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 43rd Annual Meeting of the Academy of Clinical Laboratory Physicians and Scientists, June 5-7, 2008, in Philadelphia, PA. Authors receiving a 2008 Young Investigator Award are marked with an asterisk (*).

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

1

Transfusion-Associated HLA Alloimmunization in the Renal Transplant Population.

Nancy M. Dunbar,[1] Paul R. Warner,[2] and Theresa A. Nester.[1,2] [1]Department of Laboratory Medicine, University of Washington, Seattle; and [2]Puget Sound Blood Center, Seattle.*

To explore the relationship between RBC transfusion and human leukocyte antigen HLA alloimmunization, we identified 152 patients who were awaiting kidney transplant and had accessible transfusion records and panel reactive antibody (PRA) testing done on at least 2 occasions. We investigated whether gender or prior RBC transfusion influenced the baseline PRA, whether RBC transfusion was associated with subsequent PRA increase, and whether leukocyte reduction of the RBC product was associated with decreased HLA alloimmunization. Among 114 of 152 patients with a 0% PRA at baseline, 38 (33.3%) were female compared with 28 (74%) of 38 patients with a positive PRA at baseline ($P < .0001$). Previous RBC transfusion seemed a weaker risk factor for a positive PRA at baseline than gender. Of 114 patients with a negative PRA at baseline, 32 (28.1%) had a history of RBC transfusion compared with 13 (34%) of 38 with a positive PRA ($P = .54$). Changes in PRA in the testing interval were then analyzed for any temporal relationship to RBC transfusion. Of 114 patients with a 0% PRA at baseline, 29 demonstrated an increase in PRA during the testing interval. Seven cases seemed temporally related to transfusion, 16 occurred in the absence of transfusion, and 6 increased with no temporal relation to RBC transfusion. Component modification records were then analyzed to investigate whether the use of leukocyte-reduced (LR) RBCs influenced the rate of apparent HLA alloimmunization. Of 85 patients with a 0% PRA at baseline that remained zero throughout testing, 12 (14%) received RBC transfusion during the testing interval and 8 (67%) of 12 received exclusively LR units. Of the 29 patients who experienced an increase in PRA from a baseline of zero, 13 (45%) received RBC transfusion and 10 (77%) of 13 received exclusively LR units. Among the 7 patients whose increase in PRA seemed temporally related to RBC transfusion, 6 (86%) of 7 received exclusively LR units. Although this study is limited by a small sample, it seems to support the belief that RBC transfusion is a risk factor for HLA alloimmunization. Female gender appears to be a stronger risk factor for alloimmunization than RBC transfusion, likely owing to the longer and stronger immune stimulus of pregnancy. Leukocyte reduction of RBC units may not fully protect against HLA alloimmunization in immunocompetent patients, and further investigation into the true benefits of this practice in the renal transplant population is warranted.

2

Detection of HSV in Ascitic Fluid by Immunochemistry and PCR in the Absence of Clinical Signs of HSV Infection.

M. Ianosi-Irimie, R. Camurungan, M. May, E. Cadoff, and A. Sahota. Department of Pathology and Laboratory Medicine, RWJMS, New Brunswick, NJ.*

A 57-year-old woman with a 20-year history of HIV, who was noncompliant with the AIDS medication regimen, was admitted to the hospital with hepatic failure and ascites. She developed bacterial and fungal infections, which were successfully treated. Cytopathologic analysis of ascitic fluid showed atypical cells positive for herpes simplex virus (HSV)-1 and HSV-2 by Ventana antibodies, but there was no clinical sign of HSV infection.

Because HSV can cause severe complications in immunocompromised patients, we attempted to confirm the presence of HSV in ascitic fluid by using a polymerase chain reaction (PCR) assay that we routinely use for HSV detection in cerebrospinal fluid (CSF). The ascitic fluid was centrifuged, the pellet resuspended in lysis buffer, and an aliquot of the lysate, with and without spiking with a positive control, used for PCR. A PCR product was not observed in either

sample, strongly suggesting that the ascitic fluid contained a component that inhibited the PCR reaction. The ascitic fluid contained 92 mg/dL of glucose; 1,045 IU/L lactate dehydrogenase; 4 g/dL of protein; 8 mg/dL of calcium; and 11.6 mg/dL of bilirubin. The inhibitory substance, most likely bilirubin, was removed by washing the pellet with phosphate-buffered saline before cell lysis. Using PCR primers for the DNA polymerase gene, a 179-base-pair product was observed by agarose gel electrophoresis.

Our findings indicate that a simple modification of the DNA extraction method developed for CSF can be easily adapted for ascitic fluid and possibly other body fluids. This finding could expedite the detection of HSV without using cytopathologic analyses. Early detection of HSV may be critical in immunocompromised patients such as the patient described here.

3

Choosing the Right Troponin Assay for Your Lab: Impact on Clinical Decision Making of Commercially Available Troponin I Assays.

Michael J. Bonham and Steve Miller. Department of Laboratory Medicine, University of California San Francisco, San Francisco.*

The European Society of Cardiology and the American College of Cardiology (ESC/ACC) has defined acute myocardial infarction (MI) as a troponin (cTNI) rise over the 99th percentile with symptoms of ischemia. A 4-way comparison of cTNI values from the Axsym, DxI, Centaur, and Stratus platforms was performed on 96 patient samples collected from the emergency department and inpatient settings. A retrospective chart review was carried out to determine the correlation of troponin values above the 99th percentile and an acute MI cutoff with a documented history of acute MI and/or history of significant cardiac dysfunction.

A review of patient histories showed 10 (10%) of the troponin values were from documented MIs. Of the remaining troponin values, 35 (36%) were from patients classified as having significant cardiac dysfunction and 51 (53%) were from patients with no evident cardiac dysfunction. The minimum cTNI values for clinical MI on the Axsym, Dxi, Centaur, and Stratus were 0.33, 0.37, 0.49, and 0.45 ng/mL, respectively. Using these values as an AMI cutoff gave a sensitivity of detecting MI between 38% and 47% among the 4 platforms and a specificity of 100%. Cardiac dysfunction (including MI) was present above the AMI cutoff in 87% to 94% of the samples and 44% to 68% of the samples above the 99th percentile values. The specificity of cardiac dysfunction at the 99th percentile ranged from 75% to 83%. The percentage of patients with cardiac dysfunction below the 99th percentile ranged from 20% to 32%. In at least 43 samples, at least 1 assay value was below the 99th percentile; of those, 23 (53%) were below the 99th percentile in all assays and 9 (21%) were below the 99% percentile in 1 of 4 assays.

Values of troponin between the 99th percentile and the AMI cutoff correlate with increased likelihood for the presence of myocardial dysfunction. Elevations above a defined AMI cutoff value did not indicate clinical MI 43% of the time but could be accounted for by ongoing medical problems. Patients with low-level elevations tended to have multiple medical problems, such as renal dysfunction or hypercoagulability, that could account for mildly elevated cTNI levels. While several samples were discrepant at the 99th percentile reference interval between assays, low-level elevation of troponin did not significantly affect clinical management in cases reviewed. This finding suggests that accuracy around the 99th percentile does not directly translate into changes in immediate patient care or diagnosis.

4

Prevalence and False-Negative Rate of Quantitative Glucose Testing to Detect Bacteria in Whole Blood–Derived Leukoreduced Platelets in a University Medical Center and a Comparison With Reported Culture Methods.

A.V. McKane, N. Ward, C. Senn, J. Eubanks, and R. Bowman. Sponsor: Jeffrey McCullough. Transfusion Medicine, Department of Laboratory Medicine and Pathology, University of Minnesota, Minneapolis.

After implementation of American Association of Blood Banks and College of American Pathologists standards regarding bacterial detection in platelets in March 2004, we began quantitative glucose screening of platelets to detect bacterial contamination. Single donor apheresis platelets (SDP) and whole blood–derived platelets (WB-P) were obtained from our supplier and disbursed oldest units first, with a standard outdate time of 5 days. Our institution uses approximately 60,000 platelet equivalents each year (1 platelet equivalent defined as 1 WB-P unit), of which 67% were WB-P in 2004. While our supplier used culture methods to test SDP, we thought it impractical to screen WB-P by culture. To meet the standard, we instead used a quantitative glucose testing method to screen WB-P for bacterial contamination. By using the WB-P glucometer (SureStep Flexx Meter), glucose was measured before component release (frequently storage day 4 or 5), with a positive screening cutoff of less than 500 mg/dL (established by spiking studies); the units failing screening were cultured and not issued. Samples were collected from 93,073 WB-P and tested in a 29-month period (March 1, 2004–July 31, 2006). Initially, 929 cases (0.998%) screened positively. Bacterial growth was culture-confirmed in 6 of the associated platelet units (screened storage day 5 [4 cases, 2 coagulase negative *Staphylococcus* (CONS), 1 α-hemolytic *Streptococcus,* 1 *Propionibacterium acne*], day 3 [1 case, CONS], and day 4 [1 case, CONS]), for a confirmed contamination incidence of 0.006% and a true-positive rate of 6.4 per 100,000. Two additional culture-confirmed cases of contamination were detected in transfused units causing febrile nonhemolytic transfusion reactions, for a false-negative rate of 2.1 per 100,000. The overall prevalence of platelet bacterial contamination (PBC), including those positively screened and those detected after transfusion, was 8.6 per 100,000 platelets transfused. Our findings reflect lower prevalence of PBC than ordinarily cited in the literature: 8.6 in 100,000 platelets transfused vs 33.3 to 100/100,000 (range, 20-974/100,000, various studies). This finding may be expected for a low-sensitivity method, but our results show a false-negative rate remarkably congruent to culture: 2.1/100,000 vs 2.0 to 2.4/100,000 (Eder et al. 2007). Quantitative glucose testing on WB-P prior to storage days 3 to 5 release detected a low prevalence of bacterial contamination and a false-negative rate comparable to that reported for automated culture.

5

A Novel Cytokine-Release Assay for the Evaluation of Blood Bank–Stored Platelets.

Christopher A. Tormey[1] and Gary Stack.[1] Sponsor: Brian R. Smith. [1]Department of Laboratory Medicine, Yale University School of Medicine, New Haven, CT.

The platelet-storage lesion consists of a variety of deleterious biochemical, morphologic, and functional alterations of stored platelets. The extent to which changes in platelet secretory function contribute to the platelet-storage lesion is unknown. Secretory capacity is a key platelet function because of the role granule contents play in coagulation, wound healing, and immunity. A cytokine-release assay, where platelets are stimulated in vitro to secrete one of their α-granule cytokines, RANTES, was developed to assess platelet secretory integrity.

Small volumes of platelet-rich plasma (PRP) and platelet concentrate (PC) were prepared from 20 mL of fresh, citrated whole blood (WB). On the day of preparation, 2-mL aliquots of WB, PRP, and PC were adjusted to platelet counts of approximately 250,000/μL and activated with 20 μmol/L of ADP. In addition, aliquots of stored PCs from a regional blood center were similarly activated with ADP on days 2, 5, and 7 of storage. Plasma levels of released RANTES were quantitated by EIA. Platelet aggregation was measured by single platelet counting.

ADP stimulated release of RANTES from platelets in fresh WB on average by 4.1-fold (19.9 ± 0.62 vs 4.84 ± 0.51 ng/mL [control]; n = 4). RANTES release from fresh PRP was stimulated by an average of 4.7-fold after ADP administration (26.0 ± 5.49 vs 5.50 ± 1.37 ng/mL [control]; n = 4). Following an additional processing step, fresh PC showed reduced responsiveness to ADP with only 1.3-fold stimulation of RANTES release (40.1 ± 18.4 ng/mL vs 30.3 ± 19.6 ng/mL [control]; n = 4). For stored PCs from a blood center, ADP failed to stimulate RANTES release on days 2, 5, and 7 for all PC tested (n = 10). A progressive increase in baseline levels of RANTES during storage was observed for all stored PCs examined (n = 10). Platelet aggregation studies, run in parallel, showed 95.6% ± 1.70% aggregation in fresh WB, 82.5% ± 7.00% aggregation in fresh PRP, and 60.5% ± 8.60% aggregation in fresh PC (n = 4). For stored PCs (n = 10), platelet aggregation decreased with increasing storage time: 41.0% ± 15.8% on day 2, 10.5% ± 8.10% on day 5, and 2.30% ± 2.70% on day 7.

The ability of ADP to stimulate platelets in vitro formed the basis for a novel cytokine-release assay to assess the secretory function of platelets prepared for transfusion. This study revealed a diminution in platelet secretion during the processing and early storage of PC. Platelet secretory capacity was completely eliminated by day 2 of storage. Loss of platelet aggregation during PC preparation and storage occurred more slowly as compared with platelet secretion.

6

Rapid Detection of Oleander Poisoning by Digoxin III, a New Digoxin Assay: Impact on Serum Digoxin Measurement.

Meredith A. Reyes, Jeffrey K. Actor, Semyon A. Risin, and Amitava Dasgupta. Department of Pathology and Laboratory Medicine, University of Texas Health Science Center at Houston.*

Oleander poisoning is common worldwide, especially accidental exposure to children. Despite toxicity, oleander extract is used in herbal remedies. Oleander poisoning can be detected indirectly by using fluorescence polarization digoxin immunoassays (FPIAs) owing to structural similarity between digoxin and oleandrin, the active ingredient of oleander. Recently, Abbott Laboratories released a new digoxin assay, Digoxin III, for application on the AxSYM analyzer, but application of this assay for rapid detection of oleander poisoning has never been reported before.

Aliquots of drug-free serum pool were supplemented with pure oleandrin or extract of oleander leaf to mimic concentrations of oleandrin reported in human poisoning, apparent digoxin levels were measured using the new Digoxin III assay, and results were compared with values obtained by using the FPIA and Digoxin II assay (all from Abbott Laboratories). We observed apparent digoxin values with all assays, but the Digoxin III assay showed the highest apparent digoxin values for indirectly detecting oleandrin (4.94 ng/mL by the Digoxin III, 0.72 ng/mL by the Digoxin II, and 3.31 ng/mL by FPIA in serum containing 10 μg/mL of oleandrin). In addition, when diluted extract was introduced into mice (3 different doses) by oral gavage, significant apparent digoxin concentrations were observed in all mice with all 3 doses 1 and 2 hours after feeding. For example, in 1 mouse the apparent digoxin concentration was 2.40 ng/mL 1 hour after feeding and 0.96 ng/mL 2 hours after feeding with oleander extract (50 mg/kg oleandrin), indicating that the average half-life of oleandrin is 1.1 hours. When aliquots of serum pools prepared from patients receiving digoxin were further supplemented with oleander extract, a negative interference was observed using the Digoxin II assay, while positive interference was observed with the FPIA and Digoxin III assay. Despite strong protein binding of oleandrin (>90% to serum albumin), this interference cannot be eliminated by measuring free digoxin concentrations in the protein-free ultrafiltrate using the Digoxin III assay. Digibind neutralizes digoxin-like components of oleander, and such effect can be monitored in the laboratory by measuring apparent free digoxin concentrations in the protein-free ultrafiltrate using the Digoxin III assay in patients not taking digoxin. We conclude that the new Digoxin III assay is suitable for rapid detection of oleander poisoning and also can be used to monitor progress of Digibind therapy in poisoned patients, but this assay is unsuitable for digoxin monitoring in patients taking oleander-containing herbal products.

