# abstracts of papers

## Young Investigator Awards Program Abstracts

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

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

The following abstracts were presented at the 44th Annual Meeting of the Academy of Clinical Laboratory Physicians and Scientists, June 4-6, 2009, in Redondo Beach, CA. Authors receiving a 2009 Young Investigator Award are marked with an asterisk (*).

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### 1

**Application of the Gen-Probe–Amplified Direct Test for Detection of *Mycobacterium tuberculosis* Complex in Paraffin-Embedded Tissue**

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The Amplified Mycobacterium Tuberculosis Direct Test (MTD; GEN-PROBE, San Diego, CA) amplifies rRNA from specimens containing *Mycobacterium tuberculosis* complex (MTBC) by transcription-mediated amplification and is approved for diagnostic use with acid-fast bacilli (AFB) smear-positive and smear-negative concentrated sediments prepared from sputum and bronchial and tracheal aspirate specimens. The efficacy of this test using formalin fixed, paraffin-embedded tissue specimens prepared in anatomic pathology has not been established. The goal of this study was to evaluate the diagnostic performance of the MTD test on deparaffinized tissue specimens.

From 1997 to 2007, 50 surgical pathology tissue or fluid specimens from various anatomic sites were selected from patients who had positive mycobacterial cultures for MTBC (n = 25) or *Mycobacterium avium* complex (MAC) (n = 25). One paraffin-embedded tissue block was selected from each case and five 10-µm-thick sections were shaved for MTD testing. Nucleic acids were extracted from these specimens using the EZ1 DNA Tissue Kit (QIAGEN, Valencia, CA) on the BioRobot (QIAGEN). The MTD assay was performed following the manufacturer's protocol with slight modifications.

Of the microbiology specimens that were MTBC culture–positive, 21 of 25 were MTD-positive and 4 were MTD false-negatives but came from unapproved specimen types. By comparison, the deparaffinized samples were MTD-positive in 4 of 25 MTBC culture positives and 0 of 25 MAC culture positives. The overall sensitivity and specificity values of the MTD test from deparaffinized tissue were 16% and 100%, respectively, yielding positive and negative predictive values of 100% and 54%. All MTD-positive tissue specimens were also AFB-positive, and 10% of the cases (1 MTBC and 4 MAC) had discordant samples sent for culture and surgical pathology.

This study indicates that the MTD assay should be utilized with fresh, unfixed specimens submitted to microbiology for culture and not routinely from paraffin-embedded, formalin-fixed tissue submitted for histological analysis. In order to be a useful adjunct test for fixed tissue analysis, the overall procedure must be standardized and modified to increase assay sensitivity for AFB-positive histological specimens.

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### 2

**Diagnostic Usefulness of EUS-FNAB and Flow Cytometry in the Diagnosis of a Deep-Seated Lymphoproliferative Process**

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The evaluation of deep-seated lymphadenopathy can be technically challenging. Recently, the introduction of endoscopic ultrasound–guided fine-needle aspiration biopsy (EUS-FNAB) has allowed the sampling of deep tissues, including deep-seated lymph nodes. EUS-FNAB has been demonstrated to be a highly sensitive and specific tool in the diagnosis of metastatic epithelial neoplasms in deep-seated lymph nodes. The aim of this study is to explore the use of EUS-FNAB in the diagnosis of both deep-seated lymphoma and reactive conditions with an emphasis on the number of passes required to confirm the diagnosis by flow cytometry.

A retrospective search was conducted at our tertiary care center on all EUS-FNAB specimens obtained from deep-seated lymph nodes. All specimens from 2001 to 2006 with a diagnosis of lymphoma, reactive lymph nodes, or metastatic lesions to lymph nodes submitted for flow cytometry were reviewed. Of 1,200 EUS-FNAB

specimens identified, 71 were lymph node aspirates with flow cytometry data. Papanicolaou stains, Diff-Quik stains, cell block, and immunohistochemical stains, when available, were evaluated independently by 2 pathologists. Flow cytometry data, including the number of passes and viable cells, were also evaluated.

After 2 to 3 passes, the total yield of viable cells per case diagnosed by flow cytometry ranged from $7.8 \times 10^{-2}$ million to $3.11 \times 10^{2}$ million (mean, 9.19 million). Five cases submitted for flow cytometry yielded a quantity of cells insufficient for diagnosis. While 21 patients (11 men and 10 women aged 44 to 91 years; mean, 67.8 years) were ultimately diagnosed with lymphoma, 12 patients had a reactive process, 13 were diagnosed with metastatic lesions, and 25 with a benign process. Flow cytometry diagnoses were available for 17 of the patients with lymphoma. The diagnosis of lymphoma was supported by surgical biopsy or excision in 8 cases and by immunohistochemical staining in 8 cases. Flow cytometry combined with FNAB, surgical follow-up, and/or immunohistochemistry supported the diagnosis of lymphoma in all 21 cases.

Combined use of EUS-FNAB and flow cytometry is a powerful tool for the diagnosis of a deep-seated lymphoproliferative process. Molecular studies on EUS-FNAB specimens will aid in the diagnosis and detection of minimal residual disease.

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## 4

### Evaluation of Parafibromin in Urothelial Carcinoma

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Parafibromin, the product of the *HRPT2* tumor suppressor gene, is believed to suppress tumor formation by $G_1$ phase arrest and by inducing apoptosis. Absence of parafibromin is associated with carcinomas of parathyroid, kidney, stomach, and breast. Parafibromin was detected in the normal urothelium previously. To evaluate the parafibromin staining in urothelial carcinomas, 30 urothelial specimens were selected and divided into control and cancer groups. The control group included 5 benign urothelium specimens. The cancer group included 25 urothelial carcinoma cases, 8 in the renal pelvis, 5 in the ureter, and 13 in the urinary bladder. Of 30 cases, 25 were in men. Age at time of biopsy or resection ranged between 47 and 91 years (mean ± SD, 69.89 ± 13.76). The histological grade of the renal pelvis carcinomas was intermediate to high, and that of ureteral carcinoma was low to intermediate. The bladder carcinomas were composed of both low- and high-grade tumors. Immunohistochemical staining for parafibromin was performed using SC-33638 (Santa Cruz, CA). The number of positive nuclei was assessed using a semiquantitative grading system: 1+, fewer than 25% of cells; 2+, 25% to 50%; 3+, 50% to 75%; and 4+, more than 75% staining. Intensity of staining was evaluated as weak, intermediate, or strong. The result of immunohistochemical staining was compared with histology. There was no meaningful difference in the age and sex of the control and cancer groups. The staining pattern was uniformly present and strong in the nuclei of the normal and benign urothelial lesions. In the cancer group, the staining was weaker in the renal pelvis and ureter subgroups, showing fewer positive cells (grade 1+) with weak staining. The stain showed a polar distribution in the renal pelvis, appearing stronger in the basal layer. A weaker staining pattern was more prominent if any or all of the following findings were present: high mitotic rate (>21/10 HPF), invasion, and lymphovascular permeation. In bladder carcinoma, the stain was strong and diffuse irrespective of the morphological milestones. One case with metastatic urothelial carcinoma to the lung showed strong and diffuse nuclear staining with parafibromin. In conclusion, there appears to be an association between the histological grade of urothelial carcinoma of the renal pelvis and ureter with staining grade and intensity. The less differentiated carcinomas of the renal pelvis showed less staining in this limited study. This pilot study shows that parafibromin is not promising in the differentiation of invasive and noninvasive urothelial carcinomas. The value of the stain in prediction of tumor prognosis, however, needs further elucidation.

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## 6

### Dye Cycle Violet Exclusion Defines an HSC Candidate Population From the Human Bone Marrow

*Mikhail Roshal* and Brent Wood. Department of Laboratory Medicine, University of Washington, Seattle.*

Hematopoietic stem cells (HSCs) represent a rare self-renewing population that is capable of repopulating all hematopoietic lineages. Flow cytometric isolation of murine HSCs relies on immunophenotypic markers associated with immaturity and lack of lineage commitment and the ability of these cells to efflux the DNA-binding UV excitable dye (Hoescht 33342). While side population has been shown to be present in the human bone marrow in similar proportions, its function and immunophenotype remain controversial owing to a combination of lack of good biologic assays for human stem cells, limited access to large numbers of human bone marrow samples, and limited availability of flow cytometers and sorters equipped with expensive UV laser sources capable of high-level multicolor analysis. In a recent study, Tedford et al used a violet laser excitable dye (Dye Cycle Violet [DCV]) that appeared to produce an immunophenotypically similar side population in mice and showed a side population in the normal human cord blood that was not further immunophenotypically defined. We explored the applicability of DCV for stem cell isolation from the human bone marrow. Using a combination of CD38 A594, CD34 APC, and CD45 APC-CY7 on a BD-LSR-II flow cytometer equipped with a 408-nm violet laser and excluding 7-AAD positive (dead) and anuclear events in 15 consecutive bone marrow samples, we demonstrate that the normal human bone marrow side population obtained using DCV is highly enriched for the most phenotypically immature (CD34+/CD38 low to negative) small blasts that likely represent the earliest identifiable hematopoietic precursors. Moreover, the highest dye efflux activity correlated with the least CD38 expression, suggesting that most immature cells showed the highest dye efflux activity. The DCV efflux was completely blocked by ABC pump inhibitor fumetrimorgin C. Unfortunately, the dye has complex interactions with blue laser–excitable fluorophores, producing high backgrounds in FITC and PE channels and a suppression of signal for tandem PE-CY7 and PE-CY5 fluorophores thus limiting the use of these channels to extremely brightly expressed antigens or viability dyes. Red and yellow lasers showed no background or interference from DCV. In summary, we demonstrate the feasibility of isolation of very immature DCV-defined side population HSC candidates from the normal human bone marrow using a common violet laser.

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## 8

### *KRAS* Mutational Analysis by a Rapid LightCycler Assay

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Activating mutations in codons 12 and 13 of the *KRAS* oncogene confer complete resistance to monoclonal antibody therapy targeted at the epidermal growth factor receptor (EGFR) in patients with metastatic colorectal cancer. Evidence of wild-type *KRAS* status in colon cancer tissue is increasingly required by insurance companies for reimbursement of the approximately $10,000 per month cost of anti-EGFR treatment. Many laboratories now perform *KRAS* mutation assays, but the methods often require expensive instrumentation and reagents (eg, pyrosequencing, fragment analysis, Scorpions PCR). We report a sensitive, rapid, and cost-effective strategy to detect all clinically significant mutations in *KRAS* codons 12 and 13 using the Roche LightCycler instrument (adapted from Nikiforova et al. *J Clin Endocrinol Metab.* 2003;88:2318-2326). We used formalin-fixed, paraffin-embedded cancer tissue macrodissected from glass slides to assure minimal nontumor DNA contamination. The primary assay detects mutations via PCR–melting curve analysis using a Cy5-labeled sensor probe that straddles codons 12 and 13. Multiple mixing studies using mutation-discordant pairs of patient specimens demonstrated that the melting curve assay can reliably detect down to 10% mutant DNA in a wild-type background. Validation studies on 28 tumor specimens, 13 mutant and 15 wild type, showed 100% concordance with outside laboratories and were further confirmed by direct DNA sequencing. In anticipation of cases that might be equivocal by melting curve analysis and for use in internal quality control, we developed a LightCycler allele-specific PCR reflex assay to specifically identify the 7 most common activating KRAS mutations (G12D, G12V, G12C, G12S, G12A, G12R, and G13D, representing >98% of patients). The reflex assay correctly identified the mutations determined by DNA sequencing in all samples tested, including the samples with the lowest mutant DNA signal. In summary, we have developed and validated a rapid, inexpensive, and robust clinical assay for *KRAS* mutational analysis that is ideal for laboratories with a Roche LightCycler or similar instrument.

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## 9

### Evaluation of Multimethod Vancomycin MIC Determination in Methicillin-Resistant and Methicillin-Susceptible *Staphylococcus aureus* Recovered From Clinical Specimens

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Recently, the Clinical and Laboratory Standards Institute has recommended that a minimum inhibitory concentration (MIC) method should be used to determine vancomycin susceptibility for *Staphylococcus aureus* based on the rationale that Kirby-Bauer disk diffusion analysis is not adequate to determine vancomycin susceptibility for this organism. In addition, some clinicians believe that a vancomycin MIC of 2 is a contraindication for the use of vancomycin for the treatment of *S aureus* infections and/or predicts for vancomycin heteroresistance, even though these organisms are considered to be susceptible at this MIC. The purposes of this study were to evaluate the concordance of vancomycin MIC values between different laboratory methods and to validate the use of Kirby-Bauer disk diffusion for *S aureus* vancomycin susceptibility testing.

Forty-one *S aureus* (including 21 MRSA and 20 MSSA) clinical isolates were selected. Vancomycin susceptibility testing was performed using Kirby-Bauer disk diffusion with BIOMIC V3 interpretation (Giles Scientific), Etest (bioMerieux), the VITEK2 GP67 AST card (bioMerieux), Sensititre GPALL microbroth dilution (Trek Diagnostics), and the Microscan Pos MIC Panel Type 26 (Siemens). All testing was done in accordance with the Clinical and Laboratory Standards Institute M100 document and the manufacturers' recommendations.

The *S aureus* actual MIC values varied from 0.44 or less to 3. All MIC values were rounded up to the next standard doubling dilution. All methods showed all isolates to be vancomycin susceptible (MIC ≤2) except the Etest, which identified 2 isolates as vancomycin-intermediate with a MIC of 4. Essential agreement (same class: resistant, intermediate, susceptible, and within a factor of one doubling dilution) demonstrated that the Kirby-Bauer disk diffusion method had 100% agreement (41/41) with the Sensititre and Microscan microbroth dilution methods. Essential agreement was 90% with the Etest and 85% with the VITEK2 GP67 card.

Based on these results, we believe Kirby-Bauer disk diffusion with BIOMIC V3 interpretation is a valid method for evaluating vancomycin MIC. While essential agreement is high, the intermethod variability in vancomycin MIC evaluation suggests that a MIC of 2 is neither a valid indicator of vancomycin heteroresistance nor a predictor for treatment failure.

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## 11

### Oleander Extract and Pure Oleandrin-Induced Apoptosis of Neuroblastoma Cells

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Oleander extract, used in various herbal preparations, has a potential to inhibit various kinases, transcription factors, and inflammatory mediators. There has also been some evidence supporting that oleander has anticancer properties. However, the effect of oleander extract or its active component oleandrin on the neuroblastoma cell line has never been reported before. Commercially available pure oleandrin was dissolved in ethanol (1 mg/mL) to prepare the standard solution. Oleander leaf was collected from a local nursery, and an initial oleander extract was prepared by dissolving 5 g of the leaf in 10 mL of ethanol. Then, 10 µL of the extract was added to 1 mL of drug-free serum, and the apparent digoxin concentration was measured by using fluorescence polarization immunoassays (TDX/FLX analyzer, Abbott Laboratories, Abbott Park, IL). A mathematical formula was used to calculate the oleandrin concentration of the extract. (We previously showed that oleandrin is the major digoxin-like immunoreactive component of the oleander extract.) Then, the oleander extract was further diluted with ethanol to achieve the oleandrin concentration of the extract as 1 mg/mL. We obtained purified neuroblastoma cells and incubated them with 5 different concentrations of oleandrin (0.1, 0.5, 1.0, 5.0, and 10.0 µg/mL) and also separately with oleander extract containing similar amounts of oleandrin. For controls, cells were incubated with media only. Cell survival was subsequently assessed at 24, 48, and 72 hours by trypan blue cell survival assay and by visual light microscopy and electron microscopy. Oleander extract and pure oleandrin were effective in killing neuroblastoma cells. For example, treatment of neuroblastoma cells with oleandrin at concentrations of 10 and 5 µg/mL for 24 hours showed 20% and 50% of cells, respectively, extruding trypan blue. However, after 72 hours of exposure to oleandrin at 10 and 5 µg/mL, none of the cells extruded trypan blue. In contrast with the pure compound, exposure of cells to oleander extract containing 10 µg/mL of oleandrin for 24 hours resulted in 91% of cells extruding trypan blue, and after 48 hours, 70% of cells were extruding the dye. These findings show

that neuroblastoma cell survival decreases with exposure to oleander extract and oleandrin, but pure oleandrin is significantly more cytotoxic than oleander extract. It is possible that other ingredients in the oleander extract partly neutralize the cytotoxic effect of the oleandrin. Apoptosis was confirmed by electron microscopy, and apoptosis proteins were identified by the Western blot.

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## 12

**Implementing a Massive Transfusion Protocol: Turning Ideas Into Reality**

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This project is intended to describe our experience in implementing a protocol for massive transfusion of blood products for trauma patients from literature search to preliminary results. In October 2008, a meeting with blood bank and trauma surgery staff resulted in discussing the utility of creating a massive transfusion protocol (MTP) for trauma patients. After researching existing MTPs, the decision was made to use 6 U of RBCs, 6 U of FFP, and 1 U of apheresis platelets, as this ratio approximates whole blood. We then defined specific situations in which an MTP could be initiated. The criteria for considering the use of the MTP were defined as patients requiring greater than 6 U of RBCs in 1 hour whether they were trauma, obstetric, or medical intensive care patients. For the protocol to be activated, a form was created and approved by the hospital forms committee and distributed to the trauma bay, main operating rooms, obstetric operating rooms, and the intensive care units. Although the protocol could be activated by telephone, these order sheets also include orders for CBC, prothrombin time (PT), activated partial thromboplastin time (PTT), fibrinogen level, and arterial blood gases. Once ready for implementation, we educated and trained clinicians, blood bank technologists, and pathology residents on how to recognize the need for a massive transfusion and promptly initiate and run the MTP. All involved were solicited for feedback, and the MTP has evolved from its initial inception to now include formulas for transfusion of cryoprecipitate using fibrinogen values, the administration of factor VII, and more criteria for the initiation and completion of the MTP request. Coagulation studies and CBCs were done for patients who received more than 6 U of RBCs (including massive transfusion recipients) from March 2008 to December 2008. Patients were divided into 3 risk-stratification groups based on how many total units of blood product they received in a 24-hour period. When average end point laboratory values (at up to 24 hours after transfusion) were compared between patients who received products as part of the MTP and patients who did not, MTP patients had better end point laboratory values in at least 1 of the risk-stratification groups for all 6 of the laboratory values assessed (hemoglobin, hematocrit, platelets, fibrinogen, PT, and PTT), despite having higher acuity and mortality. These preliminary results are encouraging for linking the MTP with improved outcome for patients requiring massive transfusion.

