 16.9 s (RR: 12.2-14.5 s), partial thromboplastin time (PTT) of 194 s (RR: 23-31 s), fibrinogen of 239 mg/dL, D-dimer of 956 ng/mL (RR:110-240 ng/mL), and normal immunoglobulins (IgG, IgA, IgM). Further workup revealed correction in the PT and PTT mixing studies and low factors IX (18%), X (41%), and XII (32%), and normal factor XI (82%). Since these factor deficiencies were not deemed severe enough to cause his symptoms, and in light of the "delayed" pattern of bleeding, an alpha-2 antiplasmin (A2-AP) and factor XIII level were checked. A2-AP was decreased at 34% and factor XIII was normal. In addition, since the degree of PTT prolongation could not be accounted for, we tested his prekallikrein and high-molecular-weight kininogen activities. Both were low at 3% and 23%, respectively. With these results, we concluded that his bleeding was due to a combination of multiple factor deficiencies and hyperfibrinolysis. Three months later, he required peritoneal dialysis catheter placement and a multidisciplinary team recommended epsilon-aminocaproic acid (EACA), prothrombin complex concentrate, and plasma for the procedure. Factors IX and X increased, and while A2-AP remained low post-transfusion, there was no bleeding intra- or postoperatively. At home, when the EACA dose was decreased due to dizziness and nausea, he immediately experienced bleeding from the catheter site. While clinicians are familiar with the pathogenesis of amyloidosis-induced coagulopathy due to factor X adsorption, this patient exemplifies that bleeding is multifactorial and includes hyperfibrinolysis. Clinical Pathology consultation is critical in the evaluation and management of these patients.

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A Rational Approach to Karyotyping Utilization for Multiple Myeloma

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Karyotyping analysis for multiple myeloma (MM) provides important prognostic information that may influence choice of therapy but is associated with significant laboratory costs. We performed a retrospective analysis to better understand the patterns of utilization of karyotyping and to determine if test employment is optimal at our institution. We retrieved 863 cases (630 patients) from May 2011 to June 2012 searching for a clinical history of plasma cell neoplasm-related terms, without regard to initial or follow-up status. Relevant clinical information was obtained and test results were categorized as follows: (1) aggregate morphology and flow cytometry (FC) data, (2) karyotype, and (3) concurrent paraprotein data (paraprotein level, free light chain, kappa/lambda ratio and IFE were assigned 1 point for each abnormal parameter). 789 cases had karyotypes for which only 86 (10.9%) were abnormal. We found that cases that displayed either morphology or FC positivity (M/F+, n = 386) had an increased rate of abnormal karyotypes in 72 cases (18.6%). By contrast, cases negative for both morphology and FC (M–/F–, n = 403) showed merely 14 cases (3.5%) with abnormal karyotypes. The 14 cases from 11 patients included only 2 with recognized MM-associated abnormalities (hyperdiploidy); the remainder showed likely constitutional variants (eg, inv9), age-related (eg, –Y) and changes of uncertain clinical significance/possible myeloid lineage abnormalities (eg, del 20q). Among these 403 M–/F– cases, 384 had complete paraprotein data which included 13 of 14 with abnormal karyotypes. Using a cutoff of at least 3 abnormal paraprotein parameters isolated 99 cases for which 7 (7.6%) showed abnormal karyotypes that included the 2 with recognized MM-associated abnormalities. Karyotyping is an expensive and time-consuming test for which a small subset of MM biopsy samples overall shows abnormalities. Morphology, FC, and concurrent paraprotein data (results usually available before cytogenetics) can serve as useful predictors of whether cytogenetics studies will yield an abnormal karyotype. Had we limited karyotyping to cases with M/F+ or M–/F– with at least 3 to 4 abnormal paraprotein parameters (n = 485), we would have identified 79 abnormal karyotypes (16.3%) and not performed 304 studies including 297 with normal karyotypes while missing only 7 abnormal karyotypes (2.3%) of uncertain clinical significance. If validated, such an approach could yield significant cost savings to MM patient care.

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Detection of the Antigen Excess Phenomenon in the Serum Free Light Chain Assay by Kinetic Curve Analysis: A Single Institution's Experience