7

Pharmacokinetics of Acetaminophen in Hind Limbs Unloaded Mice: A Model System Simulating the Effects of Low Gravity on Astronauts in Space.

Amanda Peterson,[1] Semyon A. Risin,[1] Govindarajan T. Ramesh,[2] Amitava Dasgupta,[1] and Diana Risin.[3] [1]University of Texas Health Sciences University at Houston; [2]Texas Southern University, Houston; and [3]NASA Johnson Space Center, Houston.*

The pharmacokinetics (PK) of medications administered to astronauts could be altered by the conditions in space. Low gravity and free floating (and associated hemodynamic changes) could affect the absorption, distribution, metabolism, and excretion of drugs. Knowledge of these alterations is essential for adjusting the dosage and the regimen of drug administration in astronauts. Acquiring such knowledge has inherent difficulties owing to limited opportunities for experimenting in space. One approach is to use model systems that simulate some of the space conditions on Earth.

In this study we used hind limbs unloaded mice (HLU) to investigate the possible changes in PK of acetaminophen, a widely used analgesic with high probability of use by astronauts. The HLU is recognized as an appropriate model for simulating the effects of low gravity on hemodynamic parameters. Mice were tail suspended (n = 24) for 24 to 96 hours prior to introduction of acetaminophen (150-300 mg/kg). The drug (in aqueous solution containing 10% ethyl alcohol by volume) was given orally by a gavage procedure, and after administration of acetaminophen, mice were additionally suspended for 30 minutes, 1 hour, and 2 hours. Control mice (n = 24) received the same dose of acetaminophen and were kept freely moving all the time. Blood specimens were obtained either from retroorbital venous sinuses or the heart. Acetaminophen concentrations were measured in plasma by the fluorescent polarization immunoassay and the AxSYM analyzer (Abbott Laboratories).

In control mice, peak acetaminophen concentrations were achieved within 30 minutes. By 1 hour, the concentrations decreased to less than 50% of the peak level, and at 2 hours, acetaminophen concentrations were almost undetectable in serum. HLU for 24 hours significantly altered the acetaminophen PK: at 30 minutes, the acetaminophen concentrations were significantly lower (statistically and medically significant) than in control mice, indicating slower absorption from the gut, and peak levels were achieved significantly later than 30 minutes. The concentrations also reduced less significantly after 1 and 2 hours, and at 2 hours, approximately 20% of the drug still remained in the circulation, indicating slower distribution and metabolism. After 96 hours of HLU, the changes in acetaminophen PK were less prominent. These data indicate that short-term HLU causes significant changes in acetaminophen PK most likely associated with HLU-related hemodynamic changes, but after 96 hours, these changes diminished.

9

vWF Multimer Analysis by Fluorescence Fluctuation Spectroscopy.

Richard Torres[1,2] and Michael Levene.[1] Sponsor: Brian R. Smith. [1]Departments of Biomedical Engineering and [2]Laboratory Medicine, Yale University, New Haven, CT.

The principal objective of this research was to demonstrate the novel application of fluorescence fluctuation spectroscopy (FFS) in the evaluation of von Willebrand Factor (vWF) multimers for diagnosis, classification, and monitoring in von Willebrand disease (vWD) and thrombotic thrombocytopenia purpura (TTP) and with the advantages of high reproducibility, simplicity, and low cost. vWF exists in peripheral blood as a distribution of multimers composed of between 2 and 80 or more monomers. Knowledge of the concentration and size distribution of vWF multimers helps in the clinical subtyping and management of vWD and could be valuable as a routine diagnostic and research tool in TTP were it more readily available. However, current gel methods for multimer analysis are laborious, technically challenging, and usually radiation-dependent. Most other traditional testing parameters for vWF also suffer from significant problems of interlaboratory variability and low reproducibility.

We are adapting the techniques of single molecule analysis from the field of biophysics to address these shortcomings. FFS monitors fluctuations in the number of freely diffusing particles within small confocal observation volumes. Our methods include the use of maximum entropy regularization fitting techniques for extraction of the relevant distribution information from the fluctuation data of antibody-tagged vWF multimers diffusing in plasma.

Results demonstrate that highly reproducible correlation spectroscopy curves and photon count histograms can be obtained from clinical samples. They show that there are marked differences and tight clustering of affected and unaffected groups in vWD. The technique also illustrates differences in the multimer distributions from vWD, patients post-DDAVP treatment, and normal controls. Parameters derived from fits of fluctuation curves correlate to antigen concentration independently from distribution measures. Analysis from TTP patients suggests variable distributions with marked decreases in total vWF.

We conclude that FFS is a practical tool for rapid vWF multimer analysis with added informational content. The development of FFS for multimer testing should prove of use in the diagnosis and monitoring of the broad range of systemic diseases that are associated with abnormalities in coagulation. This research also represents one of the first implementations of FFS for any clinical diagnostic application.

10

A Novel Liquid Chromatography–Tandem Mass Spectrometry Method for Assessing Renal Function.

Ross J. Molinaro and James C. Ritchie. Sponsor: Corinne R. Fantz. Department of Pathology and Laboratory Medicine, Emory University School of Medicine, Atlanta, GA.

We have developed and validated a novel liquid chromatography–tandem mass spectrometry (LC/MS/MS) method for the identification and quantitation of iothalamate in biological samples to better assess renal function by calculating glomerular filtration rate (GFR). Current methods using iothalamate to calculate GFR have long run times and lack the analytical sensitivity allowing a reduced iothalamate dosage.

After the addition of iohexol as the internal standard, iothalamate is isolated from plasma by methanol extraction and urine by quick-spin filtration. Gradient chromatographic separations were performed on a reversed-phase dC18 column using an ammonium acetate/formic acid mobile phase. Iothalamate demonstrated a reproducible elution time of 0.8 minutes, while iohexol, the more nonpolar compound, consistently eluted from the column at 0.9 minutes. Both iothalamate and iohexol were monitored in the multiple-reaction monitoring mode (Waters Quattro Micro) using the hydrogen adduct mass transitions. Primary (614.5 > 360.5, iothalamate; 821.6 > 652.3, iohexol) and secondary (614.5 > 486.5, iothalamate; 821.6 > 730, iohexol) ion ratios were calculated as an additional check of compound specificity. This method requires only a 3-minute run time per sample. The iothalamate standard curve for plasma and urine displayed a wide measuring range with linearity up to 600 µg/mL and a limit of quantitation at 18.75 ng/mL. Acceptable precision (coefficients of variation ≤8.6%) was demonstrated by within-run and between-run experiments using drug-free plasma and urine spiked with known low, medium, and high concentrations of iothalamate. Ion suppression was tested by sample addition and infusion assays. Recovery from plasma and urine samples ranged from 93.6% to 104.1%. Accuracy was assessed using 50 urine and plasma samples tested by LC/MS/MS and an accepted capillary electrophoresis (CE) assay. The equations of the linear regression lines were as follows: $\text{Urine}_{\text{LC/MS/MS}} = 0.959 \times \text{Urine}_{\text{CE}} - 1.351$ ($r = 0.98$, $S_{Y/X} = 11.2$); $\text{Plasma}_{\text{LC/MS/MS}} = 1.064 \times \text{Plasma}_{\text{CE}} - 0.317$ ($r = 0.89$, $S_{Y/X} = 0.94$); and $\text{GFR}_{\text{LC/MS/MS}} = 1.005 \times \text{GFR}_{\text{CE}} - 5.264$ ($r = 0.92$, $S_{Y/X} = 10.3$). GFR was calculated by using the patient's urine flow rate and plasma and urine iothalamate values.

We have developed and validated a fast, accurate LC/MS/MS assay to calculate GFR in patients that can serve to determine renal efficiency in potential kidney donors. In addition, the sensitivity of this assay holds promise for allowing a smaller dose of iothalamate to be administered to patients, thereby reducing the chances of iothalamate hypersensitivity.

11

Cytokine Production by Human and Mouse Macrophage Cultures to OSHA Extracts.

Stacia Clement-Kruzel, Shen-An Hwang, Mark C. Kruzel, Jeffrey K. Actor, and Amitava Dasgupta. Department of Pathology and Laboratory Medicine, University of Texas-Houston Medical School.*

OSHA root (*Ligusticum porteri*) extract is marketed worldwide as an immune stimulant. To test the ability of OSHA as an immune modulator, the innate effects of OSHA on cytokines produced by cultured mouse and human macrophage cell lines were examined by using 2 different brands of OSHA, OSHA-1 (root extract in 70% alcohol; Gaia Herbs, Brevard, NC) and OSHA-2 (root extract in 60% alcohol; Herb Pharm, Williams, OR). Two mouse macrophage cell lines (J774A.1 and RAW264.7; 5×10^5/well) and 2 human macrophage cell lines (THP-1 and U937; 5×10^5/well) were incubated with OSHA extracts at varying concentrations (1:5,000, 1:50,000, and 1:500,000 dilution vol/vol). Media alone, LPS, and alcohol vehicle controls (70% alcohol for OSHA-1 and 60% for OSHA-2) were also incubated with each cell line. Supernatants were collected at 24 and 72 hours postincubation and tested by enzyme-linked immunosorbent assay for the presence of secreted proinflammatory cytokines (interleukin [IL]-6, IL-10, IL-12p40, IL-1β, and tumor necrosis factor [TNF]-α).

The J774A.1 cell line for OSHA-1 demonstrated TNF-α production at the 1:5,000 dilution at 24 and 72 hours, IL-6 production at 1:5,000 dilution at 24 hours, and IL-10 production at 1:50,000 and 1:500,000 dilutions at 24 and 72 hours. The J774A.1 cell line for OSHA-2 demonstrated similar effects, with IL-1β production at 1:500,000 dilution at 24 and 72 hours postincubation. There was no IL-12p40 production by either product. The THP-1 cell line demonstrated IL-10 production at 1:50,000 dilution at 24 hours for OSHA-1 and at 1:5,000 dilution for OSHA-2. Only OSHA-1 demonstrated TNF-α and IL-1β production at the 1:5,000 dilution at 72 hours. OSHA-1 also demonstrated TNF-α production at 1:5,000 dilution at 24 and 72 hours with the RAW264.7 cell line. IL-1β was also observed at 1:50,000 dilution at 24 hours, while IL-12p40 was produced with all 3 dilutions at 24 hours. With RAW264.7 cells, OSHA-2 also demonstrated TNF-α production at 1:50,000 dilution and IL-12p40 production at 1:5,000 and 1:500,000 dilutions at 24 hours. Of interest, the human U937 cell line only demonstrated IL-10 production (OSHA-1, 1:500,000 dilution 24 hours and OSHA-2 at 1:50,000 dilution at 24 hours). No significant TNF-α, IL-6, IL-1β, or IL-12p40 production was observed for OSHA-1 or OSHA-2. We conclude that both extracts exhibit modest ability to stimulate human and mouse macrophage responses but differ in their immune stimulatory patterns, which is consistent with their predicted use as immunomodulatory herbal remedies.

12

An Evaluation of the Contribution of Multiparametric Flow Cytometric Analysis in the Diagnosis of Cutaneous B-Cell Lymphoma.

G.Y. Stetsenko and S Cherian. Department of Laboratory Medicine, University of Washington, Seattle.*

Cutaneous B-cell lymphomas often pose diagnostic difficulties using traditional clinical and histologic tools. Multiparametric flow cytometry is a well established tool in the diagnosis of B-cell lymphoma; however, few studies have evaluated the utility of this modality in the setting of cutaneous B-cell lymphoma. The aim of this study was to determine the contributions of multiparametric flow cytometry to the diagnosis of cutaneous B-cell lymphoma as compared with morphology.

Flow cytometry reports from all cutaneous specimens submitted for flow cytometry between 2002 and 2007 were reviewed, revealing 35 reports from 32 patients in which flow cytometry and morphology data were available. Antigens evaluated included κ, λ, CD19, CD20, CD5, and CD10. Within this group, the male/female ratio was 16:16, and the age of patients at the time of biopsy ranged from 26 to 86 years. For each case, the results of flow cytometry studies were compared with the final histologic diagnosis as outlined in the pathology report for the corresponding skin biopsy. On the basis of the flow cytometry result, cases were defined as true-positive (TP), true-negative (TN), false-positive (FP), or false-negative (FN) using histological evaluation as a "gold standard." Cases were categorized as "indeterminate" if no definite histologic diagnosis was provided. These data were used to determine the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of flow cytometry in the diagnosis of cutaneous B-cell lymphoma.

Of the 35 flow cytometry cases included for analysis, 16 were determined to be TP, 14 TN, 2 FP, and 2 FN. One case was categorized as indeterminate by histology and excluded from analysis. The overall sensitivity and specificity of flow cytometry were 88.9% and 87.5%, respectively. The PPV of flow cytometry was 88.9%, and the NPV was 87.5%. Of the cases within the FN category, one case had very low cellularity while no clear reason was identified in the second FN case, and the discrepancy is presumed to be due to issues of sampling. Both FP cases were secondary to presumed specimen contamination with peripheral blood.

This study confirms that flow cytometry is a reliable tool in the diagnosis of cutaneous B-cell proliferations/lymphomas and identifies pitfalls in the use of flow cytometry in the diagnosis cutaneous B-cell lymphoma.

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Value of Repeat Stool Testing for *Clostridium difficile* Toxins.

Linsheng Zhang, Lisa L. Steed, and Frederick S. Nolte. Sponsor: Christine N. Papadea. Department of Pathology and Laboratory Medicine, Medical University of South Carolina, Charleston.

Clostridium difficile is the most common cause of nosocomial diarrhea. Clinical manifestations of *C difficile*–associated disease range from mild diarrhea to life-threatening paralytic ileus and toxic megacolon. Recent increases in both the incidence of infection and the severity of clinical presentation, with recognition of an emerging hypervirulent strain, underscore the importance of prompt diagnosis and management of *C difficile* infection. Although cell culture cytotoxicity assay is well-accepted as the "gold standard" for laboratory diagnosis, toxin enzyme-linked immunosorbent assays (ELISAs) are more widely applied in clinical laboratories. Many physicians routinely order multiple toxin tests, hoping to increase the diagnostic yield, despite published reports that question this practice (Mohan et al. *Am J Med.* 2006;119:356.e7; Borek et al. *J Clin Microbiol.* 2005;43:2994; and Renshaw et al. *Arch Pathol Lab Med.* 1996;120:49).

To investigate the value of repeat *C difficile* ELISA toxin tests, we analyzed 1,079 tests performed in our laboratory from April 1 to June 30, 2007, on stool samples obtained from patients with suspected *C difficile*–associated diarrhea. The Premier Toxins A&B Test Kit, Meridian Bioscience, was used for toxin detection. Tests were considered to comprise an episode if they were ordered within 7 days of each other. There were a total of 589 episodes, 315 with only 1 test, 111 with 2 tests, 129 with 3 tests, 23 with 3 tests, and 11 with 4 or more tests ordered. *C difficile* toxin was detected in 9.5% of the episodes (56/589). Only 71% (40/56) of the total positive episodes were detected with the first test. The diagnostic yield rose to 86% (48/56) with 2 tests and to 95% (53/56) with 3 tests. The remaining 3 positive episodes (5%) required 4 or more tests for detection.