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## 13

**Antibacterial Effects of Tea Tree Oil and Cinnamon Oil Against Methicillin-Resistant *Staphylococcus aureus* and Synergistic Effects of These Essential Oils With Topical Antibiotics Bacitracin, Mupirocin, and Polymyxin**

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Tea tree oil has broad-spectrum antiviral, antibacterial, and antifungal activities, but the potential antibacterial activity of cinnamon oil is unknown. In this study, we examined the antibacterial properties of tea tree oil, cinnamon oil, terpineol, and cinnamic acid (active ingredients of these essential oils) against MRSA (methicillin-resistant *Staphylococcus aureus*). We also studied potential synergistic effects of these essential oils with the topical antibiotics bacitracin, mupirocin, and polymyxin as a possible conjugate to currently used therapy for skin infections. The presence of terpineol in tea tree oil and cinnamic acid in cinnamon oil were confirmed by thin-layer chromatography. In order to study the antibacterial properties of these essential oils and pure compounds, Mueller-Hinton agar was mixed with various concentrations of each of the pure essential oils or the active ingredients in these oils. For the pure essential oils, concentrations in each plate ranged from 0.1% to 6%. The concentrations of the 2 active components were 500 ng/mL, 1 µg/mL, 2 µg/mL, 3 µg/mL, and 4 µg/mL. The plates were allowed to set overnight and inoculated the next day with an MRSA clinical isolate positive for Panton Valentine Leukocidin toxin. Some of the plates at the various concentrations also had Etest strips containing bacitracin, mupirocin, or polymyxin. Mueller-Hinton agar with each of these compounds was used to obtain colony counts. The plates with the pure compounds terpineol and cinnamic acid demonstrated significant decreases in colony-forming units with increasing concentration of these compounds, but no growth was observed when plates were incubated with tea tree oil or cinnamon oil at a 1% concentration or more, indicating that in addition to cinnamic acid and terpineol, various other components of these essential oils have significant antibacterial properties. Cinnamic acid and terpineol dramatically reduced MICs of topical antibiotic studied as determined by placing Etest strips on the plate containing different concentrations of these compounds. For example, the MIC for terpineol (500 ng/mL) with polymyxin was only 8 µg/mL, while the MIC of pure polymyxin was 256 µg/mL. These results demonstrate that tea tree oil and cinnamon oil have strong antibacterial properties. In addition, there is a substantial increase in the efficacy of the antibiotics when used in conjunction with tea tree oil, cinnamon oil, terpineol, and cinnamic acid.

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## 15

**Utilization of an Electronic Alerting System for Critical and Corrected Laboratory Results**

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Laboratory communication with ordering physicians is important for critical values and significant changes made to laboratory results. Currently, this communication is conducted mainly by telephone calls or as a comment on the laboratory report. With the electronic medical record (EMR), electronic alerting systems are available. The aim of this study was to determine if the creation of an electronic alert (e-alert) in our EMR for critical values and significant changes in laboratory results can be used as an effective means of communication with ordering physicians.

E-alerts were created from the HL-7 message transmitted from our laboratory information systems (Sunquest v6.2 and Copath

v3.1) to our clinical information system (Cerner millennium) for all results that met our criteria for a critical value or if the result was corrected. E-alerts with details about the critical value and/or corrected result were sent to the inbox of the ordering care provider in the EMR. Inbox activity (opened, pending, deleted) was tracked for a span of 16 days for all e-alerts in a 10-month period in 2008. We analyzed 500 consecutive e-alerts stratified by their clinical relevance (ie, potential to impact and/or alter patient clinical care).

Most e-alerts (99%) went to physician inboxes with very few (1%) sent to a midwife or nurse practitioner. Only 4% (n = 18) of all e-alerts were opened, despite the fact that 48% (n = 240) of all alerts were deemed to be clinically relevant. Of the 22 e-alerts triggered by a critical laboratory value, only 1 was opened.

These data indicate that while an electronic alerting system created in the EMR for timely communication of critical and/or corrected laboratory results is feasible, laboratories should not rely solely on this method of communication as most e-alerts remain unread by ordering providers. The effectiveness of such an alerting system can be enhanced by minimizing the number of irrelevant e-alerts.

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## 16

**Performance Evaluation of the VersaTREK Blood Culture System for Quality Control Testing of Platelet Units**

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Bacterial contamination of platelet (PLT) concentrates is a leading cause of patient mortality associated with transfusion. In accordance with AABB's new proposal, an in-house validation of the BACTEC 9240, an automated blood culture system, was performed in our institution for quality control (QC) testing of PLTs. The BACTEC 9240 was recently replaced with the VersaTREK (TREK Diagnostic Systems), another non-FDA-approved blood culture system for routine PLTs QC testing, requiring a revalidation of the performance of the new system. Nine most common bacterial species associated with PLT contamination were serially diluted in 1 single-donor apheresis PLT unit per organism. Blood culture bottles (VersaTREK REDOX 1, EZ Draw, TREK Diagnostic Systems) inoculated with 4 mL of each dilution containing less than 1 to greater than $10^3$ colony-forming units (CFUs) per milliliter were incubated in a VersaTREK automated blood culture system. Four milliliters of PLT product inoculated into blood culture bottles was used as a negative control. Positive blood culture bottles were subcultured to verify the identity of bacterial growth. The VersaTREK system detected bacterial growth in less than 24 hours, with a range of 6.0 to 18.1 hours depending on the amount of initial inoculum and species tested. The detection sensitivity for a majority of the bacteria tested including *Staphylococcus epidermidis, Staphylococcus aureus, Escherichia coli, Serratia marcescens, Klebsiella pneumoniae, Bacillus cereus, Enterobacter cloacae,* and *Pseudomonas aeruginosa* was less than 10 CFUs per milliliter. The limit of detection for *Streptococcus mitis* was 20 CFU per milliliter. With the subsequent use of this system, 5 positive samples were detected from the 1,970 apheresis PLT products tested at our institution and included 3 viridans group *Streptococcus* species, 1 coagulase-negative staphylococcus, and 1 *Bacillus* species. This study validates the use of the VersaTREK automated microbial detection system for use in routine QC testing of single-donor apheresis PLTs before transfusion.

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## 17

**Evaluation of Mesenchymal to Epithelial Reverting Transition Markers in Human Primary Breast Carcinomas and Metastases**

*Yvonne Chao* and Alan Wells. Department of Pathology, University of Pittsburgh, Pittsburgh, PA.*

Metastasis contributes significantly to the mortality of breast cancer. Numerous studies have shown that loss of E-cadherin through an epithelial to mesenchymal transition contributes to tumor invasiveness and downregulation of E-cadherin in primary tumor correlates with metastasis and poor survival. However, these studies focus only on the role of E-cadherin in metastatic initiation. The ability of a cancer cell to survive and grow in a new organ environment is also critical. The nonrandom pattern of metastasis suggests that metastases form only within organs that provide the appropriate environmental cues. We hypothesize that breast carcinoma cells regain E-cadherin expression through a mesenchymal to epithelial reverting transition to enable colonization within the liver, a common site of metastases. This is based on finding activation of prosurvival signaling pathways (phospho-ERK and phospho-AKT) when E-cadherin on breast cancer cells binds to E-cadherin on hepatocytes. Previously, we have shown that coculture of E-cadherin–negative MDA-MB-231 metastatic breast cancer cells with rat or human hepatocytes results in reexpression of E-cadherin due to hypomethylation of the promoter. To query whether E-cadherin reexpression at the metastatic site is observed clinically, we surveyed tissues obtained from 25 patients with primary infiltrating ductal carcinomas. By using immunohistochemistry, we analyzed expression of E-cadherin in the primary carcinomas and the corresponding nonnodal metastases. Overall, primaries and metastases exhibited aberrant (absent or decreased compared with normal internal controls) E-cadherin expression. However, of the 3 cases with liver metastases, 2 of the 3 liver metastases expressed E-cadherin with stronger intensity than the corresponding primaries. Analyses of β-catenin, cytokeratin 18, vimentin, and pEGFR expression and E-cadherin promotor methylation status via methylation-specific polymerase chain reaction in situ hybridization are currently underway. Multiple studies have shown that microenvironmental influences can lead to the epigenetic down-regulation of E-cadherin and the initiation of metastasis. However, our work suggests that E-cadherin expression can continue to be modulated by the microenvironment of the distant metastatic site, or the liver in our model, and may be required for metastatic colonization and survival.

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## 19

**Analysis of Hyponatremia and Hypernatremia to Define Critical Sodium Values in a Pediatric Population**

*Jeannette Guarner,[1] Jay Hochman,[2] and Richard Mullins.[3] Sponsor: James C. Ritchie. [1]Department of Pathology and Laboratory Medicine, Emory University; [2]Children's Center for Digestive Healthcare; and [3]Clinical Laboratory, Children's Healthcare of Atlanta, Atlanta, GA.*

Regulatory agencies require that clinical laboratories have critical values policies, and sodium is usually included in the critical values list. Evaluation of high and low critical values for sodium has been performed in adults and showed 48% mortality with sodium levels more than 155 mEq/L and 4-hour response time to critical values in 50% of cases. We recently revised the critical values list at our pediatric institution, and several physicians suggested that

a cutoff value less than 125 mEq/L would be safer for pediatric patients compared with the 120 mEq/L currently in use.

We assessed the level at which critical values for sodium in children should be set by evaluating patient outcomes and clinician responses to hyponatremia and hypernatremia. We performed a retrospective chart review of patients with values less than 124 mEq/L and more than 155 mEq/L that occurred during a 6-month period.

A total of 53,099 sodium tests were performed, and 702 (1.32%) fell in the study reference with 166 being less than 124 mEq/L and 536 more than 155 mEq/L. There were 70 patients with sodium values less than 124 mEq/L, 99 patients with values more than 155 mEq/L, and 8 patients with values in both study ranges. In 88 patients, 1 value fell in the study range, while the remaining 89 patients had more than 1 value in the study range. Hyponatremia or hypernatremia did not appear causally related to mortality in our population; however, mortality was 55% in patients with sodium values 120 mEq/L or less, 25% for patients with values 170 mEq/L or less, and less than 15% for patients with other values. Patients with hyponatremia frequently had complications of prematurity, while patients with hypernatremia had central nervous system disorders. Of the patients, 54% were in the ICU and 23% were in the emergency department when the hyponatremia or hypernatremia occurred. Response to treatment was instituted within 1 hour in 50% of cases and before 4 hours in 80%.

Based on our mortality data, cutoff critical values for plasma sodium in children could be set at 120 mEq/L or less and 170 mEq/L or more; however, these values do not consider morbidity associated with hyponatremia and hypernatremia. Our data compared with data published for adults show faster response time by our pediatric health care providers to sodium critical values, higher mortality for hyponatremia, and lower mortality for hypernatremia.

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## 20

### What to Expect on Cerebrospinal Fluid Specimens With Less Than 5 White Blood Cells in a Pediatric Population

*Jeannette Guarner,[1] Barbara Nix,[2] Maria Atuan,[2] Christopher Mishak,[2] Connie Vejjajiva,[2] and Richard Mullins.[2] Sponsor: James C. Ritchie. [1]Emory University and [2]Children's Healthcare of Atlanta, Atlanta, GA.*

Counting WBCs in cerebrospinal fluid (CSF) is a common procedure in pediatric institutions. Elevated WBC counts in a CSF sample is the cornerstone for diagnosis of meningitis. In children with acute lymphoblastic leukemia, elevated WBC counts with presence of blasts in the CSF is of diagnostic and prognostic significance. In contrast, the relevance of low WBC counts in CSF samples is difficult to determine.

Our objective was to assess the frequency of meningitis or leukemic blasts in CSF specimens with fewer than 5 WBCs. During April through June 2008, we collected results of all CSF samples with fewer than 5 WBCs using disposable hemocytometers and correlated the counts to Cytospin preparations, patients' diagnoses, and glucose and protein results in CSF.

A total of 313 CSF samples had fewer than 5 WBCs. Clinical diagnoses included malignant neoplasia in 185 samples, possible infection in 72, neurologic process in 43, and other diagnoses in 13. A pathologist reviewed the Cytospin preparation in all but 4 samples having an oncologic diagnosis. One blast was detected in 1 sample. A specific etiologic infectious agent (such as herpes simplex, enterovirus, respiratory syncytial virus, *Haemophilus influenzae,* or *Cryptococcus*) was present in 7 patients. More than 100 RBCs were observed in 35 samples (11%). Hemocytometer results showed 0 WBCs in 125 (40%) of samples, 1 WBC in 103 (33%), 2 WBCs in 46 (15%), 3 WBCs in 22 (7%), and 4 WBCs in 17 (5%). Cytospin preparations showed 1 to 10 cells in 179 (57%) of samples irrespective of the number of cells counted in the hemocytometer. When the hemocytometer had 0 or 1 WBC, the Cytospin preparations frequently showed no cells. In oncologic patients, 85% of samples had 0 and 1 WBCs, while 53% of samples from patients with infections showed 2 to 4 WBCs. Presence of neutrophils was observed in 46 (15%) of 313 samples, and there was no correlation with the WBC count. Biochemical alterations included a glucose level more than 70 mg/L in 19 patients, glucose level less than 40 mg/L in 15, and protein level more than 40 mg/L in 80. Concomitant presence of cells in the CSF and biochemical alterations was observed in 75 (24%) of 313 patients.

Since blasts can be found in samples from patients with an oncologic diagnosis, CSF samples with fewer than 5 WBCs need to be reviewed by a pathologist. Encephalitis and meningitis caused by specific organisms occur in CSF samples with fewer than 5 WBCs. The presence of neutrophils is rare in CSF specimens with fewer than 5 cells.

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## 21

### Use of the Luminex Platform for Detection of *HFE* Mutations in Hereditary Hemochromatosis

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Hereditary hemochromatosis an autosomal recessive disorder associated with single base pair (bp) substitutions resulting in C282Y and H63D amino acid changes in the HFE protein. Genetic testing is commonly used for diagnostic confirmation in the setting of biochemical evidence of iron overload. We developed a multiplexed assay to detect wild-type and disease-associated mutant alleles of the *HFE* gene on the Luminex platform using Allele-Specific Primer Extension (ASPE) and bead hybridization. Polymerase chain reaction (PCR) primers, allele-specific probes, and universally tagged Luminex FlexMAP microspheres (Luminex, Austin, TX) were selected using the DNAsis Smart Note ASPE Probe Design Tool software (Hitachi Software Engineering America, South San Francisco, CA). Multiplex PCR yielded amplicons of 205 bp for the C282Y region and 121 bp for the H63D region. A multiplexed ASPE reaction was performed using allele-specific probes whose 5′ end contained the sequence complementary to the universally tagged microsphere and whose 3′ end defined the allele for each wild-type and mutant allele pair. Biotinylated dCTP was included during the extension reaction to serve as the reporter for ASPE. Clinical samples from 20 patients representing all possible *HFE* genotypes as determined by our current LightCycler method were analyzed on the Luminex platform. We were blinded to specimen *HFE* genotypes during the testing and interpretation process. The Luminex assay correctly identified the genotype of all 20 patient DNA samples tested. Net median fluorescent intensity (MFI) ranged between 1,893 and 7,938 for all positive alleles and between 92 and 173 for all negative alleles. Allelic ratios were used to determine genotype calls and normalize signals between samples and loci. Alleles were considered present if the allelic ratio was at least 0.27. Wild-type or mutant homozygotes had an allelic ratio of greater than 0.95. This study demonstrates the potential for the use of the Luminex platform for laboratory-developed multiplexed molecular analysis. The DNAsis Smart Note ASPE Probe Design Tool software

accurately selected primers and probes compatible for multiplexed reactions. Additional molecular targets could be added to this assay for simultaneous and cost-effective molecular genetic analyses.

## 22

**Analysis of CEA and CA19-9 in Pleural Fluid to Diagnose Malignancy**

*Jennifer Hackbarth, Kazunori Murata, Bill Reilly, and Alicia Algeciras-Schimnich. Sponsor: Amy Saenger. Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN.*

Pleural effusions are a common complication caused by a variety of diseases, including malignancies. Cytology analysis is routinely used to differentiate between malignant and nonmalignant causes of these effusions; however, this methodology has a sensitivity of only 50% to 70%. Analysis of tumor markers, including carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA19-9), has been proposed as a way to improve the classification of malignant cases. Currently, the sensitivity and specificity of these markers is not well characterized. The goals of this study were to identify the best diagnostic cutoffs for CEA and CA19-9 to distinguish between malignant and nonmalignant effusions and examine these cutoffs in specific subsets of malignancies, including lung cancers and other CEA- or CA19-9-secreting cancers.

Fluids from 66 pleural effusions in 65 unique patients seen at Mayo Clinic Rochester (Minnesota) were collected by thoracentesis for analysis. Clinical diagnosis of malignant (n = 43) and nonmalignant (n = 23) effusions was based on cytology, biopsy, and long-term follow-up. CEA and CA19-9 were analyzed on a UniCel DxI 800 using 2-site immunoassays (Beckman Coulter). Statistical analysis was performed using Analyse-It for Excel.

CEA and CA19-9 levels were significantly higher in malignant effusions. The mean and median values of CEA in nonmalignant effusions were 2.99 and 0.63 ng/mL, respectively, compared with 219 ng/mL and 4.03 ng/mL in malignant effusions. CA19-9 mean and median values were 0.85 U/mL and 1.6 U/mL for nonmalignant effusions and 31 U/mL and 4.4 U/mL for malignant effusions. A CEA cutoff of 1.78 ng/mL or more yielded a sensitivity of 58% (25/43) and specificity of 96% (26/27) for detection of all malignant effusions. When only lung cancer effusions were considered, the sensitivity was 77% (10/13). A CA19-9 cutoff of 10.1 U/mL or more yielded 41% sensitivity (16/39) and 94% specificity (16/17) for detection of malignant effusions. The sensitivity was 46% (6/13) when only lung cancer effusions were analyzed. When both analytes were combined, the sensitivity and specificity for all malignant effusions were 69% (24/35) and 91% (21/23).

Measurement of tumor markers in pleural fluid could aid in the diagnosis of malignant effusions. CEA and CA19-9 were specific markers for malignancy; however, CEA was found to be more sensitive. CEA alone or in combination with CA19-9 is a good marker for differentiating between malignant and nonmalignant pleural effusions.