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We report the incorporation of analysis of the kinetic curve of the antibody-serum free light chain (FLC) agglutination reaction, using a Siemens BNII nephelometer with The Binding Site FLC assay, as an important routine step to detect antigen excess and avoid the misreporting of results. The quantitative serum FLC assay has emerged as a critical diagnostic, monitoring, and prognostic tool for the care of patients with monoclonal plasma cell disorders, complementing and, in some contexts, potentially displacing traditional protein electrophoresis and immunofixation. The serum FLC assay is a nephelometric/turbidimetric assay that measures the binding of free kappa and lambda light chain epitopes by latex-conjugated polyclonal antibodies. An uncommon but potentially devastating shortcoming of the serum FLC assay can occur with the gross underestimation of serum FLC values when a sample concentration is extremely high, due to antigen excess. Laboratory methods to address the problem include monitoring delta changes and performing serial dilutions. To validate testing of high free light chain values, five kappa and five lambda specimens with concentrations of 1,000 mg/dL or greater were purchased and evaluated on a Siemens BNII instrument using the FLC assay from The Binding Site. For 9 specimens, analysis beginning at the manufacturer-recommended initial dilution of 1:100 triggered appropriate further dilutions which identified light chain concentrations in the anticipated range. However, for 1 kappa specimen, 10.5 mg/dL was resulted with the 1:100 dilution without the triggering of additional dilutions. With the knowledge of an aberrant result, additional titrations were carried out and, with a 1:2,000 dilution, a result of 1,220.0 mg/dL was resulted. Evaluation of the kinetic curve for the 1:100 measurement revealed an inversion. To exclude the possibility of a falsely low value due to antigen excess, we now analyze the curves for abnormalities in reaction kinetics, including curve inversion, sigmoid curves, and late curve

spikes as part of our standard procedure. Surveillance conducted over a 3-month period on over 250 assays incorporating kinetic curve evaluation revealed no additional cases of protein electrophoresis-serum FLC discrepancies. These findings suggest that routine evaluation of kinetic curves, in addition to other standard and locally implemented quality control and assurance procedures, constitutes an important mechanism for the detection of aberrantly low serum FLC results secondary to antigen excess.

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Urine Protein Electrophoresis and Immunofixation Electrophoresis in Plasma Cell Dyscrasia Workup: Utility of Evaluation of Specimens with Low Protein Concentration

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The value of urine evaluation as part of the screening for workup of a monoclonal gammopathy has been challenged by some investigators. Nonetheless, clinicians from various services (hematology-oncology, nephrology, cardiology, and neurology) continue to send urine specimens for evaluation. The University of Colorado Hospital Laboratory policy regarding urine workup had required (1) above 8 mg/dL of protein to run a UPEP and (2) a band on the UPEP gel to evaluate by IFE. The 2011 consensus recommendations from the International Myeloma Workshop Consensus Panel 3 (IMWCP3) (*Blood.* 2011;117:4701) continues to recommend urine protein electrophoresis (UPEP) and immunofixation electrophoresis (IFE) as a routine component of screening for and workup of a suspected or established plasma cell dyscrasia patient, regardless of the protein concentration of the specimen. In this study we sought to both evaluate the utility of the IMWCP3 guidelines for urine evaluation and assess the validity of employing a defined protein cutoff within our existing policy. During a 12-month period we ran UPEP and IFE evaluation on all urine specimens, random and timed, dividing specimens into low protein (8 mg/dL or less) or high protein (9 mg/dL or more) groups. 172 of 503 specimens were identified as "low protein." Of the 172 samples, 31 were interpreted as having a positive band on IFE; in most such cases, a band was not discernible on the UPEP gel. These data support the complete evaluation of urine as a component in the evaluation for a monoclonal protein, regardless of the protein concentration in the specimen. Based on the findings of this study, 18% of specimens with monoclonal bands on IFE would have been missed under the previous policy. Using these data, and following the guidelines of the IMWCP3, we now (1) run UPEP evaluation on all urine samples sent to the laboratory, regardless of total protein concentration or previous UPEP/IFE results, and (2) perform IFE evaluation on specimens from patients being evaluated for the first time as well as on specimens deemed necessary after review of current and previous UPEP in the context of serum protein electrophoresis and serum kappa and lambda free light chain data.

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Improved Presentation of Molecular Assay Results Using Self-contained Interactive HTML/JavaScript Documents

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Objectives: Interpretation of molecular assays frequently requires the analysis of charted information, such as electropherograms. Two examples are clonality assays to detect immunoglobulin heavy chain (IGH) and T-cell receptor gamma (TCRG) rearrangements. At our institution, diagnostic review occurs outside of the molecular laboratory using hard-copy charts generated by a molecular technologist using proprietary software that is licensed with the instrument. We explore the possibility of replacing the printed IGH and TCRG clonality assay charts with self-contained interactive HTML/JavaScript documents. Methods: IGH and TCRG clonality assay capillary electrophoresis fragment analysis files for polyclonal controls generated by our Applied Biosystems (ABI) 3130XL genetic analyzer are converted from their native binary ABIF format to an eXtensible Markup Language (XML) document using a custom Java program. This program also detects the peaks in the size standard data channel and assigns a fragment length to every event. The XML document is converted to an HTML/JavaScript document via an eXtensible Stylesheet Language (XSL) transformation. The resulting HTML/JavaScript document has no external data dependencies and extensively leverages the Google Charts JavaScript API for presentation. Results: The interactive HTML/JavaScript document corresponding to the polyclonal controls is generated almost instantly from the 8 data files generated by the ABI 3130XL. The final document is 3 MB and renders in approximately 5 seconds in a Google Chrome web browser on our typical departmental PC. The charts are annotated with the control ranges for each primer set, may be zoomed to a particular primer set's control range, and display the data point coordinates when the mouse cursor is hovered over a point on the curve. Conclusions: It is possible to create self-contained interactive HTML/JavaScript documents by directly processing the binary IGH and TCRG clonality assay data files generated by the ABI 3130XL genetic analyzer. This process eliminates the need for the technologist to print hard-copy documents using proprietary software and provides an electronic annotated representation of the electropherograms that is self-contained, interactive, and may be rendered in most modern web browsers.