In conclusion, our results indicate that the diagnostic yield is markedly increased by repeat testing, suggest that the yield beyond

3 tests is small, and were similar to findings in the single study that documented the value of repeat testing for *C difficile* toxins (Manabe et al. *Ann Intern Med.* 1995;123:835).

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Persistent T-Cell Monoclones in Patients With Common Variable Immunodeficiency (CVID).

Shereen Gheith, Debora R. Sekiguchi, and Adam Bagg. Sponsor: Malek Kamoun. Department of Pathology and Laboratory Medicine, Hospital of the University of Pennsylvania, Philadelphia.

CVID is a primary immunodeficiency disorder characterized by hypogammaglobulinemia and recurrent bacterial infections. Patients with CVID are at increased risk for malignancy, including B-cell lymphomas. However, T-cell lymphoproliferative disorders are extremely rare, with, to our knowledge, only 1 case of peripheral T cell lymphoma in CVID being reported.

We encountered 3 cases of CVID with monoclonal T-cell receptor γ-chain gene (*TRG@*) rearrangements that were evident in different anatomic sites; persistent over time; and occasionally identical in different anatomic sites, but without overt evidence of a neoplastic lymphoproliferative disorder.

Patient 1 is a 43-year-old man with peripheral blood (PB) lymphocytosis (WBC count, 8.3×10^9/L; 72% lymphocytes). While numerous atypical lymphocytes were noted in the PB, there was no immunophenotypic evidence of malignancy. Both bone marrow (BM) and liver showed reactive (by morphology and flow) lymphoid infiltrates. However, *TRG@* polymerase chain reaction (PCR) studies revealed the presence of an identical clone in the PB, BM, and liver.

Patient 2 is a 40-year-old man with a skin lesion and a pulmonary mass. Both the skin and the lung biopsy revealed a polymorphous infiltrate of lymphocytes without evidence of malignancy. Nonidentical monoclonal *TRG@* gene rearrangements were evident in both sites. However, subsequent analyses performed on cerebrospinal fluid and another skin biopsy revealed the presence of similar (~2-base-pair difference) monoclonal *TRG@* gene rearrangements, in the absence of overt lymphoma.

Patient 3 is a 38-year-old man with anemia, recurrent venous line infections and hypogammaglobulinemia. A BM biopsy revealed pure red cell aplasia and small reactive-appearing lymphoid infiltrates. PCR studies revealed the same monoclonal *TRG@* gene rearrangement in both the BM and PB.

We report 3 cases of CVID with the presence of molecularly detectable T-cell monoclones in the absence of morphologic or immunophenotypic evidence of lymphoma. These clones are noteworthy for their dissemination, persistence, and occasional apparent (intrapatient) identity. The significance of these observations is uncertain. They may represent an atypical T-cell response to infections and/or other stimuli in a dysregulated immune milieu or are harbingers of overt lymphoma. However, none of these patients developed lymphoma between 2 and 10 years of follow-up. Importantly, such clones should also not be overinterpreted, in the context of patients at risk for lymphoma, to represent overt neoplasia.

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Expression of Smac/DIABLO in Lymphomas Involving Ovaries and Cervix: A Retrospective Study at the University of Alabama at Birmingham.

Liz Hanson,[1] Jeffrey Medeiros,[2] Mar Garcia,[2] Youngsheng Ren,[1] and Darshana Jhala.[1] Sponsor: Marisa Marques. [1]Department of Pathology, University of Alabama at Birmingham; and [2]Department of Hematopathology, M.D. Anderson, Houston, TX.*

Inhibitors of apoptotic proteins (IAPs) are up-regulated in cancers and suppress cell death by directly inhibiting caspases. IAPs are differentially expressed in B-cell lymphomas. Some IAPs are counteracted by the cell death inducer, second mitochondrial derived activator of caspases/direct IAP binding protein/Smac. The literature shows that some drugs, along with the use of Smac, can be used for the prevention and/or treatment of human prostate cancer. Gynecologic lymphomas are rare, and their clinical presentation can mimic a nonhematopoietic malignancy, especially in ovary and cervix. Expression of Smac/DIABLO in gynecologic lymphomas has not been explored. This study will explore Smac expression in lymphomas involving ovary and cervix.

A retrospective search was performed (1994-2004) for ovarian and cervical lymphomas. All female patients with a diagnosis of lymphoma involving ovary and cervix were included in the study. The H&E sections and immunohistochemical stains were reevaluated independently by 2 pathologists. Flow cytometry data were included when available. Smac/DIABLO immunostain was performed (LSAB+ kit, DakoCytomation) and evaluated independently by 3 pathologists.

The study included 8 female patients (age range, 22-80 years; mean age, 50.6 years) with non-Hodgkin lymphoma involving ovary (6) and cervix (2). Ovarian lymphomas included secondary (4) and primary (2). The 2 cervical lymphomas were secondary. Cytology (touch prep) showed findings consistent with lymphoma in 3 cases of ovarian lymphoma. Histology showed lymphoma in all 8 cases. Ovarian lymphomas included diffuse large B-cell lymphoma (2), follicular center cell lymphoma (3), and Burkitt lymphoma (1). Cervical lymphoma (2) showed presence of CLL/SLL. Flow cytometry (3/6 cases) supported the diagnosis of lymphoma. Smac/DIABLO stain showed weak positivity in 1 case (ovarian lymphoma [follicular center cell lymphoma]) and negative in 6 cases. In 1 case, no additional tissue was available to perform the stain.

Non-Hodgkin lymphoma involving ovaries and cervix may cause confusion for the clinician with other much more frequent ovarian tumors. Smac/DIABLO expression appears to be very rare in gynecologic lymphoma. Gynecologic lymphomas expressing Smac/DIABLO may have a unique pathway for apoptosis, which, in turn, may be of value for exploring targeted drug therapy.

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Concordance of Emergency Department (ED) Rapid Point-of-Care (POC) Troponin I (TPI) Blood Levels With TPI Measured by a Hospital Laboratory Assay.

Anne M. Bartlett,[1] Christine Papadea,[1] Nina Epps,[1] Yusheng Zhu,[1] and Gary Headden.[2] Departments of [1]Pathology and Laboratory Medicine and [2] Emergency Medicine, Medical University of South Carolina, Charleston.*

The objective of this study was to determine the concordance of TPI results by 2 different methods at our hospital. TPI is initially measured on admission to the ED of our accredited chest pain center with the 10-minute i-STAT POC system utilizing 2 anti-TPI antibodies and enzymatic detection. The same (initial) or another sample drawn within 10 minutes from the patient is sent immediately to the main laboratory (Lab) for the TPI-ultra assay (TPIu) configured with 3 anti-TPI antibodies and chemiluminescence detection on the

ADVIA Centaur XP system. The Lab's turnaround time is approximately 55 minutes. The initial POC level guides early management; the initial and subsequent Lab results are used for confirmation and trending. Both assays had been extensively evaluated in our setting to validate performance characteristics prior to clinical service.

We compared the results in the initial samples of 1,080 consecutive ED cases encountered in 5 months. The initial POC sample was tested by trained ED staff; the concurrent sample was analyzed in the Lab. Each result was assigned an interpretation—normal; abnormal nonacute myocardial injury (MI) including ischemia; or acute MI (AMI)—based on cutoff values previously set for each method. Concordance was defined as the number of cases in which TPI levels by both methods had the same interpretation. Medical records were reviewed when results were discordant. Of 1,080 cases, 1,018 (94%) were concordant: 930 normal and 88 abnormal (72 MI + 16 AMI); 62 (6%) were discordant: 59 normal by POC but abnormal MI by TPIu; 3 abnormal MI by POC but normal by TPIu. Of the 59, 6 had ischemic events; of the 3, none had ischemic events. Discordant cases, most of which had coexisting chronic renal or cardiac insufficiency disorders, clustered near the cutoffs separating the interpretations. There were 94 true-positives and 986 true-negatives. Diagnostic sensitivity and specificity, respectively, were as follows: POC, 94% and 100%; TPIu, 100% and 95%.

TPI results were concordant for the majority of cases. Differences in assay configurations may account, in part, for the lack of total concordance. Rapid POC in the ED provides diagnostically specific results to screen normal cases.

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Establishing a Microarray Test for Pan-Viral Identification (Virochip) as a Core Laboratory Resource at UCSF.

Steve Miller,[1] Chris Barker,[4] Yanxia Hao,[4] David Erle,[2] Andrea Barczak,[2] Kael Fischer,[3] Joe DeRisi,[3] and Charles Chiu.[3] Departments of [1]Laboratory Medicine, [2]Medicine, and [3]Biochemistry and Biophysics, University of California San Francisco; and [4]J. David Gladstone Institutes, San Francisco, CA.*

Current methods used to diagnose viral illness fail to detect the etiologic agent in 30% to 60% of cases of acute respiratory infection and more than 75% of the time in encephalitis. The impact of these infections in terms of patient morbidity and mortality and economic costs is substantial, indicating that improved diagnostics could be both clinically useful and cost-effective. Several novel viruses causing human disease have been identified in the past 5 years, including bocavirus, metapneumovirus, and the SARS coronavirus, with this trend expected to continue. Additionally, worldwide travel makes possible exposure of a large immunologically naive population to previously regionally restricted agents.

Our research consortium is building on the previous work of DeRisi and colleagues, who have successfully used microarray platforms to identify unknown and novel viruses in human specimens. We are focusing initial efforts on acute respiratory infection, with the current platform, termed Virochip, consisting of 22,000 oligonucleotide probes derived from sequences of approximately 1,800 viruses. Previous versions of this microarray have been used to identify a novel virus (SARS coronavirus) and an unknown virus causing critical respiratory illness (human parainfluenza 4). Our group has shown this platform to compare favorably with DFA and single-agent polymerase chain reaction (PCR) for the diagnosis of acute respiratory infection in children.

Current work includes redesign of the selected oligonucleotide probes based on previous data generated and transition to a commercially available platform. Nucleic acid extraction, amplification, labeling, hybridization, imaging, and data analysis are all systematically being incorporated into clinical and core laboratory procedures, enabling samples to be analyzed without specialized technical knowledge. We are validating this process with a sample of respiratory samples from pediatric acute infections and have shown excellent correlation to the research Virochip platform, as well as DFA and PCR. Analysis is performed using E-Predict, a computational method that compares actual with theoretical hybridization patterns and yields a likelihood score for viral identification. Upon completion of this project, we expect to enable routine analysis by this method for research uses, including severe patient illness of unknown etiology and population epidemiologic studies.

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Experience With Ribosomal Gene Analysis for Identification of Clinical Bacterial and Fungal Isolates.

Gerald P. Morris and W. Michael Dunne. Department of Pathology and Immunology, Washington University School of Medicine, St Louis, MO.*

DNA sequence analysis of ribosomal genes has been used to accurately identify bacteria and fungi for 3 decades, providing insight into microbial phylogeny. More recently, it has been demonstrated that this approach is applicable to clinical pathogen identification. Several reports in recent years have demonstrated the utility of DNA sequence analysis of the 16S ribosomal RNA gene for identification of clinically relevant bacteria. Additionally, several ribosomal gene targets for fungal identification have been described, with varying degrees of success in identification; among these are noncoding internal transcribed spacer (ITS) regions 1 and 2, which demonstrate DNA sequence and length diversity sufficient for discrimination between fungal species. Analysis of ribosomal genes would likely aid in pathogen identification in the clinical laboratory, though the methodology is relatively expensive and complex, limiting its use. Therefore, we propose to use this approach to identify difficult organisms: rare organisms, unusual clinical presentations, or organisms that fail to generate high-confidence identification by automated biochemical systems.

We first tested the validity of 16S and ITS1 and ITS2 gene sequence analysis for identification of clinically relevant pathogens by analysis of well-characterized reference strains. Ribosomal gene targets were amplified from DNA from fresh subcultures using primers to highly conserved regions and subsequently bidirectionally sequenced by cycle sequencing. DNA sequences were analyzed using GenBank and Ribosomal Database Project II databases. Analysis of 18 well-characterized reference strains of clinically relevant bacteria demonstrated 100% success in species-level identification. We next tested the ability of 16S gene sequence analysis to provide an accurate identification of 33 clinical bacterial isolates referred to the Missouri State Public Health Laboratory owing to low-confidence identifications in the Barnes-Jewish Hospital clinical laboratory in an 18 month period. 16S gene analysis yielded a genus-level identification of 31 of 33 isolates identified (2 were not able to be grown from frozen stock), indicating the clinical utility of this approach. However, fungal analysis proved more problematic; analysis of 8 well-characterized laboratory type strains demonstrated only a 50% success rate in genus-level identification. The DNA sequences generated from the isolates were of high quality, and deficiencies in identifications are likely owing to deficiencies in the database used for identification. To address this,

we have begun compiling a database of both ITS1 and 2 sequence and length polymorphisms from well-characterized reference strains.

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E-Cadherin as a Chemotherapy Resistance Mechanism for Breast Cancer Metastasis.

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

Metastasis contributes significantly to the mortality of breast cancer. A key molecule that has been implicated in metastasis is E-cadherin, a cell adhesion molecule that is expressed on epithelia. Numerous studies have shown that loss of E-cadherin contributes to tumor invasiveness and metastasis. However, these studies focus only on the role of E-cadherin in detachment and dissemination from the primary tumor. The ability of a metastatic tumor cell to survive and grow in a new organ environment is critical to the development of tumor nodules and may serve as a critical target of cancer therapy. Because the pattern of metastasis to certain organs and bones is nonrandom, this suggests that metastases only form within organs that provide the appropriate signals and a hospitable environment.

We hypothesize that breast carcinoma cells require E-cadherin reexpression to maintain survival and to integrate within the liver, a common site of breast cancer metastases. Previous work in the laboratory has shown that coculture of E-cadherin–negative human metastatic breast cancer cells with rat hepatocytes results in the reexpression of E-cadherin that mediates functional ligation between the 2 cell types and activates the Erk pathway. Here we show that E-cadherin reexpression may confer a survival advantage on metastatic cancer cells by enabling them to evade chemotherapy-induced cell death. When cell death is induced by either the inflammatory cytokine TNF-α or the chemotherapeutic agent camptothecin, E-cadherin–positive breast cancer cells are more resistant to cell death than E-cadherin–negative breast cancer cells, as assayed by PI staining and a viability/cytotoxicity assay. In addition, the chemoresistance of E-cadherin–positive cancer cells is decreased after transfection with siRNA against E-cadherin or treatment with an E-cadherin function-blocking antibody. This initial cell death assay demonstrates the protective effect of E-cadherin expression and, in conjunction with future coculture studies, may reveal why chemotherapy commonly fails to treat metastatic breast cancer.

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MicroRNA Microarray Profiling in Myelodysplastic Syndrome.

John Tobias,[1] Don Baldwin,[2] James Thompson,[3] and Annette S. Kim.[4] Sponsor: Bruce Sachais. [1]Penn Bioinformatic Core, [2]Penn Microarray Facility, [3]Department of Hematology and Oncology, and [4]Department of Pathology and Laboratory Medicine, University of Pennsylvania, Philadelphia.