## 23

**Using Single-Antigen Bead HLA Antibody Profile as a Predictor of Solid Phase Platelet Crossmatch Results**

*David L. Murray, S. Breanndan Moore, Steven R. DeGoey, and Manish Gandhi. Sponsor: Bradley S. Karon. Department of Transfusion Medicine, Mayo Clinic, Rochester, MN.*

Repeated platelet transfusions can result in alloimmunization predominantly due to exposure to foreign HLA antigens. The resulting immune platelet refractoriness, a serious medical issue, requires pretransfusion platelet crossmatching or selection of HLA-compatible platelets to avoid immune-mediated platelet destruction. A study involving such platelet-refractory patients was initiated to correlate the class I HLA antibody profiles measured by single antigen bead (SAB) to the solid phase crossmatch (SPX) results for platelets from HLA-typed donors. The hypothesis was that patients with antibodies directed toward donor HLA platelet antigens (ie, a positive virtual crossmatch) would result in a positive SPX test. A retrospective review of HLA-typed donor apheresis platelets SPX results for patients who had a known SAB HLA antibody profile during a 17-month period beginning in January 2007 was performed. The ability of a virtual crossmatch to account for the SPX results was examined by calculating the positive predictive value (PPV) of positive SPX. A total of 44 clinically platelet-refractory patients were screened for HLA antibodies, and a total of 283 HLA-typed donor apheresis platelets were tested by SPX. Positive SPXs that could not be explained by the presence of HLA antibodies (ie, false-negative virtual crossmatches) were 11% (17/142). In contrast, a positive virtual crossmatch only predicted a positive SPX in 71% of the cases (125/176). Excluding positive virtual crossmatches in which the SAB-detectable antibodies were low (ie, normalized SAB values <5,000) increased the PPV from 71% to 80%. There remained a significant portion (20%) of negative SPXs that had high antibody level virtual crossmatches. Review of the clinical records yielded 9 units transfused with a positive virtual crossmatch. Patients receiving positive virtual crossmatch platelets with high SAB antibodies values (>5,000) had an average 24-hour posttransfusion count increment of 4,000 (n = 7; range, 0-12,000), while low SAB antibodies values (<5,000) averaged 17,000 (n = 2; range, 16,000-18,000). These results suggest that SAB virtual crossmatch may be more sensitive than SPX, but more data are needed.

## 24

**Choosing the Right Benzodiazepine Assay: Impact on Clinical Decision Making**

*Deborah French,[1] Judy A. Stone,[2] Judy S. Chang,[2] and Alan H.B. Wu.[1] [1]Clinical Laboratories, San Francisco General Hospital, San Francisco, CA; and [2]TPMG Regional Laboratory-Northern California, Richmond.*

A 51-year-old woman admitted for long-term care confessed to taking 1 unprescribed clonazepam tablet. Her urine sample was sent to our clinical laboratory for a drug screen; it was positive for benzodiazepines (BZDs). The clinician kept monitoring the patient's urine to detect further BZD ingestion, which would result in discharge from the care facility. The patient denied further use, but urine samples were BZD-positive for 22 days, followed by a negative and then a positive result, prompting the clinician to call for consultation. Generally, we would expect negative results within 2 to 3 days with no reexposure. We normalized the semiquantitative values from our CEDIA HS (Thermo Scientific) BZD immunoassay to urine creatinine and found a steady decrease over time, consistent with prolonged elimination from the original exposure.

Our objective was to compare results of several BZD assays for 9 urine samples from this patient (days 0, 21, 22, 23, 24, 25, 26, 30, and 31 after ingestion) to better characterize exposure(s) and illustrate the clinical significance of variability between assays for BZDs. A targeted liquid chromatography–tandem mass spectrometry (LC-MS/

MS) assay served as the "gold standard." The other assays were Biosite Triage Drugs of Abuse panel plus TCA, Syva Emit, Abbott AxSYM, and gas chromatography mass spectrometry (GC-MS).

The LC-MS/MS assay called the first urine sample positive for 7-aminoclonazepam, clonazepam, oxazepam (cutoff = 5 ng/mL), nordiazepam, and temazepam (cutoff = 2.5 ng/mL). In fact, all 9 samples were positive for oxazepam by this assay, with values decreasing over time indicating no reuse. However, the seventh sample (26 days after use) was positive for lorazepam (cutoff = 5 ng/mL) when all other samples were negative, indicating possible BZD reuse. The CEDIA HS assay called the first 3 samples positive (cutoff = 200 ng/mL; days 0, 21, and 22 after ingestion) and the rest negative; this assay uses β-glucuronidase, therefore measuring "total" drug, not only "free" drug. The GC-MS assay determined the first sample was positive (cutoff = 100 ng/mL) for 7-aminoclonazepam, and the rest were negative. The Triage and Emit assays called the first sample positive (cutoffs of 300 and 100 ng/mL, respectively) and the rest negative. With the AxSYM assay, all samples were negative (cutoff = 200 ng/mL).

These results demonstrate the advantage of high-sensitivity LC-MS/MS assays for monitoring BZD abuse and the importance of a laboratory consultation service to assist clinicians with interpretation of complex toxicology results.

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## 26

**Development of a Real-Time PCR Assay for Ultrasensitive Detection of *Plasmodium falciparum* in Human Malaria Vaccinees**

*Sean C. Murphy,[1] Jenny Prentice,[1] Patrick Duffy,[2] and Brad Cookson.[1,3] Departments of [1]Laboratory Medicine and [3]Microbiology, University of Washington, Seattle; and [2]Seattle Biomedical Research Institute, Seattle.*

To aid development of malaria vaccines against *Plasmodium falciparum*, ultrasensitive methods are required to detect subclinical parasitemia in clinical trial volunteers. Here, we developed a single-step polymerase chain reaction (PCR) assay using a specific primer set and a fluorescence resonance energy transfer (FRET) probe for detecting 16S ribosomal RNA gene amplicons from the 3D7 strain of *P falciparum*. All previously published *Plasmodium*-specific primer sets were initially compared with the fully sequenced 3D7 strain genome, and several primers sets were selected for initial evaluation. The most sensitive set was subsequently evaluated using 2 different detection modalities: a nonspecific DNA dye and a specific FRET probe. The FRET-based assay was most sensitive and specific when tested against cultured 3D7 strain *P falciparum* parasites in whole human blood. The optimized assay has a detection limit of 0.5 parasite genomes per microliter of whole blood (0.00001% parasitemia). The assay provides quantitative measurements over a range of parasitemia and will be used alongside clinical outcomes and parasitemia measured by thin blood smears to evaluate vaccine efficacy in upcoming clinical trials.

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## 28

**Assessing the Predictive Value of JAK2 V617F Allele Burden in Non-CML Classical Subtypes of Myeloproliferative Neoplasms**

*Julie Gober-Wilcox,[*1] Bharathi Vennapusa,[1] Joseph Pierce,[2] and Mohammad A Vasef.[1,2] Sponsor: Kendall P. Crookston.[1] [1]Department of Pathology, University of New Mexico, Albuquerque; and [2]TriCore Reference Laboratories, Albuquerque.*

A point mutation in exon 14 of Janus Kinase 2 (G→T at nucleotide 1849) that results in substitution of valine by phenylalanine in codon 617 (JAK2 V617F) is associated with 3 non-CML subtypes of myeloproliferative neoplasms (MPNs): polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF). The mutation is present in virtually all of PV cases, about half of ET and PMF cases, and to a lesser extent in other myeloid neoplasms. The mutational allele burden also varies among these subtypes. The purpose of this study is to assess the predictive value of JAK2 V617F allelic burden as a potential adjunct in further characterization of the subtypes of MPNs.

One hundred eight JAK2 V617F–positive cases were retrieved from the files of Molecular Oncology at the University of New Mexico/TriCore Reference Laboratories. Quantitative real-time PCR was performed on an ABI 7500 system using an allelic discrimination assay with Taqman probes on DNA isolated from whole nucleated cells in peripheral blood samples. Allele burden was calculated by using a ratio of the crossing threshold values of JAK2 wild type to JAK2 mutated and then quantified using a 6-point standard curve. MPN subtype was then correlated with the JAK2 allele burden.

An established diagnosis of MPN with subclassification was available in 55 of 108 cases, which included 28 with PV, 22 with ET, and 5 with PMF. The percentage of JAK2 V617F ranged from 2.7% to 96% (median, 12.5%) for PV, 0.4% to 65.2% (median, 2.5%) for ET, and 0.5% to 16% (median, 11.4%) for PMF. Of the 28 PV cases, 16 (57%) had a mutational load of more than 10% and 5 (18%) had a load of more than 50%; of the 22 ET cases, 4 (18%) had a mutational load of more than 10%; and of the 5 PMF cases, 3 (60%) had a mutational load of more than 10%.

Although there is some overlap, the mutational burden appears to be consistently higher in cases of PV and lower in ET. We also noticed more than 10% JAK2 V617F allele burden in 3 of 5 PMF cases. However, a definitive conclusion could not be drawn in this subtype given the low number of PMF cases. We did not perform granulocyte enrichment before DNA isolation, which may have a dilutional effect on the true allele burden, particularly in cases with possible lymphocytosis. Our findings suggest that JAK2 V617F quantitation, when used in conjunction with morphological and clinical findings, may aid in separating PV from non-PV cases.

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## 29

**Measurement of IgG and IgM in Pediatric Patients Using the VITROS 5600 and Comparison With the Siemens BN ProSpec**

*Bonnie Cole,[1] M. Rand,[2] Barbara Busby,[2] Kathy Dugaw,[2] and Rhona Jack.[1,2,] Sponsor: Joe Rutlege. [1]Department of Laboratory Medicine, University of Washington, Seattle; and [2]Seattle Children's Hospital, Seattle.*

Many patients with primary immunodeficiencies present in childhood with severe, recurrent infections. Antibody deficiencies are the most common causes of primary immunodeficiency, but quantification of immunoglobulins in childhood can be challenging. The pediatric reference intervals for IgG and IgM are below the lower limit of detection of automated analyzers not performing nephelometry. Our goal was to see if we could decrease the lower limit of detection for IgG and IgM on the VITROS 5600 (Ortho-Clinical Diagnostics, Raritan, NJ) to meet the needs in evaluating pediatric patients.

The VITROS 5600 quantifies IgG and IgM by turbidity of antigen-antibody complexes in solution, turbidity being proportional

to the concentration of IgG and IgM. The instrument automatically begins with a 1:15 predilution of patient sample with buffer. The quoted reportable ranges are 270 to 2,700 mg/dL for IgG and 25 to 400 mg/dL for IgM. Our institution currently uses the Siemens BN ProSpec nephelometer for immunoglobulins, which measures light scattering of immune complexes in solution; the intensity of light scattered is proportional to concentration of IgG and IgM. Analytical measurement ranges on the ProSpec are 7 to 23,200 mg/dL for IgG and 6 to 62,400 mg/dL for IgM.

By changing the preset autodilution and by running calibrators lower than the established range, we were able to extend the linear range of both IgG and IgM on the VITROS 5600.

Linearity was confirmed. Decreasing the autodilution to 1:5 allowed a lower limit of 50 mg/dL for IgG ($y = 0.94x + 11.2$). Within-run coefficient of variation (CV) for IgG was 1.4% at 47 mg/dL with a 1:5 autodilution. CV for IgM was 1.0% at 165 mg/dL and 7.2% at 9.8 mg/dL. Comparison of patient samples yielded a correlation ($R^2$) of 0.99 for IgG ($y = 1.0271x – 15.47$; N = 28) and an $R^2$ of 0.99 for IgM ($y = 1.076 x + 1.247$; N = 39).

In summary, we have shown that the lower limit of detection for IgG can be extended to 50 mg/dL with 1:5 predilution, and the IgM can be extended down to 10 mg/dL by extending the standard curve, improving suitability in pediatric laboratories.

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## 30

### Effect of Hawthorn on Serum Digoxin Measurements by Immunoassays and Pharmacodynamic Interaction With Digoxin

*Laura Kidd, Brian J. Poindexter, Roger J. Bick, and Amitava Dasgupta. Department of Pathology and Laboratory Medicine, University of Texas Medical School, Houston.*

Hawthorn, an herbal supplement, is indicated for congestive heart failure, angina pectoris, and chronic heart disease. We investigated the effect of Hawthorn extract on serum digoxin measurements using 2 immunoassays and the potential pharmacodynamic interactions with digoxin. Drug-free serum pools were supplemented with 2 different Hawthorn extracts (Brand 1, Herb Pharm, Williams, OR; and Brand 2, Gaia Herbs, Brevard, NC), and apparent digoxin concentrations were measured by the fluorescence polarization immunoassay (FPIA) using the AxSYM analyzer (Abbott Laboratories, Abbott Park, IL) and the Tina-Quant digoxin assay using the P-800 modular analyzer (Roche Diagnostics, Indianapolis, IN). We also prepared 2 serum pools from patients receiving digoxin and supplemented them with varying amounts of Hawthorn extract. Then digoxin concentrations were remeasured using both assays. For studying the pharmacodynamic interaction of Hawthorn with digoxin, we used isolated adult rat cardiomyocytes, measuring calcium transients by real-time fluorescence spectrophotometry. When drug-free serum pools were supplemented with varying amounts of Hawthorn extract, we observed apparent digoxin concentrations in the range of 0.30 to 0.67 ng/mL, digoxin equivalent by the FPIA assay (apparent digoxin values higher with Brand 1 extract than with Brand 2), but no apparent digoxin level was observed using the Tina-Quant assay. Similarly, when aliquots of digoxin pools were supplemented with Hawthorn extracts, only the FPIA assay showed any significant interference with digoxin measurements. For example, when a 1-mL aliquot of a digoxin pool containing 1.12 ng/mL digoxin was supplemented with 20 µL of the Hawthorn extract (Brand 1), the digoxin level was increased to 1.80 ng/mL when measured by the FPIA, but was 1.06 ng/mL when measured by the Tina-Quant assay. However, digoxin-like immunoreactive components of Hawthorn extracts are strongly bound to serum proteins, and measuring free digoxin eliminates this interference in the FPIA assay. In addition, Hawthorn extracts demonstrated pharmacodynamic interaction with digoxin in isolated cardiomyocytes with both compounds increasing intracellular calcium levels, an effect that was nullified by initial exposure to the other compound, ie, Hawthorn-treated cells did not respond to additional digoxin and vice versa. This suggests that there is binding to the same site, the Na-K, ATPase, and that binding completion could exist and be a problem in patients receiving a cardiac glycoside such as digoxin and also taking Hawthorn extracts.

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## 31

### CD8 Effector T Cells Inhibit the Development of Persistent Airway Inflammation in a Murine Model of Asthma

*Jiankun Tong,*[*1] Kimm J. Hamann,[2] and Anne I. Sperling.[2] Sponsor: Jonathan L. Miller.[1] Departments of [1]Pathology and [2]Medicine, University of Chicago, Chicago, IL.*

Human asthma is characterized by persistent airway inflammation, mucus production, and airway hyperreactivity. The role of CD8 T cells in the chronic airway inflammation of asthma is controversial. Previous studies have suggested that CD8 effector ($CD62L^{lo}CD44^{hi}$), and not CD8 central memory ($CD62L^{hi}CD44^{hi}$), T cells play an important role in the development of airway hyperreactivity and airway inflammation. Our previous studies revealed that in a murine model of asthma, mice that received Fas-deficient T cells (Lpr>Rag–/–) developed a persistent phase of airway inflammation, mucus production, and airway hyperreactivity that failed to resolve even 6 weeks after the last challenge. Examination of these mice demonstrated increased numbers of CD8 T cells in the BAL, lungs, and spleens. However, what was very surprising was the large number of CD8+ T cells that were $CD62L^{low}$ and CD69+ in the Lpr>Rag–/– mice. This CD8 population is likely to be effector cells, and the marked increase in the percentage of these cells in the Lpr>Rag–/– mice at day 42 postchallenge suggests that an active immune response is still ongoing in these animals.

The purpose of this study was to investigate the role of CD8 effector T cells in the development of chronic inflammation.

B6 and Lpr T cells from Lpr mice were harvested from lymph nodes. $10^7$ cells were adoptively transferred into Rag–/– mice intravenously at day –15. At day –14, mice were immunized by i.p. injection of 5,000 inactivated *S mansoni* eggs, which induced a natural Th2 response in the absence of active infection. At days –7 and 0, the mice were challenged with 10 µg of SEA by intranasal and intratracheal aspiration, respectively. CD8+ T cells were depleted in vivo by i.p. injection (1 mg each) of monoclonal anti-CD8 depletion antibody (YTS-169) or control antibody at days 14 to 17 for 4 consecutive days after the last challenge. Mice were sacrificed at day 28 after the last challenge, and data were analyzed.

The B6>Rag–/– mice that received anti-CD8 depletion antibody had prolonged eosinophilia, goblet cell hyperplasia, and peribronchial and perivascular inflammation. Furthermore, cytokine analysis showed that CD8 T cells produced IFN-γ, which inhibited the airway inflammation.

These results provide strong evidence that CD8 effector T cells play an inhibiting rather than a stimulating role in the development of airway inflammation.

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## 32

**Decrease in Human Blood Fetuin-A Levels in Acute Myocardial Infarction**

*William D. Ryan, Ping Gao, and Paul Wolf. Department of Pathology and Laboratory Medicine, University of California and VA Medical Centers, San Diego; and Epitope Diagnostics, San Diego.*

The goal of our study was to quantitate human blood fetuin-A levels in acute myocardial infarction (MI) patients. Fetuin-A is a circulating glycoprotein synthesized by hepatocytes. Troponin I levels were measured during a period of 3 or 4 days in patients who presented to the emergency department with chest pain suggestive of an acute MI. Human blood fetuin-A levels were obtained simultaneously. In addition, CK-MB and myoglobin levels were measured. Whole blood fetuin-A levels were determined by using an enzyme-linked immunosorbent assay technique. The assay used a 2-site "sandwich" technique with 2 specific polyclonal antibodies that bound to different epitopes of the fetuin-A molecule. Troponin I levels were quantitated by an immunoassay procedure. Increased troponin I levels, which were used to rule in acute MI, remained elevated for a period of 3 or 4 days. Fetuin-A levels decreased during the same period of time. Fetuin-A is a negative acute phase protein. Recently, neutrophilic myeloperoxidase was discovered as a plasma cardiac marker in patients developing myocardial necrosis due to acute MI. Our study showed that elevated troponin I levels in patients with acute MI correlated with a sequential decrease in fetuin-A levels. Fetuin-A is a negative acute phase protein, and we have established that levels of this glycoprotein decrease in patients with acute MI in which there is an inflammatory response to myocardial necrosis. A decrease in fetuin-A during a 3- or 4-day period in patients with acute MI may be a predictor of the inflammatory response to myocardial necrosis.