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Antiphospholipid Antibodies: Diagnostic Yield and Ordering Pattern

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Antiphospholipid syndrome (APS) is a clinicopathologic diagnosis based on laboratory findings of lupus anticoagulant (LA) and/or antiphospholipid antibodies (aPLs) in a setting of thrombotic complications. The laboratory diagnosis of APS is challenging, requiring multiple tests and repeat studies in the case of positive results. Objective: In a single institution study, we conducted an audit of ordering practices to assess compliance with the current diagnostic guidelines with an aim to develop a standardized panel of tests to optimize the investigation of suspected APS. Methods: A retrospective review of aPLs performed on 3,327 patients in various clinical settings as part of the routine workup for hypercoagulable states from October 2010 to May 2012 was completed. We investigated the rate of positivity of aPLs and LA. The cutoff for positive titers level of anticardiolipin antibodies (aCLs) and anti-β2 glycoprotein I (GPI) is >40 GPL or MPL. The positive cutoff dRVVT ratio (clot time without phospholipids excess)/(clot time with phospholipids excess) was ≥1.2. The criteria for laboratory findings supportive of a diagnosis of APS required 2 positive tests at least 12 weeks apart. Results: Of the

3,327 patients, IgG anti-β2 GPI, IgM anti-β2 GPI, IgA aCL, IgG aCL, IgM aCL, dRVVT, and hexagonal phosphate assays were performed on 1,516, 1,516, 2,722, 3,135, 3,135, 2,542, and 2,250 patients, respectively. Of 3,327, 402 (12%) of patients had at least 1 positive result. Examination of the ordering patterns showed that 330 (82%) with at least 1 positive result did not have repeat testing performed at our institution after 12 weeks. Of the remaining 72 patients, 36 (50%) had evidence of APS as demonstrated by 2 positive tests at least 12 weeks apart. Of the 36 patients, 19 (52%) were positive by serology, 8 (22%) patients were positive by LA, and 9 were positive for both LA and aPLs (25%). While not part of the diagnostic criteria, IgA aCLs were found in 4/36 (11.1%) patients. All of the patients with positive IgA aCL were also positive for dRVVT and IgG and/or IgM aCL. We found no patients in whom IgA aCL was the sole positive aPL. Conclusions: Few patients were adequately evaluated for APS with an appropriate panel that included assays consisting of LA and aPL antibodies along with confirmatory tests after 12 weeks. This likely leads to underdiagnosis of APS in approximately 50% of patients, who were positive on 2 occasions, but were not separated by 12 weeks. Despite frequent ordering, IgA aCL does not improve the rate of detection of APS and should not be ordered as a primary screening test. The inconsistency of suspected APS workups and low compliance with testing guidelines demonstrates a need for laboratory standardization to assist in the investigation of APS.

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Effects of *Nigella sativa* (Black Seed) and Honey on Patients With Normal and Abnormal Liver Enzymes

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Background and Significance: *Nigella sativa* or black seed, thymoquinone rich seed (not to be confused with black sesame or black cumin), and honeys are natural foods and are believed to play antioxidation roles in human optimum health. The purpose of this study was to establish whether these 2 natural substances play any significant role in the protection of liver in health and disease states against toxicity and fibrosis. Methods: This work was done in Sana'a, Yemen, at the National Center of Public Health Laboratories (NCPHL). Adult volunteers (n = 32) aged 18 to 55 years old were recruited and divided into 2 main groups: (1) 15 (47%) control group with liver enzymes within reference ranges, and (2) 17 (53%) patient group with elevated liver enzymes. Results: In the control group, 2 months of administration of black seeds and honey (2 g/day) resulted in a significant decrease in the levels of serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) ($P < .05$). However, in patients with liver disease, a significant decrease in all elevated liver enzymes levels was observed (AST/ALT; $P < .0001$) and (ALP/GGT; $P < 0.01$). Conclusion: This study shows that concomitant use of honey and black seeds in humans induces a powerful nutritional regimen that can be used effectively to decrease all elevated liver enzymes in patients with liver disease.

Table
Comparing effects of HN Mixture on Serum Liver Enzyme Levels in Control and Patient Groups Before and After 2 Months of Administration

Serum Liver Enzymes Level	Control Subjects (n = 15)			Patients (n = 17)		
	M0	M1	*P* Value	M0	M1	*P* Value
AST, U/L	24.44	22.11	.050*	61.58	42.83	<.0001***
ALT, U/L	20.67	16.56	.009*	105.67	66.58	<.0001***
ALP, U/L	95.11	95.78	.862	102.50	90.83	.013*
GGT, U/L14.78	15.22	.482	108.08	80.25	.003*	

*Level of significance, M0 = before administration, M1 = after administration.