MicroRNAs (miRNAs) are a form of noncoding RNA that physiologically play a key role in the differentiation and identity of different cell types through translational regulation. miRNAs have been associated with numerous cancers, with specific patterns of underexpression or overexpression, and occasionally predictive of clinical outcomes. Myelodysplastic syndrome (MDS) is a clonal hematopoietic disorder that is characterized by ineffective hematopoiesis. Although the majority of patients die secondary to their cytopenias, approximately 23% progress on to acute leukemia with an abrupt cessation in maturation. Since this transformation from MDS to acute leukemia is fundamentally both an ontologic and a differentiation aberration, miRNAs may play critical roles in the development of MDS and in the transformation to an acute leukemia. Preliminary results of an initial pilot study of miRNA microarray profiling of MDS are presented.

The study utilized 20 samples of bone marrow mononuclear cells isolated and stored at the University of Pennsylvania Stem Cell Core Facility between 2003 and 2007. Ten of these samples were obtained from patients with early MDS (subtypes RA, RCMD, and RAEB-1). The remaining samples were obtained from 10 normal controls. Total RNA was obtained from each sample and arrayed on a proprietary Agilent MicroRNA chip in a dual-channel experiment in which each sample was arrayed against a pool of all 20 samples. Class discovery ANCOVA algorithms were applied to the data to identify 14 miRNAs that differentiate the MDS samples from the normal controls. Using K-nearest neighbor cross-validation, 17 of 20 samples were correctly assigned to their appropriate group with 2 unassigned and 1 misassigned. Interestingly, the 1 misassigned sample is from the patient with the mildest disease who is currently doing well on Vidaza with no cytopenias. These classifier miRNAs are predicted to regulate some of the target mRNAs that have been implicated in previous gene expression profiling experiments in MDS. In addition, potential miRNAs were identified that distinguished the 3 patients who progressed to acute myeloid leukemia within 24 months from the 7 who did not. RT–polymerase chain reaction confirmation of these miRNAs is ongoing. Ultimately, it is hoped that miRNA profiling of MDS will provide practicing pathologists with new markers for the identification of MDS and provide clinicians with additional prognostic information to guide the use of therapeutic interventions.

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Automated Detection of High-Dose Hook Effect in a Ferritin Immunoassay.

Robyn C. Reed, Kathleen Hutchinson, Petrie M. Rainey, and Mark H. Wener.* Department of Laboratory Medicine, University of Washington, Seattle.*

The high-dose hook effect is a source of falsely low results in immunoassays, arising when excess antigen saturates antibody binding sites. Methods have been proposed to abrogate or detect hooking, but few are suitable for an automated, random-access immunoassay platform. Several diseases, including hemophagocytic syndrome (HPS) and Still disease (systemic-onset juvenile rheumatoid arthritis), result in marked hyperferritinemia and can cause hook effect in the ferritin immunoassay.

We analyzed the results of 20,540 serum ferritin tests performed on the Beckman Access platform and examined the characteristics of patient samples that showed a hook effect. Hook effect was further investigated experimentally, using specimens containing purified human ferritin. We detected 9 clinical samples that hooked (0.04%) from 5 different patients. All 5 patients had HPS; of these, 3 cases were likely related to Epstein-Barr virus or cytomegalovirus infection, while 2 were secondary to hematologic malignancies (1 T-cell lymphoma and 1 natural killer cell lymphoma). Of the 5 patients, 4 died less than a month after presentation and initial detection of high hyperferritinemia, while 1 patient with viral-related HPS is alive 2 years after diagnosis, on chronic immunosuppression. Hook effects were observed above approximately 99,000 ng/mL in patient samples and, similarly, above approximately 110,000 ng/mL in experimental samples. Above the hooking threshold, assay results

decreased predictably and reproducibly in a nearly log-linear fashion as the concentration of ferritin increased. Even at the highest concentrations of ferritin tested (600,000 ng/mL), hooking did not result in a value in the normal range. To avoid reporting falsely low results due to the hook effect, samples with results greater than 300 ng/mL can be automatically diluted and repeated. At this cutoff, ferritin concentrations up to more than 500,000 ng/mL are accurately reported, and approximately 18% of our samples are repeated.

Very high ferritin concentrations are not exceptionally rare, easily missed if not looked for, and clinically significant. In this small series, hyperferritinemia assisted in the diagnosis of HPS and was associated with a high mortality rate. We describe a method of checking for hook effect that reduces reagent and labor costs, allows random-access rather than batched testing, and retains the ability to detect the full range of clinically observed ferritin concentrations.

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Epstein-Barr Virus (EBV) LMP-1 Gene Sequence Variation to Track Transmission of EBV Infection.

Anna M. Budde,[1] Charlotte A. Romain,[1] Chiu-Ho Webb,[1] Carol J. Holman,[1] and Henry H. Balfour, Jr.[1,2*] [1]Departments of Laboratory Medicine and Pathology and [2]Pediatrics, University of Minnesota Medical School, Minneapolis.*

The incubation period and duration of contagiousness of infectious mononucleosis (mono) are not known. A limited number of sequence variations in the carboxy terminus of the EBV LMP-1 gene could be used to trace person-to-person spread of primary EBV infection and, hence, define those unknowns.

Subjects who gave informed consent participated in trials to define the natural history of EBV shedding and response to antiviral drugs. Oral cells (OC), oral supernatant fluid (OS), and whole blood (WB) samples collected at specified intervals were analyzed for EBV LMP-1 sequence variation. Our method was based on that of van Kooij et al. (*J Clin Virol.* 2003;28:85-92). The outer LMP-1 primer set was o5-168843R: CTA CAA CAA AAC TGG TGG ACT, and o12-168039F: AGA CAG TGT GGC TAA GGG AGT. The inner primer set was i8-168609R: TGC TCT CAA AAC CTA GGC GCA, and i11-168075F: TGA TTA GCT AAG GCA TTC CCA. A third polymerase chain reaction (PCR) was performed when sequences were mixed using i9-168390R AGC GAC TCT GCT GGA AAT GTA and i11-168075F. Purified PCR products were sequenced at our Advanced Genetic Analysis Center using a 3100 Genetic Analyzer (ABI) and Big Dye chemistry (version 3.1). Chromatograms were compared using Sequencher software (version 4.2.2) to find differences from the reference EBV strain B95-8.

We studied 65 subjects (39 women and 26 men); 54 were university students (median age, 21 years) with acute mono and 11 were healthy EBV antibody–positive adults (median age, 31 years). We analyzed the LMP-1 sequences present in 134 samples: OC, 77; OS, 35; and WB, 22. Sequence patterns segregated into 5 clusters, with clusters 2, 4, and 5 representing the majority of subjects (53/65). All sites sampled from the same subject had identical LMP-1 sequence patterns, and these patterns were stable over time (up to 208 days). A student and her boyfriend with acute mono both had identical LMP-1 sequence patterns. In contrast, 2 college roommates who had mono 3 months apart displayed very different LMP-1 patterns.

LMP-1 sequence patterns from students with mono and healthy adults who had been infected with EBV in the past were specific to the individual, consistently the same in the oral and peripheral blood compartments, and stable over time. Preliminary epidemiologic data suggest that our assay will be useful in tracking transmission of mono, which could enable us to determine its incubation period and duration of contagiousness.

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Microbial Contamination of Cryopreserved Parathyroid Tissue.

B. Stotler, R. Slotky, J. Schwartz, W. Inabnet, J. Lee, and J. Jhang. Sponsor: S. Spitalnik. Departments of Pathology & Surgery, Columbia University Medical Center, New York, NY.

Cryopreservation of parathyroid tissue (PT) provides patients undergoing parathyroidectomy with an option for delayed autologous heterotopic parathyroid transplantation (AHPT) to treat postsurgical hypoparathyroidism. Sterility testing is one of the standard requirements in any cellular therapy program; however, a standard protocol for sterility testing of PT has not been established, and practices are relatively unregulated. We evaluated the results of preprocessing and postprocessing cultures to identify organisms that may be potential sources of infection following transplantation.

PT was received from the operating room in a sterile container with normal saline (NS). PT was minced into 2- to 3-mm fragments under aseptic conditions and placed into cryopreservation media containing 10% freshly prepared autologous serum and 10% dimethyl sulfoxide. Sterility testing for each PT specimen was performed by inoculating individual BACTEC Peds Plus bottles with 1 mL of preprocessing fluid and 1 mL of postprocessing fluid; 1 mL of the cryopreservation medium was tested as well. All bottles were placed into a BACTEC 9240 instrument (BD, Franklin Lakes, NJ). The cultures were incubated at 37°C and continuously monitored for bacterial growth for 6 days or until microbial detection. Positive cultures were further analyzed for species identification.

From January 2005 to December 2007, 35 PT samples from 35 patients (22 women and 13 men; age 20-84 years) were cryopreserved at Columbia University Medical Center for future AHPT. All cultures of the cryopreservation medium were negative. Seven organs cultured prior to processing were positive with the following organisms: *Staphylococcus epidermidis* (5), *Staphylococcus capitis* subspecies *ureolyticus* (1), and *Staphylococcus lugdunensis* and *Bacillus pumilus* (1).

Our findings demonstrate that performing sterility testing on PT for autologous transplantation provides an essential tool for patient safety and laboratory compliance. We found that 20% of parathyroid tissues cryopreserved in our institute may demonstrate microbial contamination at the time of collection. Skin bacteria are the most common species identified. With the exception of the *B pumilus*, the staphylococcal species isolated may pose a risk for a potential subcutaneous or deep tissue infection at the AHPT site. Further studies are required to determine if AHPT of culture-positive PT increases wound infection and if administration of prophylactic antibiotics could be useful in this setting.

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Can Reflexive Testosterone Testing Improve Service and Decrease Costs?

Kimia Sobhani[1] and Daniel D. Bankson.[1,2] [1]Department of Laboratory Medicine, University of Washington, Seattle; and [2]Veterans Affairs Puget Sound Health Care System, Seattle.*

We sought to determine if the large number of testosterone panels performed annually at the Veterans Affairs Puget Sound Health Care System (VAPSHCS) could be reduced through the introduction of a reflexive panel, which would provide cost savings and service improvement.

Currently, VAPSHCS performs testosterone testing for Washington, Oregon, Idaho, and Alaska Veterans Affairs (VA) sites. Testosterone panels involve determining total testosterone (TT), a relatively inexpensive test; sex hormone binding globulin (SHBG), a relatively expensive test; and albumin (Alb). From the TT, SHBG, and Alb results, free testosterone (FT) and bioavailable testosterone (BT) are calculated. We wanted to determine a TT cutoff concentration at which 95% or more of the associated FT and BT results were normal. Reflexive determination of FT and BT would occur below this TT cutoff.

Measurement of TT and SHBG by immunoassay and measurement of Alb are performed on the Roche Diagnostics Modular Analytics E170 and P platforms, respectively. Calculated FT and calculated BT are determined using equations that incorporate TT, SHBG, and Alb values (DeVan et al. *Am J Clin Pathol.* 2008;129:459-463). Testosterone results generated from November 2006 to November 2007 were reviewed and filtered for entries that included all 5 tests: TT, SHBG, Alb, FT, and BT. The total number of panel entries was 3,075. The percentage of normal FT and BT results were then calculated at various TT cutoffs starting with the low end of our in-house TT reference range (2.8-8.0 ng/mL).

There were 1,743 panel entries of 2.8 ng/mL TT or more, for which 98.6% FT results were normal (reference range, 34-194 pg/mL), and 97.6% BT results were normal (reference range, 84-402 ng/dL). At 4.0 ng/mL TT, there were 986 panel entries, of which 99.5% FT results and 98.7% BT results were normal. Reagent, instrument, and labor costs for SHBG and Alb are approximately $7/panel. At the 2.8 ng/mL TT cutoff, the elimination of 1,743 panels would have saved approximately $12,000 annually. At the 4.0 ng/mL TT cutoff, the elimination of 986 panels would have saved approximately $7,000 annually.

A reflexive testosterone panel would save VAPSHCS up to $12,000 annually. More important, this reflexive approach could potentially decrease the need for future patient appointments and phlebotomies and accelerate the diagnosis of testosterone-related disorders.

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The Complementary Role of Molecular Genetic and Conventional Cytogenetic Detection of Diagnostically and Prognostically Important Translocations in Acute Leukemia (AL): An Analysis of Over 200 Cases.

Mojdeh Naghashpour and Adam Bagg. Sponsor: Malek Kamoun. Department of Pathology and Laboratory Medicine, University of Pennsylvania, Philadelphia.

Given the central role of genetics in the classification of AL and the recognition that conventional cytogenetics (CC) may miss important translocations, we evaluated the relative roles of molecular genetic assays and CC in patients with AL.

During a period of 3 years (July 2004-August 2007), a total of 210 consecutive diagnostic samples (peripheral blood or bone marrow) from adults with AL were analyzed by multiplex RT–polymerase chain reaction (MRP); 80.0% (168/210) of these were also successfully analyzed by CC. MRP was performed by using 1 of 2 commercially available kits (HemaVision, Aarhus, Denmark; or LTx, Asuragen, Austin, TX) that are able to detect the 7 most common AL translocations: t(8;21), t(15;17), inv(16), t(9;22), t(12;21), t(4;11). and t(1;19). CC analysis was performed on unstimulated 24-hour cultures.

Evaluable CC data were available in 168 of 210 cases (group A). The remaining 42 cases (group B) did not have CC ordered (n = 31) or CC failed (n = 11). Ninety-three cases in group A (93/168) had a clonal abnormality by CC, with 25 of 93 harboring one of the 7 translocations; all 25 of these translocations were also detected by MRP. In the remaining 68 of 93 cases with abnormal CC, 2 had a translocation detected by MRP but not by CC. Seventy-five of the cases in group A had normal CC; however, 3 of 75 had a translocation detected by MRP. In group B, 4 of 42 had a translocation detected by MRP only. Thus, a total of 9 cases (2 + 3 + 4) in this series had a translocation missed by CC but detected by MRP. The MRP data were consistent with the hematopathologic classification in all 9 cases. No cases with an MRP-detectable translocation, evident by CC, were missed by MRP (no false-negatives for the molecular assay). By contrast, depending on the denominator used, 4.9% (9/185 cases without a CC-detectable MRP translocation = 2/68 + 3/75 = 4/42) or 16.7% (5/30 cases with 1 of the 7 translocations, by either method) of AL cases had false-negative CC.

This study indicates that molecular diagnostic techniques such as MRP add crucial information by detecting therapeutically relevant gene fusions that may be missed by conventional cytogenetic analysis. However, such molecular approaches cannot currently supplant conventional cytogenetic analysis, as the latter is still able to provide a global view of important genomic aberrations that are not discernible by MRP. Thus, these approaches are best viewed as being complementary to one another.

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Genome-wide Analysis of Histone and DNA Methylation in Myelodysplastic Syndrome (MDS) With a Novel Technology: Sequential Chromatin–Immunoprecipitation Coupled Genome-wide Promoter Array (SChIP-on-chip): A Pilot Study on Refractory Cytopenia With Multilineage Dysplasia (RCMD).

Jason X. Cheng, John Anastasi, and James W. Vardiman. Sponsor: Jonathan L. Miller. Department of Pathology, University of Chicago, Chicago, IL.