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## 33

**Pediatric Reference Intervals for Adrenal Steroids and Growth Factors for Children 6 Months Through 6 Years Old**

*Kyle Rasmussen,[1] Ashley M. Bunker,[1] William L. Roberts, MD, PhD.[1,2,] [1]ARUP Laboratories and [2]Department of Pathology, University of Utah, Salt Lake City.*

The objective of this study was to establish pediatric reference intervals (RIs) for adrenal steroids and growth factors for ages 6 months (mo) through 6 years (y). The analytes included 11-deoxycortisol (11-DC), 17-hydroxypregnenolone (17OHPRGN), 17-hydroxyprogesterone (17OHPROG), pregnenolone (PRGN), insulin-like growth factor binding protein-3 (IGFBP-3), and insulin-like growth factor-1 (IGF-1). We enrolled healthy children from Primary Children's Medical Center who were undergoing elective surgeries. Blood was obtained through an IV before any fluids were administered. Results were obtained for adrenal steroids using liquid chromatography–tandem mass spectrometry and for growth factors using the Siemens IMMULITE 2000 analyzer. Data were analyzed nonparametrically. The pediatric RIs obtained for 11-DC (ng/dL) in girls 6 mo to 3 y (N = 164) were 9 to 235; girls 4 to 6 y (N = 150), 7 to 342; boys 6 mo to 3 y (N = 198), 8 to 201; and boys 4 to 6 y (N = 147), 8 to 243; the RIs for 17OHPRGN (ng/dL) in girls 6 mo to 3 y were 25 to 522; girls 4 to 6 y, 25 to 280; boys 6 mo to 3 y, 25 to 867; and boys 4 to 6 y, 25 to 290; the RIs for 17OHPROG (ng/dL) in girls 6 mo to 3 y were 5 to 211; girls 4 to 6 y, 5 to 278; boys 6 mo to 3 y, 5 to 181; and boys 4 to 6 y, 5 to 205; and RIs for PRGN (ng/dL) in girls 6 mo to 3 y were 14 to 150; girls 4 to 6 y, 15 to 154; boys 6 mo to 3 y, 11 to 239; and boys 4 to 6 y, 12 to 128. The RIs for IGFBP-3 (ng/mL) in girls 6 mo to 3 y were 1,473 to 4,189; girls 4 to 6 y, 2,169 to 4,790; boys 6 mo to 3 y, 984 to 3,982; and boys 4 to 6 y, 1,843 to 4,968; and for IGF-1 (ng/mL), the RIs in girls 6 mo to 3 y were 25 to 148; girls 4 to 6 y, 43 to 212; boys 6 mo to 3 y, 25 to 137; and boys 4 to 6 y, 37 to 192. The upper reference limit of 11-DC is significantly lower for girls 6 mo to 3 y than for girls 4 to 6 y and significantly lower for boys 4 to 6 y compared with girls 4 to 6 y. In contrast, the upper reference limits of 17OHPRGN are significantly higher for both boys and girls 6 mo to 3 y compared with boys and girls who are 4 to 6 y. They are also significantly higher for boys 6 mo to 3 y than for girls of the same age. The upper reference limit of PRGN for boys 6 mo to 3 y is significantly higher than for boys 4 to 6 y. The lower reference limits of both IGFBP-3 and IGF-1 for boys and girls are significantly lower for children who are 6 mo to 3 y compared with children who are 4 to 6 y, and the upper reference limits for both analytes are significantly lower in younger boys compared with older boys. The lower reference limits for IGFBP-3 are significantly lower in boys of both age groups compared with girls of the same ages. Age- and sex-specific RIs are necessary for these all of these analytes.

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## 34

**Reference Intervals for Seven Chemistry Analytes for Children 6 Months Through 6 Years Old**

*Ashley M. Bunker[1] and William L. Roberts.[1,2] [1]ARUP Laboratories and [2]Department of Pathology, University of Utah, Salt Lake City.*

Previous studies have determined pediatric reference intervals (RIs) for a variety of chemistry tests; however, the number of children studied is usually small. The Children's Health Improvement Through Laboratory Diagnostics study (CHILDx) at ARUP Laboratories was designed to fulfill this need. We aimed to determine the RI for children ages 6 months (mo) through 6 years (y) for the following chemistry analytes: aldolase (Ald), ceruloplasmin (Ceru), creatine kinase (CK), DHEAS, prealbumin (PAlb), pancreatic amylase (pAmy), and total amylase (tAmy). Subjects were enrolled at Primary Children's Medical Center in Salt Lake City, UT, and were already undergoing minor surgical procedures. They were selected after a brief chart overview to ensure they were healthy and not taking any prescription medications. After parental consent, serum was collected through an intravenous catheter that was placed by an anesthesiologist before the procedure(s). A total of 665 children were enrolled in this study. Specimens were analyzed using Roche Modular P and E analyzers. RIs were estimated using nonparametric or transformed parametric methods. We partitioned the data by sex and age (6 mo through 2 y and 3 through 6 y). For Ceru, we found no differences based on age or sex. The Ceru reference interval for children ages 6 mo through 6 y is 18 to 37 mg/dL. For all other analytes, data required partitioning by age and/or sex. The RIs for Ald (U/L) are 3.4 to 9.8 for girls 6 mo to 2 y, 3.0 to 8.9 for girls 3 to 6 y, 3.5 to 9.8 for boys 6 mo to 2 y, and 2.3 to 8.7 for boys 3 to 6 y. The RIs for CK (U/L) are 40 to 260 for girls and 50 to 281 for boys. The RIs for DHEAS (µg/dL) are 0 to 29 for girls 6 mo to 2 y, 1 to 47 for girls 3 to 6 y, 1 to 33 for boys 6 mo to 2 y, and 1 to 44 for boys 3 to 6 y. The RIs for PAlb (mg/dL) are 11.0 to 24.2 for girls 6 mo to 2 y, 12.0 to 22.8 for girls 3 to 6 y, 12.1 to 22.1 for boys 6 mo to 2 y, and 13.2 to 23.3 for boys 3 to 6 y. The RIs for pAmy (U/L) are 1 to 27 for girls 6 mo to 2 y, 9 to 33 for girls 3 to 6 y, 2 to 30 for boys 6 mo to 2 y, and 8 to 35 for boys 3 to 6 y. Finally, the RIs for tAmy (U/L) are 16 to 115 for girls 6 mo to 2 y, 25 to 112 for girls 3 to 6 y, 12 to 113 for boys 6 mo to 2 y, and 27 to 151 for boys 3 to 6 y. In conclusion, we have developed pediatric RIs for 7 analytes, partitioning by age and sex when necessary. In contrast with

our current study, previous studies of these analytes generally did not include as many subjects with ages of 6 mo through 6 y.

## 36

**Should Busulfan Dose Adjustments Be Made Based on First Dose Pharmacokinetics?**

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Busulfan is a cytotoxic conditioning agent that is commonly used in myeloablative regimens for patients undergoing bone marrow transplantation. Studies have shown that busulfan has wide intrapatient and interpatient variability in drug disposition, metabolism, and clearance, thus making it difficult to control drug exposure within the therapeutic range. Patients with plasma busulfan concentrations below the therapeutic range are at risk for graft rejection, whereas patients with plasma concentrations above the therapeutic range are at risk for hepatic veno-occlusive disease, which is potentially fatal. Individual therapeutic drug monitoring must be performed for each patient to ensure that busulfan concentrations are optimal for treatment. Busulfan dosing guidelines recommend dose adjustments based on first dose pharmacokinetics; however, we question whether this recommendation is appropriate for patient care.

The purpose of this study was to determine if first dose pharmacokinetics for busulfan can accurately predict the steady state concentration (Css) in order to make appropriate dose adjustments. Six patients (age, 55-66 years; 4 men) received Q6 i.v. Busulfex to achieve a target plasma concentration of 650 ng/mL. Timed plasma specimens were collected immediately after the first dose infusion and after the 13th dose to compare Css. Busulfan analysis was performed by ELISA. Pharmacokinetic analysis was performed to calculate the area under the plasma concentration vs time curve and the Css of busulfan, using ADAPT II (BMSR, Los Angeles, CA).

First dose pharmacokinetics revealed that 3 of the patients achieved close to the targeted Css after the first dose. The other 3 patients had Css more than 650 ng/mL. The busulfan dose was reduced in 3 patients at dose 3, and the other 3 patients remained on the same dose throughout treatment. Pharmacokinetic analysis after the 13th dose revealed that all 6 patients had a Css that exceeded the target plasma Css. Evaluation of other pharmacokinetic parameters showed that 5 of 6 patients had decreased busulfan clearance at dose 13.

Results from our study suggest that first dose pharmacokinetics does not accurately predict the Css at dose 13 and suggest that patients do not achieve true Css after the first dose. Dose adjustments based on first dose pharmacokinetics may underestimate plasma busulfan Css and may, therefore, put patients at risk for busulfan overdose.

## 37

**Pseudocholinesterase Activity: What Can Genotype Tell Us?**

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Serum cholinesterase, also called pseudocholinesterase (PChE), is one of two enzymes that hydrolyze choline esters. Encoded by the *BCHE* gene and synthesized in the liver, the function of PChE remains unclear, although it is implicated in the metabolism of the neuromuscular blocking agent, succinylcholine. Extended blockade and apnea with succinylcholine occur in individuals with reduced PChE activity due to genetically determined variants. Phenotypic identification of variants is achieved by measuring PChE activity in the presence of the inhibitor dibucaine, which, together with total activity, is used to calculate a dibucaine number (DN). Assignment of PChE phenotypes requires well-defined, phenotype-specific PChE and DN reference intervals. Method-specific reference intervals are not available owing to the number of biochemical methods for determining PChE activity and the lack of assay standardization.

Our objective was to perform genotype-phenotype correlations of serum PChE to validate existing reference intervals and/or establish more appropriate intervals. Cell-free DNA was extracted from 45 serum specimens for which total enzyme activity, DNs, and phenotypes were known. Phenotypes were assigned based on our laboratory criteria. Polymerase chain reaction of the *BCHE* gene coding region was performed, followed by bidirectional DNA sequencing using BigDye terminator chemistry. Sequencing data analysis was performed using Mutation Surveyor software.

Phenotype-genotype agreement occurred in 36% of specimens. Of these, 38% were wild-type (UU) and 62% were heterozygous for a common PChE variant (UA). Discordance between phenotype and genotype occurred in 64% of specimens; 48% of these had not been assigned a PChE phenotype owing to inconsistencies between the PChE biochemistry and our interpretive criteria. Of these, 51% were genotypically UU or UA. For the remaining 52% discordant results, the inaccurately assigned phenotype did not change the likelihood of succinylcholine susceptibility or implied a slightly increased risk when there was actually none. In 1 discordant specimen, the phenotype (UF) implied a slight risk, while genotypically a high-risk phenotype (AA) was present. Another was phenotyped as AS (high risk), and sequencing revealed a recently reported mutation that imparts low PChE activity.

These data highlight the inability to assign an accurate phenotype based solely on PChE activity and the DN. Method-specific biochemical reference intervals based on PChE genotype are necessary. The combination of biochemistry and genotype could improve the accuracy of PChE phenotype results.

## 38

**Detection of *Chlamydia trachomatis* Lymphogranuloma Venereum in Men With Proctitis**

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Lymphogranuloma venereum (LGV) is a sexually transmitted infection caused by serovars L1, L2, or L3 of *Chlamydia trachomatis* (CT). Although it is thought to be a disease of low prevalence, there have been several recent reports in the United States and Western Europe documenting increases in anorectal infection among men who have sex with men (MSM), particularly in HIV-infected men.

We conducted a retrospective chart review of all HIV-infected MSM diagnosed with incident CT rectal infection between September 2006 and April 2008 at Emory Crawford Long Hospital. CT diagnostic testing was performed from crude lysates of rectal swab specimens of the above symptomatic patients and 10 asymptomatic patients via

strand displacement amplification (BD ProbeTec). Genotype identification of positive samples was performed at the Centers for Disease Control and Prevention by sequencing of the CT outer membrane protein gene (ompA). Medical records were reviewed to determine clinical presentation, treatment regimen, duration of therapy, and clinical outcomes.

Eleven HIV-infected MSM (age, 26-46 years) with symptomatic proctitis were positive for CT. Presenting symptoms included rectal pain, rectal bleeding, and constipation. CD4 cell counts ranged from 112/μL to 562/μL with HIV viral load measurements from 8,600 to 121,000 copies/mL (3.9-5.1 $\log_{10}$ copies/mL) before presentation. Two men were receiving antiretroviral therapy. All but 2 men had at least 1 concurrent sexually transmitted infection. Unprotected anal receptive intercourse was documented in all men. Eight CT isolates were confirmed as LGV genotype L2b and 2 as non-LGV genotypes E and J, and 1 could not be genotyped. The 10 asymptomatic patients were negative for CT with appropriate amplification controls.

LGV can be successfully diagnosed by molecular methods from rectal swab specimens in symptomatic patients. Concurrent use of amplification controls is recommended with the BD Probetec assay because rectal samples have a high potential for inhibitors. Current commercial amplification methods provide qualitative CT results rapidly, allowing early initiation of therapy, but do not differentiate between LGV and non-LGV CT strains. Further evaluation of this specimen type for asymptomatic high-risk patients will aid in surveillance and diagnosis of this increasingly important infection.

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## 40

### A Method for Optimizing and Validating Institution-Specific Flagging Criteria for Automated Cell Counters

*Anthony Sireci, Robert Schlaberg, Woojin Yu, and Alexander Kratz. Sponsor: Steven Spitalnik. Department of Pathology and Cell Biology, Columbia University Medical Center, New York, NY.*

Blood samples submitted for WBC differentials that are flagged by automated cell counters need to be reviewed microscopically. Slide review is a labor-intensive process that delays result reporting and increases costs. Our objective was to validate a method for optimizing the cutoffs used to trigger flags on the automated cell counters used at our institution. Our goals were to reduce the percentage of automated WBC differentials requiring microscopic review ("review rate") without an unacceptable increase in the number of clinically important missed abnormalities.

Our test set consisted of 502 samples that had been flagged by 1 of 5 user-adjustable flags on the Sysmex XE-2100 (Sysmex, Kobe, Japan) instruments using factory-set default criteria. Of these samples, 117 showed a clinically significant abnormality on microscopic review (positive predictive value [PPV] of default settings, 0.23). We first adjusted the cutoff for each of the 5 flags individually to maximize the sensitivity and specificity for its respective abnormality (eg, of the atypical lymphocyte flag for atypical lymphocytes). We next sequentially adjusted the cutoffs for optimal detection of any abnormality. A combination of the maximized Youden Index and clinical judgment was used to identify the optimal cutoff values for all 5 flags.

These newly optimized rules were then validated on an independent set of 173 samples (validation set) flagged as abnormal using the default cutoffs. Of the 173 samples, 60 (35%) were flagged as abnormal using the new rules. The PPV of the new flagging criteria for the presence of any significant abnormality on microscopic review was 0.41. Twenty-two false-negative cases resulted: 3 showed 3% or more metamyelocytes and/or myelocytes (average, 6.3%; range, 3%-12%), 13 cases showed 5% or more atypical lymphocytes (average, 9.7%; range, 5%-16%), and 6 showed 7% or more bands (average, 16.2%; range, 8%-24%). Using the optimized flagging criteria reduced our review rate from 7.5% to 2.6%.

We conclude that our method can be used by laboratories to individualize review flag cutoffs on automated cell counters for their specific needs. This method may allow laboratories to safely decrease the number of WBC differential cases that require microscopic review, thereby leading to more efficient laboratory operations and savings in labor costs.

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## 41

### Medical Error Disclosure: Attitudes and Experiences of Laboratory Medical Directors

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Error disclosure is an important facet of patient care. Physician attitudes regarding medical error disclosure vary among specialties. Laboratory medicine is characterized by limited direct patient contact, generation of large amounts of patient care–related information, and disclosure opportunities to a patient's physician rather than directly to a patient. This study describes attitudes and experiences of laboratory medical directors toward error disclosure at 2 geographically separate large academic medical centers.

Surveys were mailed to 81 academic hospital laboratory medical directors. The survey, an anonymous 60-item paper document, was administered between July and December 2008. The survey contained questions about attitudes, communication, and personal experience with medical errors; evaluation of a hypothetical error vignette; communication about medical errors within the organization; and demographic factors.

Responses were received from 61 participants, 5 of which were excluded, with a combined response rate of 75%. Of laboratorians, 98% have been personally involved with laboratory errors: 71% with a near miss, 63% with a minor error, and 34% with a serious error. Only 14% of respondents disclosed a serious error directly to a patient. Laboratorians generally supported disclosure; 98% of respondents agreed that serious errors should be disclosed to patients. However, beliefs varied regarding what errors should be disclosed. Only 67% believed that minor errors should be disclosed, and 18% agreed that near misses should be revealed. Respondents were split in their beliefs regarding the cause of medical errors: 48% believed that they are due to failures of care delivery systems, not individuals, while 52% disagreed. Participants had little experience formally reporting errors to the hospital and believed that current reporting systems are inadequate.

Our study confirms that laboratorians universally encounter error but infrequently disclose and report errors. They generally support disclosure to patients, but their support diminishes as the severity of error decreases.