Although epigenetic changes such as DNA and histone methylation have been shown to play an important role in disease progress, prognosis, and therapy of MDS and leukemia, largely owing to technical difficulties there is little known at the genome-wide level about genes affected by DNA or histone methylation or the potential use of DNA or histone methylation as biomarkers in MDS or leukemia. In the present pilot study, we have developed a novel technology, SChIP-on-chip, to analyze the genome-wide histone and DNA methylation in RCMD.

In this study, age- and sex-matched specimens of bone marrow from 4 hematologically normal patients and 4 patients with RCMD were used. For the histone methylation study, the cells were first cross-linked with formaldehyde and DNA was fragmented by sonication. Immunoprecipitation with anti-H3K27me3 antibodies was performed to enrich the methylated histone/DNA complex. For the DNA methylation study, DNA was fragmented and purified with a similar method. The methylated DNAs were enriched by immunoprecipitation with anti–5-methylcytidine antibody (GeneTex). Quantitative polymerase chain reactions (Q-PCRs) were first performed with specific primers to assess histone and DNA methylation

at the previously reported methylation targets in MDS, including HIC1, p15INK4B, CALCA (calcitonin), CDH1 (E-cadherin), and ER. The methylated DNAs were amplified by ligation-mediated PCR. The amplified DNAs were labeled with fluorescent label C3 or C5 and subjected to the genome-wide promoter arrays, followed by computational analysis.

The results of the present study showed that there is a significant increase of DNA and histone methylation on the previous reported gene promoters such as p15INK4B and HIC1 in RCMD by both Q-PCR and the genome-wide promoter array analysis. More important, this new genome-wide promoter array analysis has identified a group of genes previously unknown to be hypermethylated in MDS. Some of those, such as Pu.1 and Spi-B, have been shown to play a very important role in myeloid differentiation. To our knowledge, this is the first time this technology has been used to study genome-wide DNA and histone methylation in clinical specimens. This may lead to identifying novel methylated gene signatures that can serve as biomarkers and as targets for therapy in MDS or other diseases.

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Detecting γδ T-Cell Lymphoma by Paraffin Immunohistochemistry.

Michele Roullet, MD,[1] Shereen Gheith, MD, PhD,[2] Dennis Cornfield, MD,[2] Joanne Mauger,[3] Hong Wu, MD, PhD,[4] Jacqueline M. Junkins-Hopkins, MD,[5] Shiyong Li, MD, PhD,[6] and John K. Choi MD, PhD.[1,3] Sponsor: Malek Kamoun. [1]Department of Pathology & Laboratory Medicine, University of Pennsylvania School of Medicine, Philadelphia; [2]Health Network Laboratories, Lehigh Valley Hospital, Allentown, PA; [3]Department of Pathology and Laboratory Medicine, Children's Hospital of Philadelphia, Philadelphia; [4]Department of Pathology, Fox Chase Cancer Center, Philadelphia; [5]Department of Pathology and Laboratory Medicine, Johns Hopkins School of Medicine, Baltimore, MD; and [6]Department of Pathology and Laboratory Medicine, Emory University School of Medicine, Atlanta, GA.

We have identified a commercially available antibody that can detect γδ T-cell receptor (TCR) in formalin-fixed, paraffin-embedded sections. To assess the sensitivity and specificity of the antibody, we performed paraffin immunohistochemistry (PIHC) on 23 cases of T-cell lymphomas (TCL) using the antibodies BF1 and TCRG that detect αβ TCR and γδ TCR, respectively. The TCLs included cases of angioimmunoblastic TCL, enteropathy-type TCL, hepatosplenic TCL, and peripheral TCL, not otherwise specified. Of the cases, 8 were TCRG+/BF1–, 11 cases were TCRG–/BF1+, 4 cases were TCRG–/BF1–, and 1 case was TCRG+/BF1+. Intensity of the staining with TCRG or BF1 was variable among cases. Nine cases had accompanying flow cytometry for αβ TCR and γδ TCR. Six cases had concordant flow cytometry and PIHC findings (4 γδ TCL and 2 αβ TCL). Two cases were TCRG–/BF1– by PIHC but TCRαβ (1 case) or TCRγδ (1 case) by flow cytometry, suggesting that flow cytometry is more sensitive than PIHC for the detection of TCR. One case was TCRG+/BF1– by PIHC but TCRαβ by flow cytometry and is being further studied to understand the discrepancy. These findings indicate that TCRG can aid in the diagnosis of some γδ TCLs.

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Detecting γδ T Cells in Panniculitis by Paraffin Immunohistochemistry.

Michele Roullet, MD,[1] Shereen Gheith, MD, PhD,[2] Joanne Mauger,[3] Jacqueline M. Junkins-Hopkins, MD,[4] Shiyong Li, MD, PhD,[5] and John K. Choi, MD, PhD.[1,3] Sponsor: Malek Kamoun. [1]Department of Pathology & Laboratory Medicine, University of Pennsylvania School of Medicine, Philadelphia; [2]Health Network Laboratories, Lehigh Valley Hospital, Allentown, PA; [3]Department of Pathology and Laboratory Medicine, Children's Hospital of Philadelphia, Philadelphia; [4]Department of Pathology and Laboratory Medicine, Johns Hopkins School of Medicine, Baltimore, MD; and [5]Department of Pathology and Laboratory Medicine, Emory University School of Medicine, Atlanta, GA.

In the World Health Organization (WHO) classification, subcutaneous panniculitis-like T-cell lymphomas (SPTCLs) are malignancies of mature αβ T cells or γδ T cells. The more recent WHO–European Organization for Research and Treatment of Cancer classification has recognized that SPTCL of αβ T cells and that of γδ T cells have different clinical courses, necessitating their immunophenotyping for proper subclassification and treatment. While αβ T cells can be detected in formalin-fixed, paraffin-embedded (FFPE) sections using antibodies directed against the BF1 epitope, no equivalent antibody can reliably detect γδ T cells, requiring their detection by flow cytometry, frozen tissue immunohistochemistry, or by their presumed existence based on BF1–/CD3+ T cells.

In this study, we identified a commercially available antibody (TCRG) that can detect γδ T cells in FFPE sections and examined 2 cases of flow cytometry–proven γδ T-cell lymphomas and 15 control cases of nonneoplastic panniculitis. In cutaneous γδ T-cell lymphoma and γδ hepatosplenic T-cell lymphoma, the atypical lymphocytes were CD3+/TCRG+/BF1–, while the small reactive lymphocytes were CD3+/TCRG–/ BF1+. The 15 control cases showed lobular or septal panniculitis composed of small to medium-sized lymphocytes admixed with varying numbers of histiocytes, eosinophils, or neutrophils. Most lymphocytes were CD3+, while 50% to 90% were BF1+, and fewer than 10% were TCRG+. The TCRG+ cells were scattered and did not cluster as in the 2 γδ T-cell lymphomas, suggesting that nonneoplastic panniculitis is composed of mostly αβ T cells with scattered γ/δ T cells. These results indicate that TCRG can detect γδ T cells in FFPE sections and, thus, provide a new powerful tool to characterize T cells in lymphomas and inflammation.

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Proteomic Expression Analysis of Surgical Human Colorectal Cancer Tissues: Up-regulation of PSB7, PRDX1, and SRP09 and Biomarkers of Hypoxic Adaptation in Cancer.

Michael H.A. Roehrl,[1,3] Jung-hyun Rho,[2,3] and Julia Y. Wang.[2,3] Sponsor: Michael Laposata. [1]Department of Pathology and Laboratory Medicine, Massachusetts General Hospital, [2]Channing Laboratory, Brigham and Women's Hospital, and [3]Harvard Medical School, Boston, MA.

Colorectal adenocarcinoma is one of the worldwide leading causes of cancer deaths. Discovery of specific biomarkers for early detection of cancer progression and the identification of underlying pathogenetic mechanisms are important tasks. Global proteomic approaches have thus far been limited by the large dynamic range of molecule concentrations in tissues and the lack of selective enrichment of the low-abundance proteome.

We studied paired cancerous and normal clinical tissue specimens from patients with colorectal adenocarcinomas by heparin-affinity fractionation enrichment (HAFE) followed by 2-D PAGE and tandem mass spectrometric (MS/MS) identification. We found

56 proteins to be differentially expressed, of which 32 low-abundance proteins were only detectable after heparin-affinity enrichment. MS/MS sequencing was used to identify 5 selected differentially expressed proteins as proteasome subunit β type 7 (PSB7), hemoglobin α subunit, peroxiredoxin-1 (PRDX1), argininosuccinate synthase, and signal recognition particle 9-kDa protein (SRP09).

This is the first proteomic study detecting the differential expression of these proteins in human colorectal cancer tissue. Several of the proteins are functionally related to tissue hypoxia and hypoxic adaptation. The relative specificity of PSB7, PRDX1, and SRP09 overexpression in colon cancer was validated by Western blot analysis in patients with colon adenocarcinomas and comparison with a cohort of patients with lung adenocarcinomas. Furthermore, immunohistochemistry on tissue sections was used to define the specific locations of PSB7, PRDX1, and SRP09 up-regulation within heterogeneous primary human tumor tissue. Overexpression of the 3 proteins was restricted to the neoplastic cancer cell population within the tumors, demonstrating both cytoplasmic and nuclear localization of PSB7 and predominantly cytoplasmic localization of PRDX1 and SRP09.

We describe HAFE as a powerful prefractionation tool for the study of the low-abundance human primary tissue proteome and the discovery of the colon cancer biomarkers PSB7, PRDX1, and SRP09.

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Evaluation of Five FDA-Approved Rapid HIV Antibody Assays.

A.N. Sireci, L. Herlitz, W. Yu, and M.A. Pesce. Sponsor: S. Spitalnik. Department of Pathology, Columbia University Medical Center, New York, NY.

The increase in test volume resulting from a recent Centers for Disease Control and Prevention recommendation advocating universal HIV screening in health care settings will require a shift from standard enzyme immunoassays (EIA) to more rapid HIV testing. At our medical center, a significant number of specimens test positive by EIA but show no bands by Western blot (WB). This study was undertaken to evaluate the reactivity of these EIA-positive, WB-negative specimens and control EIA-positive, WB-positive specimens with 5 Food and Drug Administration–approved rapid HIV assays.

The rapid HIV antibody assays used in this study were the OraQuick Advance HIV1/2 assay, the Multispot HIV1/2 test, the Reveal G-3 HIV-1 assay, the Clearview HIV1/2 test, and the Uni-Gold HIV-1 assay. In the study, 50 serum samples found to be HIV1/2+ with an EIA bead assay from Abbott but HIV-1– with a WB assay from Bio-Rad and 34 serum samples that were HIV1/2 EIA- and WB-positive were measured with these 5 rapid assays. HIV-2 was not detected by an ELISA assay in 29 of 50 EIA-positive, WB-negative specimens. HIV-2 was not measured in the remaining 21 WB-negative specimens.

The sensitivity and specificity of the 5 rapid HIV tests compared with WB were determined. The OraQuick by Orasure (sensitivity, 97.1%; 95% confidence interval [CI], 0.829-0.998; specificity, 96.0%; 95% CI, 0.851-0.993) and Multispot by Bio-Rad (sensitivity, 100%; 95% CI, 0.871-1.00; specificity, 92.2%; 95% CI, 0.803-0.975) tests yielded the best combinations of sensitivity and specificity. The OraQuick assay is a waived test when using whole blood or an oral fluid specimen, and the Multispot assay is a moderately complex test that uses serum but can distinguish HIV-1 from HIV-2. Our results suggest these 2 procedures will be of greatest utility in universal screening programs. The remaining tests performed as follows: Reveal from MedMira: sensitivity, 84.9%; 95% CI, 0.673-0.943; specificity, 98.0%; 95% CI, 0.878-0.999; Clearview from Inverness: sensitivity, 88.2%; 95% CI, 0.716-0.962; specificity, 97.9%; 95% CI, 0.875-0.999; and Uni-Gold by Trinity: sensitivity, 84.8%; 95% CI, 0.673-0.943; specificity, 96.0%; 95% CI, 0.851-0.993.

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Colony-Forming Unit-Granulocyte Macrophage of Cord Blood Transplants Predicts Neutrophil Engraftment and Patient Survival.

Steffini Stalos, Vincent Laroche, Darin Sumstad, Theresa Schierman, Diane Kadidlo, Todd Defor, Bruce Lindgren, Sponsors: Jeffrey McCullough and David H McKenna, Jr. University of Minnesota, Minneapolis.*

Although CD34+ and total nucleated cell (TNC) counts are common indices used to predict engraftment kinetics in umbilical cord blood (UCB) transplantation, neither assay is a true functional assessment of the UCB unit. The colony-forming unit-granulocyte macrophage (CFU-GM) is a functional assay; however, it has a less defined role in the quality assessment of UCB transplants. A retrospective analysis was performed to evaluate the role of CFU-GM (and CD34+ and TNC counts) in predicting engraftment and patient survival following UCB transplantation at our institution.

Between June 2000 and December 2004 at the University of Minnesota, 260 patients received single UCB and/or double UCB transplants (myeloablative or nonmyeloablative regimen). Some patients underwent transplant more than once. Patient demographics, diagnosis, survival, and laboratory data were obtained by institutional review board–approved medical chart review. CB units were thawed and washed according to a standardized protocol. Neutrophil engraftment was defined as day 1 of the first of 3 consecutive days with an absolute neutrophil count of 500/μL or more. Platelet engraftment was defined as the day at which a platelet count was $20{,}000 \times 10^3/\mu L$ or more without transfusion. Engraftment characteristics and Kaplan-Meier estimates were derived from postthaw CD34+ and TNC counts, CFU-GM, posttransplant neutrophil/platelet recovery, and patient survival. All UCB transplants preceded by a nonmyeloablative conditioning regimen were excluded from neutrophil and platelet engraftment. Patient survival was updated to December 2007.

CFU-GM and CD34+ and TNC doses were each independent predictors of neutrophil engraftment, while only TNC dose and CFU-GM were independent predictors of platelet engraftment. In addition, CFU-GM correlated with both TNC and CD34+ cell doses. Most interesting, Kaplan-Meier survival curves were not significantly different among CD34 or TNC doses, but CFU dose may significantly predict 7.5-year survival.

Therefore, like CD34+ and TNC counts, CFU-GM can predict neutrophil and platelet engraftment. However, unlike CD34+ and TNC, CFU-GM dose may stratify survivorship at 7.5 years and will be assessed by updated Kaplan-Meier survival curves. In addition, despite its longer turnaround time, the CFU-GM assay is a true functional assay, and it may provide additional clinically relevant information about the UCB unit.

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Characterization of Cultured Disseminated Tumor Cells From Breast Cancer Patients.

Angela M. Ferguson, Timothy P. Fleming, Mark A. Watson, and

Rebecca L. Aft. Sponsor: Mitchell G. Scott. Washington University School of Medicine, St Louis, MO.

Metastatic disease is the leading cause of mortality in breast cancer. Several studies have shown that the presence of disseminated tumor cells (DTCs) in the bone marrow of breast cancer patients is an independent predictor of metastatic disease and of overall poor survival. Despite this fact, more than half of patients with DTCs at the time of diagnosis will not develop metastatic disease even after several years. This suggests that not all DTCs are able to form a metastatic lesion.