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## 42

### A Novel Evaluation Tool for Assessing the Systems-Based Practice Competency of Pathology Residents

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*Henry A. Homburger. Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN.*

Few methods or tools exist to assess the systems-based practice (SBP) competency of pathology residents. Within the context of the clinical chemistry rotation, we assign a formal SBP project to each resident. The projects are based on unmet clinical needs. Each resident meets with clinicians who have a need not currently being met; development technologists who have data on sensitivity, specificity, turnaround time, etc; and supervisors or managers who understand regulatory, cost, and workflow implications of each potential solution. The residents are then asked to present their solution for resolving the clinical need in a 2- or 3-page report. Because no evaluation tool existed to measure resident performance in SBP, we created a novel evaluation tool that assessed resident SBP competency in 4 areas based on Richard Paul's model of critical thinking: (1) definition of the question at issue and purpose of project; (2) identification of key stakeholders, their operating assumptions, and interdependencies between stakeholder groups; (3) the completeness, accuracy, and quality of the concepts, information, and evidence presented; and (4) the rationale and evidence behind the proposed solution and insight into the strengths and weaknesses of alternative solutions. For each of the 4 areas evaluated, a 5-point Likert scale was developed with standard definitions of what constituted performance of 1 through 5. Five reviewers independently evaluated 7 resident projects. The internal consistency of the 4 evaluables in the tool was measured using the Cronbach $\alpha$. The Cronbach $\alpha$ scores for the 4 evaluable areas ranged from .88 to .91, with an overall value of .92. In general, Cronbach $\alpha$ scores of .8 to .9 are considered ideal as they reflect internal consistency between questions without significant duplication or overlap. Interrater reliability, assessed by intraclass correlation coefficient (ICC), varied from 0.25 to 0.37, with an overall ICC of 0.29. ICC values between 0.2 and 0.3 reflect "fair" agreement between raters, while values of 0.6 to 0.8 reflect "substantial" and values 0.8 to 1.0 "almost perfect" agreement. In conclusion, we have developed a method and evaluation tool for assessment of the SBP competency during the clinical chemistry rotation. The method involves assignment of a formal SBP project based on current clinical needs within the practice. The evaluation tool was found to be internally consistent, with none of the 4 evaluable areas significantly duplicating constructs assessed by the others. Interrater reliability was only fair, demonstrating a need to improve the standard definitions associated with each evaluable area to allow for more objective evaluation.

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## 43

**Prevalence of Human Parechovirus in North American Children Originally Tested for Enteroviral Meningitis**

*Masha Catherine Nzabi,* Suresh Babu Selvaraju, and Rangaraj Selvarangan. Department of Pathology, Children's Mercy Hospital, Kansas City, MO.*

Human parechovirus (HPeV), previously considered a member of the genus Enterovirus, was recently reclassified into a new genus, Parechovirus, within the family Picornaviridae. Recent reports from Europe and South America associate HPeV with a wide spectrum of clinical disease in children, including serious illnesses such as febrile seizures, meningitis, neonatal sepsis, and acute flaccid paralysis. Study of cerebrospinal fluid (CSF) from children in Amsterdam detected a prevalence of 0.4% to 8.2% during 2004 to 2006. To date, there are no data on the prevalence of HPeV in children from North America.

The aims of this study were to detect the prevalence of HPeV in children who originally tested negative for enterovirus meningitis and to understand the epidemiology of HPeV infection in North America. Frozen aliquots of enterovirus-negative CSF specimen extracts from 2006 and 2008 were tested by 2 different HPeV real-time RT-PCRs. The pediatric patients were seen at the Children's Mercy Hospitals and Clinics, in Missouri and Kansas. A total of 460 CSF samples comprising 242 from 2008 and 218 from 2006 were analyzed. The integrity of the RNA was tested by randomly testing frozen CSF extracts for GAPDH mRNA. Demographic and clinical data were abstracted from medical records.

HPeV was detected in 4 (1.8%) of 218 CSF samples from the year 2006, while none (0/242) of the CSF samples from the year 2008 tested positive. Among the 4 children testing positive for HPeV, 3 were boys and 1 was a girl. All 4 children were admitted in the months of September and October and were younger than 10 weeks of age. They presented clinically with fever and irritability; 2 presented with meningitis and 2 with erythematous rash. All of them were hospitalized for a median duration of 3 days and received a course of antibiotics for 48 hours during hospitalization.

The prevalence of HPeV in North American children who originally tested negative for enterovirus meningitis was low compared with Europe. There seems to be an age, sex, and time preference in the prevalence of HPeV in children, with a high incidence in newborns, boys, and the fall season in the limited positives detected in our study. Further studies at other sites in North America are required to determine if testing for HPeV as part of a viral test panel is warranted for children with meningitis.

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## 44

**Detection and Analysis of Nanoscale Tumor Membrane Biomarkers**

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In addition to soluble mediators, cancer cells communicate within their microenvironment via biological nanoparticles derived from cancer cell membranes. These nanoscale messengers (~100 nm) are known as exosomes and are released into the extracellular matrix and blood. Exosomes contain and transmit protein biomarkers and RNA transcriptomes of the cancer cells from which they are derived.

In basic science laboratories, exosomes are traditionally isolated via differential centrifugation followed by technically challenging and time-consuming confirmation via electron microscopy of exosome pellets. While exosomes have great potential as biomarkers, there is a need to develop rapid methods that overcome the technical challenges associated with confirming exosome isolation and can be adapted to clinical settings for patient exosome screening purposes. Thus, we have adapted dynamic light scattering (DLS) technology, a tool traditionally relied on for the sizing of synthetic nanoparticles, to rapidly (<10 minutes) confirm the presence or absence of exosomes in biological samples before exosome isolation. DLS also provides new knowledge about exosomes. For example, we found that exosome size distribution is not necessarily unimodal but can be multimodal. This demonstrates the presence of exosome subtypes within specific cell-derived populations (mouse melanoma, human breast carcinoma, and mouse endothelial) and suggests differences in function that may correlate to pathological status.

Having adapted DLS for exosome analysis, we next sought to evaluate whether exosomes can serve as biomarkers of a specific tumor process such as angiogenesis. We hypothesized that melanoma exosomes contain angiogenic messages in the form of mRNA. By using an $RT^2$ PCR array (SABiosciences), profiling the expression of mouse genes involved in modulating angiogenesis, we isolated mouse B16 melanoma cell and exosome mRNA for comparison. The melanoma exosomes contained the majority of mRNA for angiogenic proteins expressed in the parent cell, including mRNA for genes not detected in the parent cell indicating selective sorting of mRNA into exosomes. The same may hold true for other tumor types as well and will require confirmation using blood samples from patients with cancer. Ultimately, adapting DLS technology for confirmation of exosome isolation will expedite analysis of normal and pathological exosome biomarker profiles for individual patients.

## 46

### Microparticle Quantitation in Patients With and Without Systemic Lupus Erythematosus

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Patients with systemic lupus erythematosus (SLE) frequently experience Libman-Sacks endocarditis, stroke or transient ischemic attack (TIA), and neuropsychiatric SLE (NPSLE). It is believed that stroke or TIA and NPSLE may result from ischemic brain injury due to a hypercoagulable state rather than from cardioembolism. These 2 pathogenetic factors pose a different diagnostic and therapeutic approach for prevention of neurological events in patients with SLE.

In a well-characterized cohort of 29 patients and 15 age- and sex-matched healthy control subjects, we measured cellular microparticle levels determined using flow cytometry according to cell lineage and presence of tissue factor and phosphatidylserine (PS) on the outer membrane. In addition, we compared microparticle levels among patients with SLE with and without a history of stroke or TIA and neuropsychiatric SLE.

Mean measurements (per microliter) in the patient and control groups were as follows: total microparticles, 2,735 and 2,874 ($P$ = .79); total PS+ microparticles, 543 and 504 ($P$ = .85); tissue factor+, 171 and 102 ($P$ = .36); platelet-derived, 534 and 552 ($P$ = .93); monocyte-derived, 609 and 760 ($P$ = .48); and endothelial-derived, 200 and 224 ($P$ = .72). Mean measurements among patients with and without past stroke or TIA were as follows: total microparticles, 2,646 and 2,797 ($P$ = .84); tissue factor+, 180 and 165 ($P$ = .92); platelet-derived, 565 and 513 ($P$ = .86); monocyte-derived, 561 and 643 ($P$ = .79); endothelial-derived, 218 and 188 ($P$ = .80); total PS+ microparticles, 537 and 547 ($P$ = .98); PS+ tissue factor+, 120 and 104 ($P$ = .90); PS+ platelet-derived, 478 and 411 ($P$ = .81); PS+ monocyte-derived, 242 and 279 ($P$ = .87); and PS+ endothelial-derived, 114 and 99 ($P$ = .89). Mean measurements among patients with and without NPSLE were as follows: total microparticles, 3,002 and 2,449 ($P$ = .43); tissue factor+, 159 and 182 ($P$ = .87); platelet-derived, 489 and 577 ($P$ = .75); monocyte-derived, 494 and 717 ($P$ = .45); endothelial-derived, 192 and 208 ($P$ = .88); total PS+ microparticles, 462 and 618 ($P$ = .60); PS+ tissue factor+, 102 and 117 ($P$ = .91); PS+ platelet-derived, 411 and 464 ($P$ = .84); PS+ monocyte-derived, 208 and 316 ($P$ = .62), PS+ endothelial-derived, 98 and 112 ($P$ = .86).

Levels of microparticles were similar among patients with SLE with and without stroke or TIA or NPSLE and in healthy control subjects. Therefore, ischemic brain injury in patients with SLE may result from cardioembolism rather than from hypercoagulability.

## 48

### Increasing Blood Center Donor and Employee Morale and Retention by Informing Them of the Disposition of Collected Blood

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Important goals of blood centers include increasing the morale and retention among donors and employees. Blood is usually transfused without feedback to the donors or employees involved in the process. We hypothesized that sharing the recipient's story with the donor and employees who "touched" the blood would increase donor and employee satisfaction, morale, and retention through their involvement in the process.

Information was elicited from transfusion services about interesting patients who received blood products with a successful outcome. Maps to track the path of these blood products were made. Our initial pilot indicated that an average of 10 blood center employees were involved in each donation. A notification system was set up to send a letter to employees who had contact with the product as well as the blood donor. These letters described, in an HIPAA-compliant manner, the recipient, indication for transfusion, the blood products received, and the outcome. Two surveys were prepared, one for employees and the other for donors. The employee survey contained 6 questions, and the donor survey contained 5 questions. Each survey question used a Likert scale of 0 to 10 regarding the respondent's judgment of the value of the notification system.

Surveys were sent to 250 blood center employees and 85 donors. An initial return response was received from 32 employees and 38 donors (13% and 45% response rates, respectively). The highest rated employee response was 9.4/10 to the question, "Do you feel that [this blood center] should continue informing donors and staff what happens to the blood?" The lowest rated response was 7.8/10 to "Do you feel that receiving information like this periodically would influence you to more likely continue working for [the blood center] rather than seeking another job?" The highest rated donor response was 9.5/10 to, "Describe your overall experience with donating blood [here]?" The lowest rated response was 7.7/10 to the question, "Was your time spent reading the story worthwhile?" Collection of additional data is ongoing to permit thorough analysis of donor and employee response.

Initial results indicate that donors and employees enjoy the feedback and strongly favor continuation of the notification program. Donors subjectively are more likely to donate in the future as a result of the feedback. Evaluating the employee survey results raised a complex question in differentiating employee morale vs employee retention. Data on employee turnover could be incorporated into future studies.

## 49

### Use of an Online Learning Management System to Teach a Clinical Lab Course for Family Medicine Residents

*Tim Amukele, Misbah Keen, Zehava Chen-Levy, and Michael Astion, University of Washington School of Medicine, Seattle.*

To date, 54% of all CLIA-certified laboratories in the United States are in physician offices. Laboratory training equivalent to 20 CME hours commensurate with director duties is required for nonpathology physicians to direct a moderate complexity laboratory. We did not find any examples of training programs specifically targeted at residents.

We derived 32 competencies using a SMART paradigm and have designed a pilot hybrid (online + experiential) course to fulfill the CLIA requirements for directors of moderate complexity laboratories. The current Web-based content covers basic concepts in laboratory medicine, including introduction to laboratory medicine, method validation, QA/QC, and how to order and interpret laboratory tests. These represent 6 hours of narrated PowerPoint lectures converted into flash files. Flash files allow viewing on all online devices, including cell phones. In addition to the lectures, we have assessment quizzes and 12 pdf articles that can be downloaded from the site. We deliver the content using a content management system called Moodle.

Moodle is interactive and easy to update and has embedded evaluation tools, including question statistics such as percentage correct, standard deviation, question discrimination index, and question discrimination coefficient. In addition, Moodle has several features that make testing reliable, including scrambling the order of questions and answers for each attempt and opening the quiz in a secure window.

Since it was deployed in September 2008, 5 of a possible 8 family medicine residents have taken the pilot course and completed a 15-question competency assessment. The results show an overall grade of 99%, indicating that the participants are completing the training. However, the current sample size precludes any conclusions about the quality of the assessment tool. Obtaining laboratory credentials will improve testing practices across the United States and increase the earning potential of future primary practice residents.

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## 51

### Monitoring Patient Compliance With Fluticasone Propionate Therapy by LC-MS/MS

*N.L. Korpi-Steiner,[*1] R.J. Singh,[1] B.C. Netzel,[1] J.C. Seegmiller,[1] M.R. Matthews,[2] and J.B. Hagan.[3] Sponsor: A.K. Saenger.[1] Departments of [1]Laboratory Medicine/Pathology, [2]Family Medicine, and [3]Allergic Diseases, Mayo Clinic, Rochester, MN.*

Inhaled corticosteroids such as fluticasone propionate (FP) are the most effective treatment for persistent asthma. However, noncompliance with therapy by 20% to 80% of treated patients is associated with substantial costs, morbidity, and fatalities. A noninvasive test to assess FP treatment compliance is needed. Our aims were to optimize measurement of FP-17β-carboxylic acid (17βFP), the major metabolite of FP, in urine by liquid chromatography–tandem mass spectrometry (LC-MS/MS) and to conduct a preliminary evaluation of its efficiency in monitoring patient compliance with FP.

The 17βFP was extracted from urine using acetonitrile protein precipitation and liquid-liquid extraction followed by LC-MS/MS analysis (ABI 4000). The linearity, limit of quantitation (LOQ), and imprecision were determined. Measurement of 17βFP in urine samples collected daily from patients before (day 1), during (days 2-5; total dose FP, 110 µg 2 puffs daily), and following cessation of FP therapy (days 6-14) was conducted (n = 4). Patients with asthma receiving witnessed administration of FP (n = 9) 16 to 24 hours before urine collection served as the "gold standard" for compliance, whereas patients with asthma not receiving FP (n = 10) served as control subjects. Patient 17βFP results were normalized to urine creatinine values.

The linear range of 17βFP measured by LC-MS/MS was 10 to 9,510 pg/mL, with an LOQ of 10 pg/mL. Within-run and between-run imprecision testing indicated maximum coefficients of variation of 9.3% at 10.3 pg/mL and 10.6% at 11.1 pg/mL, respectively. Urine 17βFP was below the LOQ in patients before FP therapy (day 1) and was detectable at concentrations ranging from 854 to 1,991 ng 17βFP/g creatinine following 2 doses of FP wherein 17βFP levels remained elevated throughout the dosing period, and 17βFP concentrations were again below the LOQ 5 days after ceasing therapy (days 11-14). The 17βFP metabolite was detected in all 9 patients receiving witnessed administration of FP and was not detected in 10 control subjects, indicating 100% clinical sensitivity and specificity.

Measurement of 17βFP by LC-MS/MS has acceptable analytical performance for clinical use. Preliminary clinical evaluation demonstrates 100% clinical sensitivity in the detection of 17βFP in patients receiving low-dose FP therapy and 100% specificity in patients with asthma not receiving FP therapy. These data support the clinical utility of measuring 17βFP in urine samples to monitor patient compliance with FP therapy.

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## 52

### D-Dimer Assay Adaptation on an Automated Chemistry Analyzer to Reduce Turnaround Times

*Alex C. Chin, Francoise Brierre, Corinne R. Fantz, and James C. Ritchie. Department of Pathology and Laboratory Medicine, Emory University, Atlanta, GA.*

Pulmonary embolism (PE) and acute coronary syndrome are characterized by dyspnea and thoracic pain, which are common clinical symptoms exhibited by patients presenting at emergency care centers and require prompt diagnosis by the clinical team. In the laboratory, the small D-dimer fibrin degradation fragment and cardiac troponin I are measured to exclude PE and detect acute coronary syndrome, respectively. Currently in our laboratory, plasma D-dimer is quantified from sodium-citrate tubes on the Dade-Behring BCS coagulation analyzer, while plasma cardiac troponin I is assessed from lithium-heparin tubes on the Beckman LX-20 chemistry analyzer. Development of a high-throughput automated method that measures both D-dimer and cardiac troponin I concentrations from the same specimen would be advantageous in improving turnaround times and patient management.

Our objective was to compare method performance of an automated chemistry with an automated coagulation D-dimer assay. D-dimer assays from Roche Diagnostics and Diagnostica Stago were modified for use on the Beckman LX-20 and the Dade-Behring BCS, respectively. Method comparisons and clinical outcomes were assessed on 43 citrated plasma samples. Next, comparisons between patient-matched citrated and heparinized plasma samples were performed on the Roche/LX-20 assay.

Low (429 ng/mL) and high (1,815 ng/mL) control interassay precisions of the Roche/LX-20 assay were 5.32% and 1.15%, while intra-assay precisions were 2.12% and 2.02%, respectively. Linearity was assessed by dilution of the manufacturer's calibrator (range, 0-4,290 ng/mL). Initial evaluations between the LX-20/Roche (y) and the BCS/Stago (x) methods on citrated plasma samples yielded a Deming correlation of $y = 0.746x + 18.178$, $r = 0.9504$. The BCS/Stago cutoff of less than 260 ng/mL corresponded to an LX-20 Roche cutoff of less than 212.14 ng/mL, which yielded sensitivity and specificity values of 91.2% and 100%, respectively. Of the 9 patients who

 

had D-dimer levels of less than 212.14 ng/mL in both methods, 5 had thoracic pain and PE was ruled out of the final diagnosis.

The assay on the chemistry analyzer is fully automated and exhibits good performance characteristics regarding interassay and intra-assay precision and acceptable correlation with the coagulation analyzer. Adaptation of a D-dimer assay on a chemistry analyzer allows simultaneous testing with cardiac markers from the same specimen that, in turn, should lead to better turnaround times and, ultimately, accurate and quicker patient diagnosis.

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## 53

**Reevaluation of Turbidity as a Method for Screening Lipemic Blood Specimens**

*Kazunori Murata, PhD,* Jean M. Hornseth, Joseph P. McConnell, PhD, and Amy K. Saenger, PhD. Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN.*

Lipemic specimens pose an analytical challenge in clinical chemistry because the presence of lipids can interfere with the measurement of numerous analytes. One such analyte is sodium, which may be falsely low in lipemic specimens when quantified using the indirect ion-specific electrode (ISE) method. Current chemistry analyzers screen for lipemic specimens by measuring specimen turbidity, referred to as the L-index. However, L-indices and serum triglyceride concentrations have been found to correlate poorly with each other, necessitating reevaluation of methods used to screen lipemic specimens.