We are characterizing gene expression in bone marrow DTCs from breast cancer patients to identify predictive biomarkers of metastatic disease. Primary bone marrow DTCs are rare and difficult to analyze without enrichment. Therefore, we have attempted to culture DTCs directly from bone marrow aspirates for further genomic and biological studies. Bone marrow cells were cultured with and without preselection for the tumor epithelial marker EpCAM. Cells were harvested after various passages in culture, and RNA was isolated and hybridized to gene expression microarrays.

Expression analysis of the cultured cells revealed that they express a very different set of genes as compared with primary cell samples and that gene expression changes as these cells are passaged. One cultured cell line, however, demonstrated an expression profile reminiscent of primary breast tumor cells, and this line is being characterized further. Culturing DTCs will allow us to learn more about their biology and should aid in the development of diagnostic and treatment strategies for patients with metastatic breast cancer.

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Evaluation of the Siemens DCA Vantage Hemoglobin A_{1c} Point-of-Care Analyzer.

A. Charnot-Katsikas, S.R. Hui, and K.T. Yeo. Sponsor: Jonathan L. Miller. Department of Pathology, University of Chicago, Chicago, IL.

The Diabetes Control and Complications Trial (DCCT) established that tight control of hemoglobin A_{1c} (A_{1c}) can dramatically reduce the development and progression of microvascular complications in diabetic patients, thereby improving long-term morbidity and mortality. Subsequently, laboratory A_{1c} assays have aligned with the DCCT method. Recently, use of point-of-care A_{1c} devices (POC) have become widely popular because of their ease of use and because they provide immediate feedback of glycemic control to patients. It is therefore important that these A_{1c} POCs are evaluated and compared with a DCCT-traceable method to justify their use in clinical practice.

Our objective was to examine the analytical performance characteristics of the new Siemens DCA Vantage POC A_{1c} method and compare it and the older DCA 2000+ POC to the Bio-Rad Variant II A_{1c} reference method.

Whole blood was collected from 46 patient samples and analyzed for A_{1c} by the Bio-Rad Variant II, the DCA Vantage, and the DCA2000+ for a period of 11 days. The Variant II uses a cation-exchange high-performance liquid chromatography method, while A_{1c} determinations in the DCA 2000+ and the DCA Vantage utilize a monoclonal anti-A_{1c} antibody immunoagglutination assay.

Between-run precision for the DCA Vantage showed a coefficient of variation of 1.1% at 5.5% A_{1c} and 3.6% at 12.7% A_{1c}. The analyte measuring range, as determined by a linearity set ranging from 4.1% to 15.6%, demonstrated a least squares regression of $y = 0.93x + 0.028$ ($r^2 = 0.97$; $S_{y/x} = 0.65$; range, 4.1%-14.0%). Comparison of the Vantage with the Variant II demonstrated a least squares regression of [Vantage] = 0.82 [Variant II] + 0.94 ($r^2 = 0.98$; $S_{y/x} = 0.21$; range, 5.5%-12.2%). Comparison of the DCA 2000+ with Variant II revealed a least squares regression equation of [DCA 2000] = 1.04 [Variant II] – 0.72 ($r^2 = 0.94$; $S_{y/x} = 0.62$; range, 5.3%-13.8%). The bias between the Vantage and Variant II methods was not statistically different in the presence of hemoglobin AS and AC variants as compared with nonvariant samples ($P = .4889$, Mann-Whitney test).

The DCA Vantage demonstrated excellent precision and showed good correlation with the Variant II. The Vantage POC provides a quick and easy way to monitor A_{1c} in an outpatient setting and to provide immediate education and treatment interventions. This is especially valuable for use in children, for whom repeated venipuncture is challenging.

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Genotype-Phenotype Relationship of Thiopurine S-Methyltransferase (TPMT) in a Reference Population.

K.L. Johnson-Davis,[1] R. Melis,[2] E. Lyon,[1] J. Juenke,[2] and G. McMillin.[1] *[1]Department of Pathology, University of Utah, Salt Lake City; and [2]ARUP Institute for Clinical and Experimental Pathology, Salt Lake City.*

Azathioprine and 6-mercaptopurine (6-MP) are commonly used as anticancer and immunosuppressant agents. Azathioprine is metabolized to 6-MP. TPMT catalyzes the primary inactivation pathway in which approximately 90% of 6-MP is converted to an inactive metabolite, 6-methylmercaptopurine (6-MMP). Life-threatening adverse effects are documented when standard dosing is administered to individuals with TPMT deficiency due to accumulation of 6-MP. TPMT deficiency may relate to genetic mutations of *TPMT*. Approximately 11% of Caucasians are heterozygous for *TPMT* mutations, and intermediate enzyme activity is predicted; 0.3% are homozygous for *TPMT* mutations, and enzyme deficiency is predicted. Correlation of genotype and phenotype and performance of laboratory tests to identify patients at risk for adverse effects prior to drug administration could minimize the incidence of toxicity and improve dose selection. The purpose of this study was to develop a phenotype assay to measure TPMT activity and compare results with the *TPMT* genotype for a reference population.

TPMT activity and genotype were determined with blood collected from 146 healthy volunteers. The most common alleles associated with impaired TPMT activity (**2, *3A-C*) were detected by a single nucleotide primer extension assay (SNaPshot). To detect TPMT enzyme activity, RBCs were lysed, 6-MP and S-adenosylmethionine were added, and then the reaction was incubated for 1 hour at 37°C. The concentration of 6-MMP in the lysate was determined by HPLC-UV, and results were converted to units of activity per milliliter of lysed packed RBCs (U/mL).

No TPMT variants (**2, *3A-C*) were identified in 126 patients; 20 were heterozygous for the **3A* (n = 15) and **3C* alleles (n = 5). No homozygous mutations were identified. By using the data from patients in which no variants were detected, assuming a unimodal distribution and 90% confidence intervals, a parametric reference interval was determined: 28 to 65 U/mL. The TPMT activity for 19 of the 20 patients with heterozygous mutations fell within the reference interval.

An assay to measure TPMT activity was developed, and a reference interval was determined that could be used to identify TPMT-deficient patients. However, a poor relationship between

heterozygous mutations of *TPMT* and enzymatic activity was observed. Further study will be required to evaluate the clinical utility of the enzymatic activity relative to predicting dose requirements of azathioprine and 6-MP.

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Clinical Testing for Mutations in the *PAX2* Gene and Diagnosis of Renal Coloboma Syndrome.

Matthew A. Bower,[1,2] Chengsu Xu,[3] Randolph Peterson,[3] Lisa A. Schimmenti,[1,2] and Xinjing Wang.[2,3] Sponsor: John Eckfeldt. [1]Division of Genetics and Metabolism, [2]Institute of Human Genetics, and [3]Department of Laboratory Medicine and Pathology, University of Minnesota Medical Center-Fairview, Minneapolis.

Renal coloboma syndrome (RCS) is an autosomal dominant disorder characterized by ocular and renal malformations. Mutations in the paired-box gene, *PAX2*, have been associated with RCS.

Our laboratory developed the first clinically available testing service for *PAX2* mutations in 2007. Testing involves bidirectional sequencing of all 12 coding exons of the *PAX2* gene and adjoining intronic sequences. In the first year the service was offered, we completed testing on 20 probands with ocular and/or renal findings. In 9 of 20 cases, the probands presented with classic findings of RCS (bilateral optic nerve coloboma and hypoplastic, dysplastic, or multicystic kidneys). Mutations were identified in 5 of 9 families with these findings. Mutations were not identified in any of the 11 probands with atypical findings, such as iris coloboma. Consistent with prior observations, all of the mutations identified in this sample were frameshift mutations that would be expected to result in a truncated PAX2 protein. Two of the mutations identified in our laboratory are novel mutations (c.836delG and c.894delTinsGC). Additional findings in mutation-positive families included short stature, elevated pancreatic amylase, and hearing loss. Of the 5 mutation-positive cases, 4 occurred in the absence of any family history, suggestive of a de novo origin for the mutation. In the remaining case, the familial mutation was present in 2 affected siblings and absent in their father, who had bilateral papillary anomalies. This latter case suggested the possibility of somatic mosaicism, though this has not yet been confirmed by molecular analysis.

Mutations were found in approximately half of cases with classic findings for RCS. The majority of *PAX2* mutations appear to be de novo. There is growing evidence that mosaicism, both germline and somatic, may not be uncommon in RCS. The possibility of somatic mosaicism should be strongly considered in mutation-negative cases with suggestive clinical findings.

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Chromogranin A Fragments Are Responsible for an Apparent High-Dose Hook Effect Observed With a Sequential, Noncompetitive ELISA.

M. Laura Parnas,[1] J. Alan Erickson,[2] and David G. Grenache.[1,2] [1]Department of Pathology, University of Utah Health Sciences Center, Salt Lake City; and [2]ARUP Institute for Clinical and Experimental Pathology, ARUP Laboratories, Salt Lake City.*

Chromogranin A (CgA) is a 439-amino-acid glycoprotein widely expressed in neuroendocrine tissue that functions as a prohormone, giving rise to biologically active peptides as a result of posttranslational proteolytic processing. The serum concentration of circulating CgA has been demonstrated to be a useful marker for the detection and monitoring of neuroendocrine tumors.

Our laboratory quantifies CgA using a home-brew, sequential, noncompetitive, enzyme-linked immunosorbent assay (ELISA) that involves the binding of CgA to a solid-phase polyclonal rabbit capture antibody. This is followed by washing and then the addition of an HRP-labeled monoclonal mouse antibody that allows for direct quantification. By using this assay, we have observed an apparent high-dose "hook" effect in approximately 15% of specimens following specimen dilution, which was unexpected given the sequential format of the assay. We hypothesized that the presence of CgA fragments that express the epitope recognized by the capture antibody but not the epitope recognized by the labeled antibody was responsible for the observed hook effect.

Specimens with various concentrations of CgA that did and did not show the hook effect were examined by Western blotting. Serum was diluted 1:20 with phosphate-buffered saline, and proteins were separated by SDS-PAGE, transferred to nitrocellulose, and blotted with either the polyclonal capture or the monoclonal label antibody. The presence of a prominent band located at approximately 78 kDa is consistent with intact CgA and was observed in all the samples with both antibodies. A second band at approximately 45 kDa was detected in all the samples and is consistent with the presence of prochromacin, a large C-terminal active peptide of CgA (aa 79-439). Additional distinct CgA fragments with molecular masses between 40 and 60 kDa were detected exclusively in the samples that demonstrated the apparent hook effect. These data strongly suggest that, in our assay, circulating fragments of CgA can saturate the capture antibody but are not detected by the labeled antibody. This prevents binding of the intact molecule, causing an apparent low concentration of the circulating marker. Dilution of these specimens prior to quantification by ELISA decreases the relative abundance of the fragments and allows the capture and detection of intact CgA, thus producing an apparent hook effect.

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An LC-MS/MS Method for the Measurement of Androstenedione in Serum.

Evrim Erdogan,[1] Mark M. Kushnir,[2] John Hughes,[3] William L. Roberts,[1] Wayne A. Meikle,[1,2,4] and Alan L. Rockwood.[1,2] Departments of [1]Pathology and [4]Medicine, University of Utah, Salt Lake City; [2]ARUP Institute for Clinical & Experimental Pathology, Salt Lake City; and [3]Agilent Technologies, Pleasanton, CA.*

Androstenedione, produced by the adrenals and gonads, acts as a dual precursor for testosterone and estrone. Elevated concentrations can be observed in women with hirsutism, polycystic ovarian syndrome, congenital adrenal hyperplasia, and tumors of the adrenals or gonads.

We developed a liquid chromatography–tandem mass spectrometry (LC-MS/MS) assay for androstenedione in serum that uses 0.1 mL of sample. Calibration standards were prepared in 10 g/L of BSA at concentrations ranging between 0.1 and 2.0 ng/mL; human serum samples were used as controls. Calibrators, controls, and serum samples were spiked with 20 µL of internal standard (2.5 pg/µL), d_3-testosterone. Samples were derivatized using aqueous hydroxylamine, and the derivatives were then extracted with 2 mL of methyl tert-butyl ether. The organic layer was transferred into new tubes; the residues were reconstituted and injected in the

instrument. Instrumental analysis was performed on an Agilent 6140 triple quadrupole tandem mass spectrometer equipped with a series 1200 HPLC system (Agilent). Analysis was performed in the multiple-reaction monitoring mode using mass transitions 317 → 124 and 317 → 112 for androstenedione and 307 → 124 and 307 → 112 for d_3-testosterone. The chromatographic separation was performed on a 4.6 × 50-mm Rapid Resolution HT C_{18} column with 1.8-μm particles (Agilent), the oven temperature was 50°C, and the injection volume was 15 μL. The mobile phase, methanol with 10 mol/L of aqueous formic acid (70:30), was delivered at a flow rate of 0.6 mL/min. The total instrumental analysis time was 3.5 minutes. The instrument was operated in positive-ion mode with optimized voltages: ion spray, 4000 V; fragmentor, 160 V; collision energy, 30 V. The limit of detection, limit of quantification, and upper limit of linearity for the method were 0.17, 0.33, and 92 ng/mL, respectively.

Within-run coefficients of variation (CVs) were 15.9% (0.3 ng/mL) and 3.8% (2.3 ng/mL), and between-run CVs were 5.0% and 3.6% at concentrations of 0.3 and 2.3 ng/mL, respectively. The method was compared with chemiluminescent immunoassay (Siemens) using a set of serum samples (n = 31). The regression equation for the comparison was $y = 0.59x + 2.02$; $r = 0.41$; $S_{y/x} = 0.80$. None of the 22 steroids evaluated interfered with the analysis. This LC-MS/MS method has acceptable performance for the measurement of androstenedione in serum.

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Crimson Project: Supporting High-Throughput Sample Collection From Pathology Departments: A New Paradigm for Enabling High-Throughput Pipelines of Discovery in Academic Medical Centers.

Lynn Bry and Neil C. Herring. Department of Pathology, Brigham & Women's Hospital, Boston, MA.*

The Crimson application taps into discarded sample streams from pathology departments for collection of materials in a high-throughput and cost-effective manner through integration with laboratory information systems. The application can support multiple banking sites within an institution while enabling full oversight of activities by a local internal review board (IRB) as every collected sample remains associated with a valid IRB protocol. Other core functions include a rule-set manager through which investigators can create queries to collect samples meeting defined criteria, an "honest broker" to manage release of samples with respect to patient consent status, online sample submission for research testing, a workflow engine for handling downstream processing and testing, shipping functions, and a billing system to recoup costs associated with services provided.

After 1 year of operations, the specimen bank within the BWH Clinical Laboratories successfully collected and managed more than 15,000 research samples for IRB-approved studies, with more than 95% of samples being released to investigators for downstream analyses. Use of the application has also centralized research processing within a 24X7 operating clinical laboratory, providing standards and infrastructure to support clinical trials and other studies that often present with nonstandard tube types and processing protocols. Most important, Crimson has uniformly reduced the costs associated with sample collection, often providing an order of magnitude or more reduction in cost/sample, while also providing a 1 to 2 order of magnitude increase in the throughput of materials collected. For many studies, this dually beneficial trend results in savings of tens to hundreds of thousands of dollars each year.