The objective of our study was to determine whether the poor correlation between L-indices and serum triglycerides could be explained by the presence of chylomicrons and very-low-density lipoprotein (VLDL) particles in serum. An additional objective was to determine which of the measures of lipemia—the L-index, triglyceride concentration, total cholesterol, or high-density lipoprotein (HDL) cholesterol—was most predictive of the degree of pseudohyponatremia seen in lipemic specimens.

L-indices and triglyceride concentrations were measured for 32 lipemic serum specimens on a Roche Cobas 6000 modular chemistry analyzer, followed by analysis on a Bruker-Biospin 400MHz HNMR using the LipoScience NMR LipoProfile method to measure the total VLDL and chylomicron particle concentration. An additional 46 lipemic and nonlipemic serum specimens were analyzed for the L-index and triglycerides, total cholesterol, HDL cholesterol, and sodium levels, measured by indirect and direct ISE.

L-indices correlated poorly with the serum triglyceride concentrations ($R^2 = 0.497$) and with the combined chylomicron and total VLDL particle concentration ($R^2 = 0.280$). The percentage difference in sodium concentrations between values obtained by indirect and direct ISE was also not found to correlate with the L-index, triglyceride concentrations, total cholesterol level, or HDL cholesterol level.

We conclude that the L-index in lipemic specimens is not always reflective of the presence of lipid and/or lipoprotein particles and may be due to the presence of other unknown constituents. Furthermore, the extent of pseudohyponatremia is not always predictable based on the various indices of lipemia.

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## 54

**Performance Validation of QIASymphony DNA Extraction for the Artus/Qiagen RealArt CMV TM PCR Assay: Method Comparison With the MagnaPure Total Nucleic Acid Kit**

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Human cytomegalovirus (CMV) can cause severe clinical manifestations in neonates, HIV-infected people, and solid organ and bone marrow transplant recipients. Quantitation of CMV plasma viral load can be used to predict development or relapse of disease and to monitor therapy in patients with AIDS and transplant recipients.

We compared the performance of the Qiagen QIASymphony and Roche MagnaPure automated extraction platforms for extraction of CMV DNA for use in a quantitative real-time CMV PCR assay (QPCR). Extraction of CMV viral nucleic acid was performed using the MagnaPure and QIASymphony, and CMV target amplification and detection were performed using the Artus/Qiagen RealArt CMV TM PCR assay on the ABI 7500. Linearity was assessed by 5-fold serial dilutions of a known high-positive patient EDTA plasma sample, and within-run precision (%CV) was determined with 8 sample replicates at each dilution. The limit of detection was determined by probit analysis of multiple replicates of serial 2-fold dilutions of the Acrometrix CMV high-positive control. Patient sample comparison within the dynamic range of the assay was performed using 45 stored positive patient samples. Evaluation of bias included linear regression and determination of the mean difference and SD between extraction methodologies.

Quantitative CMV PCR results using the QIASymphony extraction method showed a mean positive bias of 0.19 $\log_{10}$ copies/mL compared with QPCR following MagnaPure extraction. By using QIASymphony extraction, the CMV QPCR assay was determined to be linear for a range of 64 to $5.0 \times 10^6$ copies/mL. Terminal dilution studies demonstrated 95% detection at 9.5 copies/mL. The CV near the limit of detection was 7.96% at 2.26 $\log_{10}$ copies/mL and 0.44% at 6.79 $\log_{10}$ copies/mL.

We found the performance between the QIASymphony and MagnaPure automated extraction platforms to be comparable when used with the Artus CMV PCR assay. Also, the difference in CMV viral loads between extraction methods was clinically insignificant and did not require rebaselining of patient results. Finally, we found that the QIASymphony platform offered more flexibility in batch size, allowing for more cost-effective utilization of reagents and consumables and improved work flow.

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## 55

**Compound Heterozygosity for Hemoglobin S [β6(A3)Glu6Val] and Hemoglobin Korle-Bu [β73(E17)Asp73Asn]**

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We report a case of compound heterozygous hemoglobin S [β6(A3) Glu6Val] and Korle-Bu [β73(E17)Asp73Asn] in a 2-year-old girl. This hemoglobin genotype is associated with a benign clinical course, much like sickle cell trait. However, its laboratory characteristics are very similar to compound heterozygous hemoglobin S and D-Los Angeles [β121(GH4)Glu121Gln], which results in severe sickling hemolytic anemia.

Cation-exchange high-performance liquid chromatography (HPLC), cellulose acetate (pH 8.6) electrophoresis, citrate agar (pH 6.2) electrophoresis, and β-globin gene sequencing were used to establish the patient's hemoglobin phenotype. Three-dimensional

modeling was used to predict the location of mutant amino acid residues on the hemoglobin molecule.

HPLC and electrophoresis were suggestive of hemoglobin S and Korle-Bu but could not exclude hemoglobin S and D-Los Angeles completely. β-Globin sequencing revealed compound heterozygosity for [β6(A3)Glu6Val] and [β73(E17)Asp73Asn], which established the diagnosis of hemoglobin S and Korle-Bu. Molecular modeling demonstrated that Korle-Bu and D-Los Angeles mutations both alter the surface of hemoglobin, although at different locations, and, thus, could affect interactions with sickle hemoglobin differently. The interactions between hemoglobin S and these variant hemoglobins could account for the different clinical phenotypes in compound heterozygotes.

It is clinically important to distinguish compound heterozygous hemoglobin S and Korle-Bu from hemoglobin S and D-Los Angeles because the former is a benign trait condition, while the latter is a severe sickling disorder. Given the relative gene frequencies of hemoglobins S, Korle-Bu, and D-Los Angeles, this critical differential diagnosis should be recognized, particularly in people of African ancestry.

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## 57

### Diagnostic and Prognostic Utility of Procalcitonin in ICU Patients After Major Trauma or Surgical Event

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Sepsis is a major cause of death among critically ill patients. Detection of serum procalcitonin (PCT) is associated with disease severity and prognosis and aids in diagnosis and monitoring of sepsis. PCT is elevated after surgery or trauma in the absence of sepsis; therefore, the diagnostic utility of PCT is questionable in this situation. Monitoring changes in PCT over time may improve its use as a sepsis marker in ICU patients.

Our objective was to evaluate the diagnostic and prognostic utility of PCT in trauma and surgical ICU (TICU and SICU) patients. All patient samples were surplus serum specimens collected from physician-ordered chemistry panels performed in the Vanderbilt University Core Laboratory; 113 samples from 56 ICU patients were used to determine population-specific cutoffs. Specimens from 100 separate patients (51 in SICU and 49 in TICU), each collected for 7 consecutive days, were used to evaluate the prognostic strength of PCT. PCT assays were performed on the BRAHMS Kryptor. Optimal cutoffs were determined by receiver operating characteristic (ROC) analysis.

Patients were categorized as septic (n = 34, 61%) or nonseptic (n = 79). Areas under the ROC curves were 0.7 (95% confidence interval, 0.59-0.8), 0.84 (0.71-0.96) and 0.62 (0.48-0.76) for the combined population, TICU, and SICU respectively. Optimal cutoffs were 0.5 ng/mL (sensitivity = 74%, specificity = 65%), 0.5 ng/mL (sensitivity = 56%, specificity = 94%), and 15 ng/mL (sensitivity = 27%, specificity = 6%) for the total, TICU, and SICU populations, respectively, with corresponding odds ratios for developing sepsis of 0.2 ($P < .0001$), 20.3 ($P = .001$), and 3.3 ($P = .044$). The mean PCT and the maximal and total change in PCT over 7 days did not correlate with overall survival ($P = .78$, $P = .19$, and $P = .098$ respectively). PCT did not correlate with bacterial culture positivity (relative risk = 0.79; $P = .32$). Mean PCT values were significantly different in samples collected fewer than (mean ± SEM = 6.1 ± 0.95) or more than (mean ± SEM = 3.3 ± 0.67) 48 hours after trauma or surgery ($P = .026$).

PCT is a poor predictor of infection and prognosis when evaluated singly or over 7 consecutive days in both TICU and SICU patients. PCT values were correlated with number of days after trauma or surgery. Thus, single or consecutive measurements of PCT cannot be used in isolation to diagnose infection in SICU and TICU patients. PCT may be useful in combination with other clinical parameters and other laboratory values to evaluate sepsis in these populations.

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## 58

### Human Chorionic Gonadotropin: Do You Know the Specificity of Your Immunoassay?

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Human chorionic gonadotropin (CG) is a heterodimeric glycoprotein hormone with considerable molecular heterogeneity. CG has clinical utility in the diagnosis of pregnancy, as a Down syndrome risk marker, and as a tumor marker. The relative abundance of specific CG isoforms produced during pregnancy can differ from those produced in nonpregnancy disorders. As such, the analytical specificity of CG immunoassays should be well described. However, there is uncertainty regarding which CG isoforms are detected by different CG immunoassays.

Our objective was to determine the analytical specificity of 8 commercially available CG immunoassays. Purified first World Health Organization reference reagent preparations of CG, nicked CG (CGn), β-subunit (CGβ), nicked β-subunit (CGβn), and the β core fragment (CGβcf) were obtained from the National Institute for Biological Standards and Controls (Hertfordshire, England) and reconstituted with phosphate-buffered saline buffer. Test specimens were prepared by adding each isoform to aliquots of CG-negative, human female serum to create 5 concentrations (target range of 50-100,000 IU/L). Specimens were analyzed in duplicate using 8 CG immunoassays (Siemens Immulite 2000 HCG, Beckman DxI Total βhCG, Siemens ADVIA Centaur Total hCG, Abbott Architect Total βhCG, Roche Elecsys 2010 HCG+ β, Vitros ECi Total βhCG II, Tosoh AIA-1800 Total βhCG, and the Siemens Dimension RxL HCG FLEX). General linear models comparing each assay with the Immulite, controlling for the 5 different concentrations of each isoform, were used to calculate the given $P$ values using SAS software, version 9.1 (SAS Institute, Cary, NC).

The Immulite assay detected all CG isoforms. The Elecsys was able to detect CGβcf, but not within the 95% confidence limit ($P = .045$). The Dimension RxL was unable to detect CGβ ($P = .036$), CGβn ($P = .027$), and CGβcf ($P = .037$). Assays detected all other isoforms with the exception of the following that, while detected, were not within the 95% confidence limit ($P < .05$): DxI: CG and CGn; Centaur and Architect: CGn, CGβ, and CGβn; Elecsys: CGn; ECi: CG and CGβ; Tosoh: CG and CGβ; and Dimension RxL: CG.

Immunoassays for CG demonstrate considerable variability in their ability to detect specific CG isoforms. The inability of the Dimension RxL assay to detect CGβ and CGβn is acknowledged by the manufacturer, yet laboratorians may be unaware of this limitation. When the detection of CGβcf is necessary, only the Immulite and the Elecsys CG assays should be utilized.

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## 60

### Discordant Roche Elecsys Thyroid Function Tests due to Interference From Antibodies Against Ruthenium-Tag and Streptavidin

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Thyroid function tests (TFTs) on the Roche Elecsys are based on the electrochemiluminescence immunoassay format (ECLIA). Antibodies (Abs) against the signal generating ruthenium (Ru) tag or the capturing complex (biotin tag or streptavidin-coated paramagnetic beads) are potential sources of assay interference, despite blocking agents in reagents. In case 1, the Elecsys TFT for a 50-year-old woman with well-controlled Hashimoto thyroiditis revealed an elevated total T4 level (168 µg/dL), a low thyroxine binding index of 0.32, and an elevated fT4 index (53) that were discordant with a normal TSH level (0.90 mIU/L). In case 2, a 76-year-old woman with aortic stenosis who was being evaluated for cardiac catheterization showed an elevated total T4 level (19.8 µg/dL) and a high fT4 index (99.0) and normal TSH level (0.40 mIU/L). These were considered likely laboratory artifacts rather than thyroid resistance (rare).

Aliquots of original samples were retested in multiple alternative laboratories that used different immunoassay formats (Siemens Advia Centaur and Immulite) and sent to Roche Diagnostics in Germany for further workup of potential interfering factors. Roche pretreated the respective patient's serum samples (1:5 diluted) with specific adsorbing target solutions, followed by remeasurement using Elecsys. The groups were as follows: no pretreatment, excess streptavidin beads, nonspecific sheep polyclonal Abs, and nonspecific Abs with Ru-tag. Abs removal by PEG precipitation was also performed.

Retesting by non-ECLIA methods showed normal TFTs in both cases. In case 1, only pretreatment with nonspecific antibodies with Ru-tag led to a significant depression of fT4 (1.30 to 0.89 ng/dL) and total T4 (4.2 to 2.5 µg/dL). In case 2, the streptavidin bead pretreatment group gave the largest decrease in total T3 (3.36 to 1.68 nmol/L) and total T4 (17.4 to 6.5 µg/dL), and elevation in TSH (0.32 to 0.77 µU/mL) and Tup (<0.22 to 0.77). In both cases, PEG precipitation significantly reduced the TFT interferences.

These 2 cases illustrate the occurrence of discordant TFTs due to the presence of Abs against the signal-generating Ru complex and capture complex (streptavidin beads), respectively. These occasional interferences can occur for any given immunoassay; when discordant results are suspected, it is important to confirm them with alternative methods using a different assay format.

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### Heparin-Induced Thrombocytopenia Antibody ELISA Turnaround Time and Direct Thrombin Inhibitor Use: A Single Institutional Experience

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The purpose of this study is to determine if implementing in-house heparin-induced thrombocytopenia (HIT) ELISA testing with a more rapid turnaround time (TAT) decreases unnecessary direct thrombin inhibitor (DTI) use. We retrospectively gathered data from our hospital database for all inpatient HIT antibody ELISA assays from June to October 2008 (referral testing period) and October 2008 to January 2009 (in-house testing period). During the study period 315 inpatient assays were ordered and results reported (142 referral and 173 in-house). The mean TAT measured from the day of sample collection to the day results were available was statistically different: 2.5 ± 0.7 days (range, 1-4 days) during the referral testing period and 1.4 ± 0.9 days (range 0.5-5 days) during the in-house testing period. DTI therapy was initiated in 17% of patients during the referral testing (21/125 patients) and in-house testing (26/150 patients) periods. During the referral testing period, 90% (19/21) of patients treated with DTIs had therapy started before results were reported and 10% (2/21) had therapy started after results were reported. Similarly, 81% (21/26) of patients had therapy initiated before results were reported and 19% (5/26) of patients had DTI therapy started after results were reported during the in-house testing period. The mean number of days that a patient with a negative result received DTIs was not statistically different during the referral and in-house testing periods, 3.3 ± 1.2 days and 2.9 ± 1.5 days, respectively. Although in-house HIT antibody testing decreases TAT, the results of this study suggest that it may not lead to fewer days on DTIs in patients with a negative test result. It may also suggest that a greater reduction in TAT is required to see an impact on patient management or that decreased TAT alone is not sufficient to change DTI use in the absence of physician education and hospital-wide policies to reduce heterogeneity in management of HIT.

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## 62

### A Multidisciplinary Approach Leads to Proper Diagnosis and Treatment of Cryoglobulinemia: A Case Report

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With the ever-increasing pressures to cut health care costs, the clinical pathologist and laboratory staff have a significant role in optimizing patient care through close collaboration with the clinical team. We describe a case in which the diagnosis and management exemplify this concerted effort. The patient was a 68-year-old white man with a history of non-Hodgkin lymphoma who was transferred to our hospital with an 8-day history of severe epistaxis and bilateral "sausage" engorgement of retinal vessels. While cryoglobulinemia was in the differential diagnosis, laboratory studies had been thus far inconclusive. On admission, his serum viscosity was reported as normal and the serum immunofixation could not be done owing to lack of protein migration from the point of origin. Owing to persistent epistaxis, plasma exchange was requested. During the first procedure, protein precipitation caused occlusions and multiple instrument alarms. Under the direction of the clinical pathology group, blood samples were drawn and kept at 37°C during transport to the laboratory and while testing was performed.

The patient's serum viscosity was elevated at 2.1, the "cryocrit" was 11%, and serum immunoglobulin levels were decreased (IgG, 456 mg/dL; IgM, 42 mg/dL; and IgA, 16 mg/dL). Type II cryoglobulinemia was diagnosed by the presence of a monoclonal IgM κ and a single IgG polyclonal band in the serum and a κ light chain–restricted cryoglobulin. His urine immunofixation was also positive for a free κ light chain. While many patients with type II cryoglobulins have chronic hepatitis C, there was no evidence of such infection. Bone marrow biopsy showed involvement by a B-cell lymphoma, small cell type, with cryoglobulin production. In order to successfully perform plasma exchange, we warmed the patient's

room, used a blood warmer, and entered a falsely high hematocrit in the instrument setting in order to avoid red cell spillage.

Immune complex–mediated vasculitis is commonly seen in mixed cryoglobulins and most likely caused the patient's epistaxis and visual changes. His diagnosis was delayed by improper collection and handling of the blood sample, which caused partial precipitation of the cryoglobulins and false-negative results. Clinical pathology consultation was essential to the establishment of the diagnosis and prompt management of his symptoms.

## 63

**Vitamin D Measurement: Cost-Effectiveness of In-House Testing Using LC-MS/MS**

*Brandie L. Firetag, Deborah French, Kara L. Lynch, and Alan H.B. Wu. Department of Laboratory Medicine, University of California San Francisco.*

The functions of vitamin D have drastically expanded from the traditional role in bone mineralization to functions in autoimmune diseases, cancer, diabetes, and cardiovascular disease. Consequently, the volume of 25-hydroxy vitamin D (25-OH D) testing has dramatically increased in the last several years. At our institution alone, the number of annual tests performed has risen from 568 in 2006, to 1,205 in 2007, and 2,655 in 2008. Last year, we spent nearly $78,500 in send-out testing on 25-OH D. The 2 most common platforms used for determination of plasma concentrations of 25-OH D include liquid chromatography–tandem mass spectrometry (LC-MS/MS) and the DiaSorin LIAISON, which is a chemiluminescent assay. Whereas the LC-MS/MS is more precise and is used in comparison studies as the standard, it is technically demanding. In contrast, the DiaSorin assay is automated and requires less technical expertise.

Our objectives were to develop an LC-MS/MS method for 25-OH D and examine the cost-effectiveness vs bringing in the DiaSorin LIAISON chemiluminescent assay and determine which platform would be more feasible given our test volume. We have developed an LC-MS/MS assay that can be used to identify 25-OH D2 and D3 in plasma and requires only a 4.5-minute run time per sample. After the addition of D6–vitamin D3 (internal standard), 25-OH D2 and D3 were isolated using solid-phase extraction. Gradient chromatographic separations were performed on a Luna C18, 5-µm (50 × 2mm) column maintained at 65ºC using an ammonium acetate/formic acid mobile phase (Agilent 1200 Series LC). Both 25-OH D2 and D3 were monitored in MRM-IDA-EPI mode (Applied Biosystems 3200QTRAP MS/MS) using transitions of 397.3>271.3 and 385.3>259.3, respectively. The method is currently being validated.

With the current volume of approximately 250 samples of 25-OH D testing per month, in-house test using LC-MS/MS would save approximately $51,000 per year over send-out testing. Using the DiaSorin LIAISON, we would save about $30,000 per year. Other considerations include volume; with LC-MS/MS, we can manage current demand by batching 2 runs per week for a total of 60 tests per week. A test volume exceeding 475 tests per month would require an additional LC-MS/MS. The DiaSorin LIAISON can perform about 90 tests per hour, which is significantly more efficient.

Given that our laboratory currently has experienced personnel and an LC-MS/MS instrument, we have been able to develop a method for 25-OH D that is currently being validated that would be economically sound to perform 25-OH D testing in-house with a maximum volume of 475 tests per month.

## 64

**An Evaluation of a Rapid HIV Test on Neonate Blood as a Potential Surrogate Sample for Mothers of Unknown HIV Status**

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Although HIV testing in pregnant women is recommended by the Centers for Disease Control and Prevention guidelines, current point-of-care (POC) tests are Food and Drug Administration approved only for the testing of people older than 16 years. Since there is a 48-hour window of opportunity postpartum to identify HIV-exposed neonates and prophylactically administer antiretroviral therapy, it becomes critical to know the HIV status of the mother. Unfortunately, as we have found in our institution, occasionally the mother is unavailable or refuses to consent to an HIV test. In this situation, rapid testing of the neonate provides a crucial opportunity to assess perinatal exposure and, thus, trigger treatment with antiretroviral therapy in time to greatly reduce perinatal HIV transmission to the neonate. Accordingly, the focus of this study is to correlate the neonate's HIV serological results with those from the mother in order to establish that the neonate's results adequately reflect the HIV status of the mother and that our rapid POC HIV testing device (OraQuick Advance HIV-1/2 test) correlates with our more established Siemens Centaur method and the methods being used at Mayo Laboratories.

The study population consisted of 56 children, 42 younger than 4 days and 14 from 14 days to 11 years old. When neonates were compared with mothers for HIV serologies, we observed the following: OraQuick, 13 negative/13 negative; 1 positive/1 positive; and Centaur, 7 negative/7 negative; 4 positive/4 positive. When the OraQuick results are compared with the Centaur results, we observed the following: 51 negative/51 negative and 4 positive/4 positive. On comparison of OraQuick results with Mayo results, we observed the following: 25 negative/25 negative and 2 positive/2 positive. The latter 2 positive results were confirmed by Western blot. Summarizing, when rapid HIV test results were compared between neonates and mothers regardless of the method used, 100% agreement was observed.

## 65

**Identification of Platelet and Monocyte Microparticles**

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Microparticles (MPs) are cell fragments released in response to activation, injury, or apoptosis. MPs retain the characteristics of the parent cell and are thought to contribute to a prothrombotic state in many different clinical settings, including trauma, cardiac disease, infection, and cancer. MPs have been defined as cell fragments 0.5 to 1.0 µm in size, 1.0 µm selected to separate MPs from the normal platelet size distribution. There has been little agreement on the normal number of MPs in plasma (published mean normal levels of MPs range from 237/µL to 2,014/µL). Our objectives were to evaluate different commercial beads used to calibrate size based on forward scatter (FSC) and to determine whether platelet and monocyte MPs could be identified using CD41a and CD14, respectively, on an LSRII flow cytometer.

Beads were evaluated from 2 companies. Company A (NIST size-certified) 1.0-µm latex beads overlapped with only the lower

5% of the normal platelet size distribution consistent with a 1.0-μm size. Company B (non–size-certified) 1.0-μm beads overlapped with approximately 40% of normal platelet size distribution, suggesting that the bead was actually larger than 1.0 μm. The 0.5-μm beads from both companies gave similar FSC results. We selected the NIST-certified beads to calibrate the flow cytometer with respect to size. To determine whether CD41a could be used to identify platelet MPs, we first stained platelet-rich plasma with CD41a, which produced a clear positive peak that correlated with the number of platelets in the sample by automated cell counter. We then produced artificial platelet MPs by repeated freeze-thaw resulting in platelet fragments in the 0.5- to 1.0-μm range. There was a clear separation (1.5 log difference in the fluorescence brightness) of the artificial platelet MPs vs CD41a– MPs in the same samples. For monocytes, CD14 clearly separated monocytes from other normal WBCs.

We then purified CD14+ cells using a cell sorter. Artificial monocyte MPs were prepared by repeated freeze-thaw of these purified CD14+ cells. The CD14 fluorescence of the artificial monocyte MPs was 10-fold less than the original cell and partially overlapped the CD14– population, possibly owing to reduced monocyte MP surface area. The typical monocyte has a diameter of 20 μm and a surface area of about 1,000 $\mu m^2$, whereas the MPs based on our size gate had an average diameter of 0.75 μm and a surface area of about 2 $\mu m^2$, 500-fold smaller than the original cell.

We recommend using NIST-certified size beads to calibrate MP size on flow cytometers as other beads were not the size-indicated. CD41a could be used to identify platelet MPs prepared from platelet-rich plasma, but CD14 only partially separated MPs prepared from purified monocytes from other MPs in the plasma.

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## 66

**Comparison of Immunofixation Electrophoresis Band Detection by Examination of Physical Gels vs Electronic Gel Imaging**

*Shree Sharma, Kellen Sweat, Gina Pesek, John Theus, and Joshua Bornhorst. Department of Pathology, University of Arkansas, Little Rock.*

Examination of digital images of physical specimens is an increasingly attractive option for the modern laboratory owing to the potential instant recall, ease of storage, and possible diagnostic advantages stemming from image enhancement. One such technique in which electronic imaging may be of use is serum immunofixation electrophoresis (IFE), which is commonly used in the identification and monitoring of monoclonal gammopathies. The storage and retrieval of physical IFE gels can be time-consuming. We systematically compared concordance of the observation of bands on physical gels with electronic images.

A total of 43 IFE gels submitted from a myeloma clinic were randomly selected. Gels were produced using a Sebia Hydrasys Hydragel kit. Gels were scanned on the Sebia-provided Epson Perfection V700 photo scanner with 6400 DPI resolution and visualized using the Sebia Capillarys imaging software (v.6.1.2). No image alteration was undertaken. Two experienced pathologists and a clinical chemist independently reviewed the images and gels. Reviewers were blinded to any previous interpretation or patient identification. Scan and gel examination were separated by a period of more than 1 day. The gels were examined for distinct and indistinct IgG, IgA, and IgM bands with associated κ and λ chains and free κ or λ bands.

The average number of bands per patient for all observers for scans was 0.76, which was less than the observed number of bands per patient of 1.5 for gels. The difference in the set of the mean number of bands for each patient by gel vs the set of the mean number of bands for each patient by scan was statistically significant by paired *t* test ($P < .001$). The mean number of bands per patient for the 3 individual observers were 0.81, 0.69, and 0.81 for scans and 1.67, 1.57, and 1.26 for gels. No significant ($P < .05$) interobserver difference was established by ANOVA analysis for gels or digital scans.

The number of bands observed in the digital images was significantly lower than was observed on gels, which may preclude their suitability in clinical use. The differences between images and gels were greater than interobserver differences. While the cause is unknown, there are continual improvements in digital imaging, and no attempt was made to enhance the images in the case. Similar systemic studies should be undertaken before the adoption or alteration of a digital imaging system in routine practice.

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## 67

**Cost-Effectiveness of Pharmacogenomic Testing for Clopidogrel**

*Sarah M. Calkins and Alan H. Wu. Department of Laboratory Medicine, University of California, San Francisco.*

The 2006 update of AHA/ACC Guidelines recommends that clopidogrel in combination with aspirin should be initiated in patients after acute coronary syndrome or percutaneous coronary intervention (PCI) with stent placement. Clopidogrel is a prodrug that undergoes cytochrome P450 metabolism in order to exert antiplatelet effects. Recent studies have shown that patients with loss-of-function polymorphisms in cytochrome P450 2C19 (CYP2C19) poorly metabolize clopidogrel and do not gain the complete antiplatelet benefit. These patients have an increased risk for myocardial infarction (MI), stroke, and death.

We investigated the situations in which genotypic testing of the CYP2C19 would be a cost-effective strategy based on primary efficacy outcomes including acute MI, stroke, and death. Based on outcome data published in 3 recent publications, the search was limited to principal diagnosis of diagnosis related groups (DRGs) related to MI, stroke, and death due to cardiovascular causes. The mean dollar amounts for these 9 diagnoses ranged from $19,442 for circulatory disorders with acute MI without major complications, discharged alive (DRG 122) to $55,797 for PCI with drug-eluting stent with major cardiovascular disorders (DRG 557).

Simon et al reported (*N Engl J Med,* 2009) an additional 4 patients out of 2,208 could theoretically undergo alternative therapy and avoid MI, stroke, or death by identifying risk based on having 2 loss-of-function alleles. If we assume these patients to have DRG 122, the genetic testing would have to cost $35 or less to be cost-effective. On the opposite end of the spectrum, Collet et al reported (*Lancet,* 2009) that genetic testing could have identified 8 additional patients out of 259 who would fail clopidogrel therapy and have an adverse event. If we assume these patients to have DRG 557, testing of the 259 subjects would need to be less than $1,723 to be cost-effective. These are theoretical extremes. Clinically, a combination of DRGs would be seen among affected individuals. By combining results from the 3 studies and using an average charge for the DRGs, CYP2C19 testing of all 3,926 subjects would have to be less than $266 per patient to be cost-effective.

Current reimbursement for pharmacogenomic testing is in the range of $250 to $450. Based on mean data calculated, the cost of genotype testing would fall very near the break-even point for the cost of treating adverse events. Further study will be necessary as this analysis did not evaluate secondary diagnoses, quality life years gained, or the impact on other therapeutics that also use this metabolic pathway.

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## 68

**Screening for HLA-B Alleles Associated With Drug-Induced Severe Cutaneous Reactions**

*Kara L. Lynch,* Kathy Chen, Debbie A. Kane, and Alan H.B. Wu. San Francisco General Hospital/University of California-San Francisco, San Francisco, CA.*

Stevens-Johnson syndrome, toxic epidermal necrolysis, and drug-hypersensitivity syndrome are severe cutaneous adverse reactions (SCARs) that have high morbidity and mortality. Recent studies have identified a strong genetic association between HLA-B alleles and susceptibility to SCARs, such as HLA-B*5701 and abacavir and HLA-B*1502 and carbamazepine. These findings have led the Food and Drug Administration to make changes to the black-box warnings urging health care professionals to screen patients in genetically at-risk populations for B*5701 and B*1502 before initiating treatment with abacavir and carbamazepine, respectively. Methodologies used for HLA-B typing are labor intensive, costly, and not readily available in routine clinical laboratories.

This study was designed to develop and evaluate methods for in-house screening of high-risk HLA-B alleles and determine the cost savings of implementing a screening method and reflex send-out confirmation for positive screens compared with sending all testing out to a reference laboratory. A flow cytometry screening method was developed and validated using an antibody that detects all subgroups of HLA-B57/B15/B40/B13 (provided by S. Mallal). Blood samples from 200 patients were tested. Intermediate resolution typing was determined for a subset of the samples using a reverse PCR/SSOP method (LABType SSO, One Lambda) and Luminex xMAP technology. Results from these 2 in-house methods were compared with sequenced-based typing results.

The sensitivity and specificity of the flow cytometry method were 100% and 78% with a negative predictive value (NPV) of 100%. The low specificity of the antibody is due to its ability to detect more than one HLA-B serological group and all HLA-B*57 subgroups. The flow cytometry method using this antibody had a sensitivity of 100% and an NPV of 100% for HLA-B*1502. The PCR/SSOP method also had a sensitivity and an NPV of 100% for HLA-B*5701 and HLA-B*1502. At the SFGH laboratory in 2008, there were 307 requests for B*5701 typing. All tests were sent to a reference laboratory, which cost the SFGH lab $47,160. Using the flow cytometry method or PCR/SSOP typing for screening and reflex send-out confirmation for positive screens would have resulted in laboratory savings of $32,018 and $35,093, respectively.

A screening method used to rule out the presence of an HLA-B allele before prescribing a high-risk drug needs to be 100% sensitive to eliminate the risk of SCARs. The flow cytometry and PCR/SSOP methods were 100% sensitive for the detection of B*5701 and B*1502. These methods offer a fast, sensitive and cost-effective screening assay for routine use in the clinical laboratory. As new associations are discovered, these screening methods could be expanded for the detection of other high-risk alleles.

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## 69

**Hyperbilirubinemia in Plasma Samples Can Be Misinterpreted as an Autoimmune Inhibitor in ADAMTS-13 FRET-VWF73 Assay**

*Shiu-Ki R. Hui, Sharniece C. Covill, and Kenneth D. Friedman. Sponsor: Alan E. Mast. Blood Center of Wisconsin, Milwaukee.*

A low ADAMTS-13 activity with the presence of autoimmune inhibitor (by mixing study) is found in many patients with thrombotic thrombocytopenic purpura (TTP). Assay of ADAMTS-13 by FRET-VWF73 (FRET) uses optical end points to measure activity. A recent article (*J Thromb Haemost.* 2007;5:866) suggested that hyperbilirubinemia can interfere with the FRET assay, falsely decreasing ADAMTS-13 activity. In addition, others (*J Thromb Haemost.* 2007;5:1330) have suggested that bilirubin oxidase (BOD) treatment could restore the integrity of the assay. The objective of this study is to determine the effect of hyperbilirubinemia in our in-house FRET assay, the potential of hyperbilirubinemia to influence the inhibitory titer results, and the efficacy of BOD treatment to eliminate such interference.

To determine effect of hyperbilirubinemia interference, normal plasma is diluted with heat-treated plasma (NTP) to produce final plasma with ADAMTS-13 activities of 50% or 25%. These samples were then spiked with synthetic or patient-derived bilirubin (heat-inactivated plasma from a patient with hyperbilirubinemia at 53 mg/dL) at increasing bilirubin levels up to 26 mg/dL. ADAMTS-13 activities of these spiked samples were then measured by FRET assay. ADAMTS-13 activities assay showed dose-dependent interference with synthetic and patient-derived bilirubin; residual activity (RA) at 26 mg/dL was 50%. This level of interference was significantly lower with our in-house assay compared with the literature (50% vs 10% RA at 26 mg/dL). The inhibitory effect of hyperbilirubinemia by FRET assay was evaluated via mixing study using normal plasma and hyperbilirubinemia plasma from patients before and after BOD treatment. At a bilirubin level of 25 mg/dL, the RA was 54% calculating to an inhibitor level of 0.8. Post-BOD treatment, the RA was restored to 90%, showing elimination of the inhibitory activity. Finally, the FRET assay was done on several hyperbilirubinemia (up to 50 mg/dL) samples from patients before and after BOD treatment. Post-BOD samples showed elimination of inhibitor activities and some increases in ADAMTS-13 activities.

In conclusion, the sensitivity to hyperbilirubinemia interference on the FRET assay appears to be laboratory- and method-specific. Because inhibitor activity is determined by mixing study, high bilirubin interference can be misinterpreted as the presence of an autoimmune inhibitor. Finally, BOD treatment can potentially reduce hyperbilirubinemia interference.

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## 70

**Point-of-Care Testing Is Not Required to Meet the New CDC HIV Testing Guidelines: Evaluation of the First FDA-Approved Random Access HIV Laboratory Assay**

*Matthew P. Thompson, Darnely Hidalgo, and M. Qasim Ansari. Department of Pathology, University of Texas Southwestern Medical Center, Dallas.*

In 2006, the Centers for Disease Control and Prevention (CDC) issued new guidelines on HIV testing, greatly increasing the number of people requiring HIV screening. Current studies suggest that approximately one third of patients do not return for the results of traditional HIV testing. Thus, the CDC has recommended point-of-care testing for HIV to reduce turnaround time and attempt to reverse this alarming trend.

The VITROS company recently introduced the first Food and Drug Administration (FDA)-approved HIV assay able to be performed on a "true" random access analyzer in a laboratory setting. In an effort to provide timely and accurate HIV test results to our clinicians at Parkland Hospital without incurring the additional cost and manpower required to perform routine point-of-care testing, we decided to evaluate the VITROS HIV assay. We compared

our current HIV testing methodology, the Abbott HIVAB HIV-1/HIV-2 recombinant DNA EIA test performed in batch on the Abbott COMMANDER system, with the VITROS Immunodiagnostics Products Anti-HIV 1+2 Assay performed on the random access VITROS ECi/ECiQ Immunodiagnostic System in a head-to-head comparison of 300 patient samples.

Our data indicate that the VITROS Immunodiagnostics Products Anti-HIV 1+2 Assay is equally as sensitive (100%; n = 298) and more specific (98% VITROS vs 83% Abbott HIVAB; n = 298) than the Abbott HIVAB while decreasing the HIV status result reporting time from an average of 14 hours to approximately 1 hour.

This is the first report of the performance of the VITROS Anti-HIV 1+2 Assay in a large hospital setting. The VITROS assay outperformed our current HIV testing methodology while meeting the FDA guidelines for a shortened turnaround time without incurring the increases in cost, manpower, and trained human interpretation that accompanies point-of-care testing. It is our belief that the VITROS assay will ultimately decrease the percentage of HIV-infected people who are unaware of their HIV status in the Parkland Hospital population, hopefully translating into a decrease in the incidence of HIV.