Existing efforts are integrating Crimson within national infrastructure including that of i2b2 (Informatics for Integrating Biology to the Bedside). Discussion will include how integration with external research systems can further support high-throughput studies using clinical materials for key analyses.

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FasL Expression on T Cells Is Sufficient to Prevent Persistent Airway Inflammation in Murine Model of Asthma.

Jiankun Tong,[1] Kimm J. Hamann,[2] and Anne I. Sperling.[2] Sponsor: Karen Frank.[1] [1]Department of Pathology, [2]Department of Medicine, Committee on Immunology, Committee on Molecular Medicine, University of Chicago, Chicago, IL.

Our previous studies revealed that in a murine model of asthma, mice that received Fas-deficient T cells developed a persistent phase of airway inflammation, mucus production, and airway hyperreactivity that failed to resolve even 6 weeks after the last challenge. These studies implied that dysregulation of the Fas signaling pathway on human T cells could be an important factor in the development of the chronic nature of asthma.

The purpose of this study was to investigate how Fas-FasL interaction occurs between T cells and other cells in vivo and how Fas-FasL interaction is involved in the resolution of persistent airway inflammation. T cells from B6 or Gld mice with abnormalities of the FasL signaling pathway were harvested from lymph nodes; 107 cells were adoptively transferred into Rag–/– or FasL-deficient Rag–/– mice intravenously. At day –14, mice were immunized by intraperitoneal 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. The mice were studied between 4 and 28 days after the last challenge.

We found that while B6 or Gld T cells were transferred to Rag–/– or FasL-deficient Rag–/– mice, only FasL-deficient Rag–/– mice that received Gld T cells had persistent airway inflammation, goblet cell hyperplasia, and decreased interferon-γ production after the last challenge.

These results demonstrated that FasL expression on T cells or other cells is sufficient for the resolution of airway inflammation and that the absence of Fas-FasL interaction signaling on T cells can lead to persistent airway inflammation in this model.

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Immune Hemolysis due to Minor Rh-Incompatible Solid Organ Transplant in Two Recipients From the Same Donor.

A. Victoria McKane,[1] Michael Hughes,[2] Mark Hill,[2] Ty Dunn,[2] Abhinav Humar,[2] and Nicole D. Zantek.[1] Sponsor: S. Jeffrey McCullough.[1] Departments of [1]Laboratory Medicine and Pathology and [2]Surgery, University of Minnesota Medical Center, Minneapolis.

Passenger lymphocyte syndrome (PLS) causes immune-mediated hemolysis after transplant due to viable donor B-lymphocyte antibody production targeting recipient blood group antigens. The majority of cases are due to ABO incompatibility; however, there have been rare reported cases attributed to other blood groups antigens. We report herein the development of immune-mediated hemolysis in 2 group A Rh(D)+ organ transplant recipients from a group A Rh(D)– cadaveric organ donor.

Anti-D and anti-C were detected in the donor's serum 5 days before the transplants. A 56-year-old man received the liver, and a 59-year-old woman received a kidney and the pancreas. Fourteen days after transplant, the liver recipient was noted to be anemic with a hemoglobin of 6.1 g/dL, which was decreased from 11.9 g/dL immediately after transplant. Anti-D and anti-C were newly detected in the patient's serum. The kidney and pancreas recipient had progressive anemia posttransplant with a hemoglobin decrease from 11.2 g/dL after transplant to a nadir of 7.9 g/dL on posttransplant day 9 when anti-D was detected in her serum. Both patients had evidence of hemolysis. Both patients required transfusion of group A Rh(D)– RBCs. In addition, the liver recipient received prednisone.

While PLS has been associated with immunosuppressive regimens that predominately suppress T lymphocytes, thus permitting B-cell proliferation and subsequent antibody production, both patients received combined B- and T-cell immunomodulation. Patients receiving minor-Rh incompatible transplants, particularly in the setting of known donor alloantibodies, should be monitored carefully for signs of hemolysis.

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B-Type Natriuretic Peptide as a Biomarker of Disease Severity in Critically Ill Pediatric Patients.

N.L. Korpi-Steiner,[1] E.G. Seferian,[2] Y. Ouellette,[2] A.S. Jaffe,[1] G.M. Arteaga,[2] and A.K. Saenger.[1] [1] Department of Laboratory Medicine/Pathology and [2]Pediatric Critical Care, Department of Pediatric and Adolescent Medicine. Mayo Clinic, Rochester, MN.*

Brain or B-type natriuretic peptide (BNP) is a diagnostic marker of ventricular dysfunction, and an automated immunoassay permits rapid measurement. BNP values help predict the prognosis of patients with chronic heart failure and adults with idiopathic pulmonary hypertension. The pediatric literature reported similar utility of BNP testing in outpatient management of left ventricular dysfunction; however, its role in the critically ill pediatric population is not well characterized. We hypothesized that serial measurements of BNP may be a marker of disease severity and presage outcomes in pediatric patients with critical illness.

A prospective, observational study was developed to successively measure BNP levels on days 1, 2, and 5 after admission to the pediatric intensive care unit (PICU). BNP concentrations were measured on the Beckman Coulter UniCel DxI 800 using plasma EDTA samples. Patients with respiratory dysfunction were included with ages ranging from newborn through 18 years. Patients admitted to the PICU postoperatively were excluded.

In a 6-month period, a total of 28 patients were evaluated with the following diagnoses associated with respiratory dysfunction: respiratory infection, 10; primary or secondary pulmonary hypertension, 6; congenital heart disease, 4; septic shock, 4; and acute heart failure secondary to myocarditis, 4. BNP levels were within the normal reference range (<64 pg/mL) in patients with respiratory infections who were previously healthy or with repaired congenital heart disease. In patients with pulmonary hypertension, a mean percentage reduction in BNP levels correlated with response to medical management as measured by echocardiogram and cardiac catheterization (57% ± 7% difference between days 1 and 2). Patients with nonrepaired congenital heart disease had elevated levels of BNP, decreasing after initiation of medical treatment and prior to surgery (50% ± 2.5% difference between days 1 and 2). Significant BNP elevations in patients with septic shock were present on day 1 and decreased as the patient's clinical condition improved (19% ± 5% difference between days 1 and 2). This was noted in all patients except one, who died within 48 hours after admission.

Serial BNP determinations may be helpful in the initial evaluation of critically ill pediatric patients who develop a respiratory infection. Elevations often suggest underlying heart disease or sepsis. In addition, the response of pulmonary hypertension to medical management is associated with a reduction in BNP levels. More studies are warranted to determine a diagnostic and/or prognostic role for serial BNP determinations in the pediatric acute care setting.

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Neutrophil CD64 (FcγR1) Expression in the Postoperative Patient Population.

Sara Beth Kilborn and Michael Icardi. Sponsor: Ann M. Gronowski. Iowa City Veterans Affairs Medical Center, Iowa City, IA.

Expression of CD64 (FcγR1) has been reported to increase on neutrophils in patients with systemic bacterial infections. A recent study has suggested that neutrophil CD64 could be used as a marker for infection in postoperative patients. Our objective was to determine the usefulness of neutrophil CD64 in the postoperative population.

The neutrophil CD64 index was measured in preoperative and postoperative blood samples from 23 surgical patients for 7 days after surgery. Specimens were leftover from physician-ordered testing. Retrospective chart review was performed for all patients. The CD64 index was determined by flow cytometry using a Leuko64 kit (Trillium Diagnostics, Maine). The patients were all men and ranged in age from 27 to 83 years. Surgeries included the following: open AAA repair, 2; distal gastrectomy, 1; vagotomy with pyloroplasty and ulcer cautery, 1; colectomy, 2; open cholecystectomy, 1; radical prostatectomy, 4; total hip arthroplasty, 2; total knee arthroplasty, 2; esophagectomy, 3; cranioplasty, 1; ORIF of mandible with iliac bone graft, 1; foraminotomy, 2; and lumbar diskectomy, 1. Eight patients were determined to have postoperative infections by chart review. Institutional review board approval was obtained for this study.

The CD64 index modestly increased by an average of 0.398 in all but 1 patient in the noninfected group, whereas the CD64 index increased in infected patients by an average of 1.30125 ($P = .0251$). A CD64 index cutoff of 1.2 predicted the presence of infection with a sensitivity of 100% and a specificity of 87% ($P = .0001$).

These results demonstrate a significant increase in the CD64 index in infected patients compared with noninfected patients. Furthermore, a CD64 index above 1.2 is both sensitive and specific for infection in a postoperative setting. These findings suggest that the CD64 index can be used to detect postsoperative infection. Future studies with larger numbers of patients will be necessary to investigate this further.

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Interpretation of Methadone Concentration in Postmortem Vitreous Humor.

Jill Adamski,[1] Hillary Zalaznick-Kimbrell,[1] Michael Milone,[1] Mark Lichtenwalner,[2] Leslie Shaw,[1] and Frederic Hellman.[3] Sponsor: Donald S. Young.[1] [1]Department of Pathology and Laboratory Medicine, Hospital of the University of Pennsylvania, Philadelphia; [2]DrugScan, Warminster, PA; and [3]Delaware County Medical Examiner Office, Lima, PA.

A 53-year-old man with a history of polysubstance abuse and cirrhosis was admitted to our hospital for treatment of bacterial sepsis. Throughout hospitalization, the patient's maintenance methadone dose of 60 mg/d was continued. On day 25 of hospitalization, the patient developed acute respiratory failure and died. Approximately 24 hours prior to death, the patient was noted to have received a dose of 600 mg of methadone orally with no reported adverse events noted upon evaluation.

An autopsy was performed revealing bilateral serosanguineous pleural effusions (3.3 L) with grossly underinflated lungs and cirrhosis. The only postmortem specimen available for toxicologic analysis was vitreous humor. By using GC-MS, the level of methadone in the vitreous specimen was determined to be 2.28 μg/mL. A premortem blood specimen that was collected approximately 3 hours prior to the accidental methadone overdose was also analyzed and found to have a methadone concentration of 0.77 μg/mL. A search of published literature revealed no consensus reference levels with regard to the relationship between methadone concentrations in blood and vitreous humor in postmortem specimens.

Given the limited data available, we conducted a study for analysis of paired postmortem blood and vitreous specimens in decedents who were found to have measurable levels of methadone on routine postmortem toxicology studies. A study of 19 cases from the Delaware County Medical Examiner's records indicates that the ratio of blood to vitreous methadone levels ranges from 0.57 to 16.7 with a median of 4.9. Linear regression of the data set reveals the relationship: BLOOD (μg/mL) = 4.84*VITREOUS (μg/mL) – 0.007 with a SE for the slope estimate of ± 0.505 μg/mL and $r^2 = 0.84$. The level of methadone found in the vitreous humor of our patient was 2.28 μg/mL, which is well above the highest value in our data set; however, extrapolation suggests that the blood concentration for methadone was 8 to 13 μg/mL.

Multiple factors, including individual metabolism and time between drug ingestion and death, could contribute to the variability in blood to vitreous concentrations for methadone. Our study shows that methadone concentrations in vitreous humor are correlated with blood. Vitreous can therefore be used to estimate blood concentrations for postmortem methadone analysis in cases where blood is not available.

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Unexpected Antibody Pattern in C4d-Negative Renal Allograft Rejection Responds to the Use of Plasma Exchange: A Case Report.

Corinne L Stobaugh,[1] Deborah J. Novak,[1] Barbara J. Masten,[1,2] Margaret W. Chambers,[2] and Kendall P. Crookston.[1,2] [1]Department of Pathology, University of New Mexico, Albuquerque; and [2]Tricore Reference Laboratories, Albuquerque.*

The use of plasma exchange is well documented as a category II treatment for acute humoral rejection in renal allografts. In this abstract, we report the HLA findings of an unusual case of C4d-negative acute renal allograft rejection successfully treated with plasma exchange.

A 24-year-old Hispanic man with end-stage renal disease secondary to idiopathic membranoproliferative glomerulonephritis received a living donor-related kidney transplant. The donor and recipient shared a 1 haplotype HLA match. The mismatched HLA antigens were A2, B62, and DR4. The recipient's pretransplant antibody screen was positive for a low-titer donor-directed antibody against A2. However, the T cell (class I) and B cell (class I and II) crossmatches were negative. The patient received intraoperative and postoperative thymoglobulin.

Posttransplant, a rising creatinine prompted a renal biopsy on postoperative day (POD) 5. The renal biopsy showed an acute infarct, significant acute tubular necrosis, and no acute cellular rejection and was negative for C4d staining. An HLA single antibody screen obtained on POD 10 showed moderately increased levels of previously undetectable donor-directed B62 antibody. Other non–donor-directed specificities were also identified such as B57 and B75. At this time, the T-cell crossmatch became positive, and plasma exchange was initiated for treatment of possible acute humoral rejection. The patient was given a total of 10 plasma exchanges, beginning POD 14, followed by IVIG. Not only did the patient's creatinine level respond to this treatment (8.49 mg/dL at initiation of treatment to 1.95 mg/dL at discharge from the hospital), but so did his unique combination of HLA antibodies. The HLA A2, B62, B57, and B75 antibody levels all decreased to low levels, and the patient's T cell crossmatch returned to negative. Since only the A2 specificity was identified prior to transplant, it was hypothesized that the other antibodies were reacting to epitopes closely resembling those possessed by the donor. HLA Matchmaker was used to investigate this possibility and identified 2 epitopes shared by the A2, B62, B57, and B75 antigens.

We have concluded that the mechanism for this patient's rejection is primarily cellular, with a possible humoral component. The use of plasma exchange may have aided in mitigating these processes by removing not only donor-directed antibodies, but also cytokines responsible for stimulating cellular immunity.

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Cytokine Storm in a Mouse Model of IgG-Mediated Hemolytic Transfusion Reactions.

Eldad A. Hod,[1] Chantel M. Cadwell,[2] James C. Zimring,[2] Set Sokol,[1] Jeffrey Jhang,[1] and Steven L. Spitalnik.[1] [1]Department of Pathology, Columbia University Medical Center, New York, NY; and [2]Department of Pathology and Laboratory Medicine, Emory University School of Medicine, Atlanta, GA.*

Prior in vitro studies support the hypothesis that cytokines play a central role in the pathophysiology of IgG-mediated hemolytic transfusion reactions (HTRs). However, little evidence is available from in vivo studies. Therefore, after establishing well-defined mouse models of IgG-mediated and IgM-mediated HTRs, we examined whether cytokines were involved.

To this end, RBCs from human glycophorin A transgenic mice or wild-type mice (as negative controls) were transfused into nontransgenic recipients passively immunized with anti–glycophorin A or negative-control IgG mouse monoclonal antibodies. Only transfusion of incompatible RBCs induced IgG-mediated HTRs, exemplified by the rapid clearance (ie, within 15 minutes) of the transfused RBCs. In addition, using a multiplex, flow cytometric assay markedly elevated plasma levels of monocyte chemoattractant protein-1, interleukin (IL)-6, and tumor necrosis factor-α were seen within 2 hours of transfusion of incompatible RBCs. Significant increases in these cytokines were seen even after transfusion of only 50 μL of incompatible blood (with a hematocrit of 40%). No significant changes in plasma levels of IL-10, IL-12, or interferon-γ were observed under any conditions.