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## 71

### Validation of a Novel Ultraperformance Liquid Chromatography–Tandem Mass Spectrometry Method for Direct Monitoring of Argatroban Anticoagulation

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Argatroban, a direct thrombin inhibitor approved for prophylaxis and treatment of venous thromboembolism in patients with heparin-induced thrombocytopenia, is most commonly monitored using clotting-based methods, such as the activated partial thromboplastin time (aPTT). Unfortunately, the aPTT can overestimate the degree of anticoagulation in patients with clotting factor deficiencies or inhibitors, other nonspecific inhibitors (eg, lupus anticoagulants), and patients being bridged to oral anticoagulants. The aPTT response to argatroban also varies with different manufacturers' reagents and argatroban concentrations. For these reasons, a direct measure of plasma argatroban concentrations is preferred and may also be used to establish concentration-based therapeutic ranges.

Our objective was to evaluate a novel, highly sensitive and specific UPLC-MS/MS method for direct quantification of plasma argatroban concentrations and determination of argatroban therapeutic ranges, in comparison with established (aPTT) and newly developed (Hemoclot Thrombin Inhibitors [HTI], HYPHEN BioMed) automated clotting-based methods for argatroban monitoring. Argatroban was isolated from 100 µL of plasma after internal standard–spiked methanol extraction and centrifugation. The supernatant was injected into the UPLC-MS/MS (Waters ACQUITY UPLC TQD) and quantified using a 7-point standard curve. The aPTT, prothrombin time (PT), and HTI clotting-based assays were all performed on the Dade Behring BCS automated analyzer.

Our UPLC-MS/MS method is linear from 0.04 to 2.0 µg/mL, with interassay and intra-assay precisions of less than 10% over the entire linear range. Our method correlates well with the HTI method, yielding a slope of 0.98, an intercept of 0.1, and $r^2$ of 0.99 for spiked drug-free patient plasma. Plasma argatroban levels also correlated well with PT and aPTT, although the responses differed for patients receiving concurrent warfarin therapy and patients with baseline-prolonged aPTTs.

By using our novel UPLC-MS/MS method, we demonstrate a good correlation between plasma argatroban mass- and activity-based assays. Although the aPTT and HTI methods offer quick alternatives, our UPLC-MS/MS argatroban assay is preferred in patients with underlying factors leading to inaccurate estimations of the extent of argatroban anticoagulation using clotting-based assays. This novel assay was also useful for determining argatroban concentration–based therapeutic ranges.

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## 72

### LC-MS/MS Method for Simultaneous Measurement of Vitamins $D_2$, $D_3$, 25(OH) $D_2$, and 25(OH) $D_3$ in Human Serum

*Michael A. Burson,[1] Jennifer Puhl,[2] and Geza S. Bodor.[1,2] [1]UCHSC, Department of Pathology, Aurora, CO; and [2]VA Medical Center, Denver, CO.*

Our objective was to develop a liquid chromatography–tandem mass spectrometric (LC-MS/MS) method for simultaneous measurement of vitamins $D_2/D_3$ (D2, D3) and their 25-hydroxy derivatives, 25(OH)-$D_2/D_3$, (OHD2, OHD3) in human serum. Current clinical practice is to measure $OHD_2$ and $OHD_3$ to assess vitamin D status although knowing D2 and D3 concentrations may facilitate our understanding of physiologic functions of these vitamins.

Analytes were separated on a C18 column (100 mm × 2.1 mm × 3 µm, RESTEK) and then introduced into a triple quadrupole mass spectrometer (ABI 3200 Q-trap) via an APCI source in the positive ion mode. Compound-specific optimizations were performed for the 6 most abundant MRMs for each analyte, and the 3 most abundant MRMs were selected for the final method. Hexadeuterated D2, OHD2, and OHD3 (D2d6, OHD2d6, and OHD3d6, Medical Isotopes) were optimized as above and used as internal standards (IS). Calibrators were prepared in acetonitrile (ACN) at 5, 10, 20, 50, 100, and 150 ng/mL for each analyte. Samples and calibrators (500 µL) were spiked with 75 ng IS, extracted in 1 mL ACN, and centrifuged. Thirty microliters of supernatant was injected into a Shimadzu HPLC at 70% H2O:30% ACN at 350 µL/min flow. The analytes were eluted at 100% ACN in a 13-minute run. Preliminary studies addressed linearity, precision, analytical sensitivity, and analyte stability. Accuracy and precision were assessed by triplicate injection of samples over time. Level of detection (LOD) and level of quantitation (LOQ) were defined as the concentration in which the S/N ratios exceeded 3 and 10, respectively. Analyte stability was assessed by measuring peak area of analytes and IS in serum samples and calibrators that were stored for up to 1 month at –70°C. Postextraction stability was assessed by retesting extracted samples, stored at 4°C over the course of 1 month.

Our assay was linear from 5 to 150 ng/mL. This method had an LOD and LOQ of 5 ng/mL for each analyte, except D3, which had an LOQ of 10 ng/mL. Vitamin D2 and D3 concentrations were at or below the LOQ for the samples analyzed; therefore, precision could not be calculated. OHD2 and OHD3 concentrations, from LOQ to 120 ng/mL, demonstrated imprecision of 39% to 10.4% coefficient of variation, respectively.

We have developed a practical LC-MS/MS assay for simultaneous measurement of vitamins D2 and D3 and their OH metabolites. We are currently developing protein-based calibrators for similar processing of samples and calibrators. Data suggest that this modification provides similar if not improved precision.

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## 73

**Relative Concentrations of NT-proBNP and BNP vs Estimated Glomerular Filtration Rate**

*Michelle Cox,[1] Susan LeSourd,[1] Ronald Faught,[1] Ryan Calloway,[1] Crystal Pennington,[1] Amy K. Saenger,[2] and Joshua Bornhorst.[1] [1]Department of Pathology, University of Arkansas, Little Rock; and [2]Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN.*

Circulating concentrations of B-type natriuretic peptide (BNP) and N-terminal pro-BNP (NT-proBNP) have been shown to correlate with the severity of congestive heart failure and also have been used as markers for other disorders. Both BNP and NT-proBNP have been recognized to be elevated in patients with renal dysfunction. The relative extent of elevation of these markers has yet to be fully stratified by degree of renal impairment.

Our aim was to delineate the relationship between BNP and NT-proBNP by comparing the ratio of measured concentrations of these 2 peptides vs chronic kidney disease (CKD) stage and estimated glomerular filtration rate (eGFR). Samples of EDTA plasma from 146 patients (age range, 25-91 years; 90 men) representing all CKD classifications were tested for BNP (Beckman Coulter Access) and with a new NT-proBNP assay (Ortho-Clinical Vitros). The majority of samples (118) were frozen (–80°C) immediately after initial analysis and subjected to subsequent testing for NT-proBNP using the Roche Elecsys assay, allowing comparison of the NT-proBNP methods. An eGFR was calculated for each patient using standardized serum creatinine measurements.

A positive relationship was observed between the ratio of NT-proBNP(Vitros)/BNP and eGFR for all 146 samples. The median ratio was 3.44 for CKD stage 1 (N = 43), 4.03 for stage 2 (N = 42), 8.63 for stage 3 (N = 30), 11.26 for stage 4 (N = 17), and 27.71 for stage 5 (N = 14). This can be described by the best-fit power function of NT-proBNP/BNP = $106.2(\text{eGFR})^{-0.74}$ ($R$ = 0.66). Patient age, sex, and race had little effect on this correlation. Importantly, only weak relationships were observed between the NT-proBNP/BNP ratio and NT-proBNP (range, 13-175,000 pg/mL) or BNP (range, 7-5,002 pg/mL) concentrations. Comparison of 118 samples in the Vitros and Roche NT-proBNP assays produced a correlation of Vitros NT-proBNP = 1.12 (Roche NT-proBNP)-272 pg/mL ($R$ = 0.997).

Our data demonstrate that eGFR appears to be a predominant determination of the NT-proBNP/BNP ratio, with NT-proBNP exhibiting higher relative elevation in cases of reduced eGFR. This relationship was observed for 2 analogous NT-proBNP assays. The development of this potentially predictive quantitative description of this phenomenon may be of value in the clinical interpretation of these markers for patients with reduced renal function.

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## 75

**Comparison of Three PCR-Based Methods for the Detection of *Mycoplasma pneumoniae* (Mp) in Varied Clinical Specimens**

*Jose Lima, Li Xiao, Amy Ratliff, Lynn Duffy, and Ken B. Waites. Department of Pathology of the University of Alabama at Birmingham.*

Polymerase chain reaction (PCR) approaches have been found to be useful for the rapid, sensitive, and specific detection of Mp in clinical specimens. Our objective was to compare the sensitivity and specificity of 3 PCR-based methods for detection of Mp in clinical specimens.

The first method consisted of a primer/probe set targeting the Mp ornithine transcarbamylase (*argI*) gene. A real-time PCR (RT-PCR) program (*argI*-PCR) was developed using the Roche LightCycler 2.0 instrument. The assay had been previously validated and analytical sensitivity and specificity determined using a collection of 36 Mp type strains and clinical isolates that included representative strains from subtype 1 and subtype 2 and 26 other mycoplasmal and ureaplasmal species, as well as 22 bacterial species, 4 respiratory viruses, and human genomic DNA. The second method consisted of a published RT-PCR method (repMp1-PCR) targeting the repetitive sequence repMp1 (*J Clin Microbiol.* 2007;45:2726-2730). Both RT-PCR methods were compared with a traditional PCR (T-PCR) targeting the 16S rRNA and/or culture in detection of Mp in 2 sets of clinical specimens. One set contains 91 specimens from the upper and lower respiratory tract collected and cultured between 1992 and 1999. Another set contains 78 specimens from respiratory tract, blood, and various tissues and body fluids that have been tested by T-PCR after 2003. PCR assays were repeated on samples yielding discrepancies.

All 3 methods amplified all Mp type strains and clinical isolates. There was no cross-reactivity with any of the other DNA preparations tested. Among the first set of 91 clinical specimens, 52 were culture positive and 39 were negative. Mp DNA was detected in 55 samples, including all of the culture positives by the 3 PCR methods. Using culture as the "gold standard," the sensitivities of rep-, *argI*-, and T-PCR were 100% and the specificity was 92.3%. Among the second set of 78 specimens, 10 were T-PCR positive and also positive by the 2 RT-PCRs. repMp1-PCR detected 1 additional positive sample. When compared with T-PCR, *argI*-PCR was 100% sensitive and 100% specific vs repMp1-PCR that showed 100% sensitivity and 98% specificity.

The *argI*-PCR method is specific and more sensitive for detection of Mp in clinical specimens compared with culture and is as sensitive and specific as T-PCR. The repMp1-PCR is a good first target for clinical Mp detection, and *argI*-PCR could serve as a second target for verification of positive results. Using 2 gene targets standard in clinical Mp diagnoses by RT-PCR would help to reduce the false-positive rate.

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## 76

**Evaluation of Predicted Exchange Efficiency in Red Cell Exchange Procedures: A Useful Tool in Evaluating Central Line Performance Failure**

*Kelley E. Capocelli,[*1] Myra A. Killeen,[2] Whitney B. Smith,[3] Eileen M. Jata,[3] Kimberly M. Hirst,[3] and Edmund N. Orsini.[3] Sponsor: Mary P. Berg. [1]Department of Pathology, the Children's Hospital and University of Colorado Denver Health Sciences Center, Aurora; [2]CaridianBCT, Lakewood, CO; and [3]Department of Pathology, the Children's Hospital, Aurora, CO.*

Efficiency of a red cell exchange in the setting of sickle cell disease is defined as percentage reduction of sickle hemoglobin and depends on the total volume of blood exchanged. Functioning double-lumen central venous access devices (CVADs) are as effective as peripheral venous catheters for achieving an adequate reduction in the percentage of sickle hemoglobin following erythrocytapheresis. In the setting of inefficient exchange results and normal conventional port studies, mechanical and/or flow issues cannot be ruled out.

From February 2008 through October 2008, we compared predicted vs actual post–exchange hemoglobin S levels in 10 patients undergoing a total of 123 exchange procedures on the Spectra (CaridianBCT, Lakewood, CO) for sickle cell disease—6 with

CVADs, 3 with peripheral venous access (PVA), and 1 with a port and PVA. Inefficient exchanges with CVADs, seen in 3 patients, were further investigated with port studies that showed no evidence to suggest catheter disruption; however, suspicion of blood mixing or a closed circuit could not be ruled out. Therefore, subsequent exchanges in these patients were achieved with port and PVA or PVA only.

Comparison of exchange efficiencies through calculation of the ratio of predicted vs actual postprocedural hemoglobin S levels among the various modalities confirmed that PVA only provides the most efficient exchange (mean = 0.89; 95% confidence interval [CI] = 0.836-0.950); however, significant variation from the mean is not demonstrated when comparing the efficiency of procedures achieved through other forms of access, as long as a peripheral vein is involved. The efficiency of the procedures performed through a CVAD only showed a mean ratio of 0.84 with a 95% CI of 0.756 to 0.928. When this ratio falls below 0.756, there is a significant difference in the exchange efficiency when compared with that achieved with PVA only ($P$ =.0055; Student $t$ test) or with port and PVA ($P$ < .0001).

Clinicians performing erythrocytapheresis for reduction of the percentage of sickle hemoglobin need to be cognizant of the efficiency of the exchange procedure. Collection efficiency below a calculated threshold can indicate mechanical or flow failures not diagnosable by conventional port studies and may indicate the need to include PVA in the red cell exchange procedure or consider replacement of the CVAD.

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## 79

**Chronic Viral Disease and Acquired Hemophilia: An Association With Prognostic Significance?**

*R. Darrell Sanders, Patricia Tichenor, Jeremy Henderson, and Marisa B. Marques. UAB Hospital, Birmingham, AL.*

Acquired hemophilia (AH) is a rare and life-threatening hemorrhagic condition seen in adults. AH is caused by an autoantibody to factor VIII (FVIII) and is associated with other autoimmune diseases such as rheumatoid arthritis, but also malignancy, pregnancy, and drugs. We have recently observed chronic viral infections and AH that appear to behave differently than other types of underlying conditions.

In the last 8 years, we encountered 10 patients (5 men, 5 women; mean age, 66.6 years; range, 56-76 years) with AH. Three patients had a history of HIV or hepatitis C infection before the development of AH. Seven had a variety of other conditions. At diagnosis, all patients had low FVIII activity ranging from less than 1% to 4% due to an inhibitor of 2 to 350 Bethesda units (BU). In addition to an activated prothrombin complex concentrate for bleeding control, they received a variety of immunosuppressive regimens, mostly steroids alone or in combination. We arbitrarily defined recovery as the number of days from diagnosis to the first FVIII activity of 50% or more (with loss of inhibitor). None of the patients with chronic viral infections (n = 3) have reached 50% (maximum FVIII, 1%-3%; and inhibitor, 190, 215, and 800 BU) after a mean follow-up of 241 days (range, 111-322 days). Among patients without infections, all but one survived hospitalization for AH and another has not yet recovered (FVIII of <1% at 138 days of follow-up). The remaining 5 patients recovered on average at 76 days (range, 12-198 days). Compared with patients with viral infections, the recovery time of the patients without infections was significantly faster ($P$ = .051; Student $t$ test).

AH should be included in the differential diagnosis of a sudden and severe bleeding diathesis in patients with chronic viral infections. Unlike more common autoimmune conditions, the autoantibody in AH is expected to disappear in most patients independent of the immunosuppressive regimen. Our data suggest that AH associated with chronic viral infections is more refractory to treatment, which is likely related to the underlying altered immune system in such cases. We suggest that AH is a classic example of how clinical pathologists can actively participate in the laboratory diagnosis, management, and treatment follow-up of affected patients.

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## 81

**Development and Validation of a Laboratory Test for Glycosylated Ferritin**

*Timothy Amukele* and Mark H. Wener. University of Washington School of Medicine, Seattle.*

Ferritin, the major iron-binding protein in nonerythroid cells, exists as glycosylated (GlyFer) and nonglycosylated forms. Normal serum ferritin is predominantly GlyFer (50%-80% glycosylated). Circulating tissue ferritin released during inflammation, liver disease, preeclampsia, and certain malignancies is mostly nonglycosylated (20%-40% glycosylated). With infection, the plasma concentration of ferritin is increased but the glycosylation pattern remains the same as in healthy control subjects. Circulating ferritin in patients with active Still disease (systemic rheumatoid arthritis) and hemophagocytic syndrome has a very low percentage of GlyFer (<20% glycosylated), and this can be used for diagnosis and monitoring.

GlyFer binds to concanavalin A, whereas the nonglycosylated form does not. To detect GlyFer, serum is mixed with Con-A sepharose and incubated, and then the sepharose-bound GlyFer is centrifuged. The unbound fraction (nonglycosylated ferritin) in the supernatant is quantified using an immunoenzymatic assay. The percentage of glycosylated ferritin is calculated from the total and nonglycosylated ferritin concentrations. We used ceruloplasmin as an internal control to be certain that an excess amount of Con-A sepharose was available to maximize GlyFer binding. Ceruloplasmin is fully glycosylated and shows binding to its limit of detection under the conditions of the experiment.

The coefficient of variation (CV) of the assay was 5.5% at a GlyFer percentage of 54% and a mean GlyFer concentration of 13.2 ng/mL. The reportable range was established by studying the precision of the assay over a range of glycosylation of ferritin. The precision of the assay varied with the total amount of ferritin and with the percentage of GlyFer. The precision was lowest at low amounts of total ferritin *and* a low percentage of GlyFer. The CV was less than 20% at a total ferritin of more than 43 ng/mL and a GlyFer of more than 22%. There was no saturation of the Con-A column at a total ferritin of up to 1.55 mg/mL and 98% GlyFer.

This reference range was based from a study of 120 blood bank donors. Since the distribution of "percent glycosylated ferritins" in this population was gaussian, the reference range was based on the central 95% of the distribution curve. Our reference range is 47% to 78% glycosylated ferritin. This is consistent with that found in prior studies of healthy volunteers in whom 50% to 80% of the serum ferritin is glycosylated.

Citrate, EDTA, and heparin specimens; a glucose level of 1,000 mg/dL or less; a haptoglobin level of 780 mg/dL or less; and a ceruloplasmin level of 194 mg/dL or less do not interfere with this assay.

We have validated an assay for the measurement of glycosylated ferritin in serum and plasma. This assay may be useful in the diagnosis and monitoring of patients with Still disease and hemophagocytic syndrome.

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