The proinflammatory cytokines elaborated in this in vivo mouse model are also implicated in the systemic inflammatory response syndrome and confirm the hypothesis that HTRs induce cytokine storm.

Future studies will examine the role that individual cytokines play in this pathological process.

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Increasing Limitations of Drug Screens in an Era of Expanding Molecular Diversity.

Matthew D. Krasowski[1] and Sean Ekins.[2] [1]Department of Pathology, University of Pittsburgh, Pittsburgh, PA; and [2]Collaborations in Chemistry, Jenkintown, PA.*

Laboratory assays aimed at rapid and qualitative detection of drugs are widely used in medicine. Traditionally, drug screens are immunoassays performed on urine samples that target classes of drugs such as amphetamines, barbiturates, benzodiazepines, opiates, and tricyclic antidepressants. A challenge to this strategy has been the expansion of available medications within existing drug classes and also the development of novel drugs that do not fit within existing classes.

We utilized different molecular descriptors (functional class fingerprints [FCFP], MDL public keys) and the Tanimoto similarity coefficient. By using historical data on drugs implicated in emergency room visits and trends in prescription drug use, we show that over time, drugs that would be clinically useful to detect increasingly fall outside the specificity of existing drug screens owing to increasing molecular diversity. Even within existing drug classes, some drugs are not well detected based on differences in similarity to the template drug within the class. Examples of this within the opiate class include buprenorphine (similarities, FCFP = 0.31 and MDL = 0.79, relative to morphine) and oxycodone (FCFP = 0.31 and MDL = 0.80), which are increasingly important clinically but poorly detected by most commercially available opiate immunoassay screens.

The structural diversity of the new generation of psychiatric medications suggests that it would be very difficult to develop screens that would detect multiple therapeutic drugs. We also utilize similarity and pharmacophore analysis to try to predict cross-reactivity of assays with compounds not previously tested, using the cross-reactivity of tricyclic antidepressant screens with structurally related molecules such as quetiapine as a model system. The computational methods used are amenable to rapid screening of large databases of drugs, drug metabolites, and endogenous compounds and may be useful for identifying cross-reacting molecules that would be otherwise unsuspected and yet to be tested. Funding was provided by National Institutes of Health grant K08-GM074238.

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Archived Online Videos of Laboratory Medicine and Pathology Core Curriculum Lectures Are a Valuable Educational Resource.

L. Walden Browne, Kuang-Yu Jen, Joan Etzell, and Enrique Terrazas.* Departments of Pathology and Laboratory Medicine, University of California, San Francisco.*

Core curriculum lectures for residents in both laboratory medicine and anatomic pathology at our institution (~5-6 per week) have been made available online at a password-protected Web site for more than 4 years. Six months preceding this study, the screen-capture software program Camtasia (TechSmith) was implemented instead of a consumer video camera to improve sound and image resolution, decrease document size, and facilitate recording. Only anecdotal information to support the perceived value of the online lectures was available. We developed a survey primarily directed at residents, fellows, and faculty in our departments to evaluate the quality of the video service and frequency of use.

All residents, fellows, and faculty in both departments were invited via email to participate voluntarily in an anonymous, non-remunerated online survey consisting of 15 questions and a comment section. The majority of questions were answered as follows: strongly agree, agree, neutral, disagree, or strongly disagree. Web server logs were examined to tabulate access to the video lecture files.

Respondents to the survey included 20 (74%) of 27 eligible residents, 7 (47%) of 15 fellows, 19 faculty members, and 3 "others." Online lectures were viewed by 85% of resident/fellow (trainee) respondents with 1 to 5 lectures per month reported by 48% and 6 to 10 lectures per month by 15%. The majority of trainees strongly agreed or agreed that they were likely to view lectures online they had attended (74%) or missed owing to illness or vacation (93%) or conflicts (74%). The likelihood of using the service to comply with work-hour regulations received varied responses. Most trainees opined that their attendance would not be affected by the knowledge the lecture was being recorded for later use (81%). Most respondents thought that the lecturers' effectiveness was unaffected by knowledge that the lectures are recorded (78% trainees; 74% faculty). Responses were mixed regarding whether the lectures should be made available to individuals outside the department. The change to Camtasia was perceived as an improvement. During a 6-month period, an average of 140 videos per month were viewed.

The use of archived online video-recorded resident lectures is a useful, relatively inexpensive educational resource for pathology residents that supplements the live lecture format, without perceived adverse impact.

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Evaluation of Intermethod and Intraindividual Variation in Serum Ferritin Concentrations.

Bradley A. Ford,[1] Karl G. Hock,[1] Charles S. Eby,[1,2] Daniel W. Coyne,[2] and Mitchell G. Scott.[1*] Departments of [1]Pathology and Immunology and [2]Medicine, Washington University, St Louis, MO.*

The 2006 National Kidney Foundation Kidney Disease Outcomes Quality Initiative (KDOQI) Clinical Practice Guidelines and Clinical Practice Recommendations for Anemia in Chronic Kidney Disease recommend that patients on hemodialysis maintain a serum ferritin concentration greater than 200 ng/mL; above 500 ng/mL, the KDOQI guidelines recommend that administration of intravenous (IV) iron should be done only after careful consideration of laboratory markers of iron status and clinical response to treatment with IV iron and erythropoiesis-stimulating agents. Studies have shown month-to-month intraindividual coefficients of variation (CV_I) for ferritin in healthy subjects to be 14% to 26% and a CV_I of 25% to 43% among hemodialysis patients.

We examined the short-term day-to-day and week-to-week CV_I for ferritin in dialysis patients and showed that individual variability in 60 stable hemodialysis patients ranged from 3% to 172% (interquartile range, 8%-17%) for a 6-week period. In addition, proficiency testing data suggest considerable variability between different commercial ferritin immunoassays. We demonstrated that between-method bias using pooled human serum is consistent with proficiency testing data and indicates that ferritin values can differ by as much as 33% when different immunoassays are used.

The combination of between-method bias and large intraindividual variability suggests that serum ferritin values should not be used as a strict guide for clinical decisions regarding administration of IV iron to patients on chronic hemodialysis.

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A Novel Lipid Nanoparticle for Detecting the Procoagulant Nature of Anti–β_2-Glycoprotein-1.

Joshua L. Hood and Charles S. Eby. Department of Pathology and Immunology, Division of Laboratory and Genomic Medicine, Washington University, St Louis, MO.*

Research was undertaken to determine whether a nanoscale-sized lipid reagent could be established to investigate the procoagulant nature of anti-B2GP1 (β_2-glycoprotein 1) in vitro. Recent evidence suggests anti-B2GP1 antibodies are responsible for the majority of thrombotic complications of antiphospholipid antibody syndrome. To date, laboratory diagnostic testing for antiphospholipid antibody syndrome has relied on qualitative enzyme-linked immunosorbent assays for detection of anti-B2GP1 and paradoxical prolongation of clotting test (lupus anticoagulants).

A nanoscale lipid reagent, based on the relationship between B2GP1, platelets, and thrombosis was constructed using standard liposomal preparation methods of lipid solubilization, evaporation, and ultrasonication. The novel particle, designated NPMP for nanoplatelet micro particle, was found to have a ζ potential of approximately –40 mV and an effective diameter of 59 nm, providing it with a high degree of colloidal stability. The particle was tested in clot-based reactions (silica clotting time) with normal pooled plasma with or without polyclonal anti-B2GP1 antibody. When NPMP was used in the presence of anti-B2GP1, the clotting time was shorter compared with NPMP alone such that the clotting ratio [(anti-B2GP1 + NPMP)/(NPMP alone)] was less than 1, demonstrating procoagulation.

This is the first example of a functional clot-based testing particle specific for anti-B2GP1 that reveals the procoagulant nature of the disease in vitro. NPMP is a novel tool for studying and understanding the pathophysiology of APS in vitro. Further testing and validation with patient samples will be performed to establish the potential use of NPMP as a diagnostic APS test reagent.

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Prevalence and Distribution of XMRV, a Novel Retrovirus, in Neoplastic and Nonneoplastic Human Prostate Tissues.

Robert Schlaberg, Daniel Choe, and Ila Singh. Department of Pathology, Columbia University Medical Center, New York, NY.*

Xenotropic murine leukemia virus–like retrovirus (XMRV) was recently discovered in prostate cancer tissue from men homozygous for a common polymorphism in the RNase L gene. RNase L is a constitutively expressed nuclease that mediates antiviral and proapoptotic effects of interferon. A relatively common variant (R462Q) with a 3-fold reduced enzymatic activity is associated with prostate cancer. Homozygous carriers of this mutation mount a less efficient antiviral response, which might render them more susceptible to XMRV infection and persistence. We are investigating the prevalence and distribution of XMRV in normal and malignant prostatic tissue.

Genomic DNA was extracted from banked, formalin-fixed, paraffin-embedded (FFPE) and frozen tissues. A real-time polymerase chain reaction (PCR) assay amplifying part of a human single-copy gene (vesicle-associated membrane protein 2 [VAMP2]) was developed to confirm DNA integrity and absence of inhibitors. RNase L genotyping was performed with a real-time PCR allelic discrimination assay. In addition, we developed a sensitive real-time PCR assay to test for the presence of XMRV. A fluorescence in situ hybridization assay was designed to investigate the distribution of integrated proviral DNA. To detect replicating virus in tissue sections, we developed an immunohistochemical assay with a polyclonal antibody that we raised against XMRV.

DNA extracted from banked FFPE tissue was sufficiently intact for efficient PCR amplification of amplicons shorter than 200 base pairs (bp). Amplification of a 168-bp region of VAMP2 was similarly efficient from DNA extracted from FFPE and frozen tissue. The allelic discrimination assay was validated by direct sequencing of homozygous wild type (RR), heterozygous (RQ), and homozygous variant (QQ) cases and found to be 100% accurate and reproducible. We found that 96 samples can be genotyped in less than 2 hours. The real-time PCR assay targeting a 138-bp-long, conserved region of the viral *pol* gene is highly sensitive, allowing detection of 10 genomic copies per reaction in greater than 50% of the time. In addition, 96 samples can be tested within less than 1 hour.

XMRV has been associated with the R462Q RNase L polymorphism in prostate cancer tissue. We developed sensitive and efficient tools for testing of readily available, banked tissue to investigate the prevalence and distribution of XMRV in human prostatic tissue. Data will be presented from 200 prostate cancer cases and 200 nonmalignant prostates.

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A Novel and Economical Method to Monitor the Internal Temperature of Plate-Based Real-Time PCR Machines.

Megan E. McNerney and Loren Joseph. Sponsor: Jonathan L. Miller. Department of Pathology, University of Chicago, Chicago, IL.

The College of American Pathologists guidelines require clinical molecular laboratories to regularly monitor the internal temperature of individual wells within polymerase chain reaction (PCR) machines to ensure temperature accuracy. Monitoring of traditional end-point PCR machines is straightforward, including manual thermometers and computer-linked probes. Plate-based real-time (RT)-PCR machines pose a problem because they do not readily accommodate temperature monitors. The objective of this study was to develop a method to monitor plate based RT-PCR machine temperatures.

Double-stranded oligonucleotides with defined melting temperatures (Tm) have been employed as internal controls for complete PCR reactions (Seipp et al. *J Mol Diagn.* 2007;9:284). We hypothesized that the Tm of an oligonucleotide could similarly be used as a surrogate measurement of the temperature of plate-based RT-PCR machines.

In this study, we found that the SYBR Green dissociation curve of a double-stranded oligonucleotide run in a Stratagene MX4000 RT-PCR machine compares consistently with the Tm derived from a parallel reaction in a LightCycler 2.0. We have also run a biplex assay successfully, permitting simultaneous monitoring of 2 temperatures. Finally, we established that the sample plate can be reused in multiple tests.

Simplified buffer conditions, including the absence of enzyme, make this economical. The potential to tune this method to multiple

temperatures of interest suggests the general applicability of this method. This method is a simple and economical approach to measuring RT-PCR machine temperature accuracy and could permit development of one or more widely available protocols for interlaboratory standardization.

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Increasing Blood Donor and Employee Satisfaction by Informing Them of the Disposition of Their Blood.

Mark W. Hubbell, John P. Burns, and Kendall Crookston. Department of Pathology, University of New Mexico, Albuquerque.*

Important goals of blood collection centers include increasing the public's desire to donate blood, retaining new donors for future donation, and increasing morale and retention among employees. Blood products are typically transfused without ex post provision of recipient information to the donor or to the employees involved in the procurement, testing, and distribution of those products. The "genealogy" of a blood product begins with the donor and includes many people who "touch" the unit before it is transfused to the recipient. We hypothesize that sharing the recipient's story with the donor and with the people who touched the blood will increase satisfaction with their involvement in the process.

Information was elicited from transfusion services about recipients of blood products who had a successful outcome. We identified all employees who handled or processed the product between a specific donor and the ultimate recipient. Our initial pilot indicated that an average of 10 blood center employees were involved substantively in each product donation. A notification system was established that transmits a letter to donors and employees who had contact with the product and to hospital blood banks and administrators involved in the recipient's care. These letters describe, in a Health Information Portability and Accountability Act–compliant manner, the recipient, his or her medical circumstances, the products received, and the outcome. An accompanying Likert scale (0 to 10) questionnaire assesses opinion concerning the perceived value of this notification to donors, employees, and administrators.

The genealogy of 144 blood products collected from a high school blood drive in 2007 was presented to a group of 250 volunteer donor coordinators. Initial response indicates considerable enthusiasm for this project. Collection of additional data is ongoing to permit further analysis of donor and employee response.

We hypothesize that informing donors of the fate of their contributions and communicating to employees and administrators the outcome of their efforts will increase employee and donor retention, recruitment, and morale. Initial results indicate a favorable response, but continued data collection is needed to enable thorough analysis.

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Comparison of a Clinical Capillary Electrophoresis System With Agarose Gel Electrophoresis for Evaluation of Serum Protein Abnormalities.

N. Sertac Kip, Colleen Lamb, and Anthony A. Killeen. Sponsor: Leo T. Furcht. Department of Laboratory Medicine and Pathology, University of Minnesota, Minneapolis.

We evaluated the Capillarys capillary electrophoresis (CE) system (Sebia) for performance of serum protein analysis relative to the Hydrasys agarose gel electrophoresis system (Sebia). We compared performance of both methods for determination of protein fractions in serum and for use in identification of monoclonal proteins in B-cell disorders.

We analyzed 95 clinical samples by both methods. Relative to agarose gel electrophoresis, albumin measured by CE showed a mean bias of –0.33 g/dL. The α_1 and α_2 fractions both showed positive mean biases when measured by CE of 0.18 g/dL and 0.03 g/dL, respectively. The β fraction showed a mean bias by CE of –0.02 g/dL and the γ fraction, a mean bias of +0.15 g/dL. Forty-four samples had monoclonal proteins that were detected by both systems. There were 9 samples in which a monoclonal protein was seen by CE that was not seen by agarose gel electrophoresis. In all 9 cases, the monoclonal protein was small and estimated to be less than 0.15 g/dL. Among samples with larger monoclonal proteins (>2 g/dL), there was some variability in the measurement of the monoclonal protein by the 2 systems but values agreed to within 10% of each other. In 1 sample, obvious β-γ bridging was seen by both systems.

The CE system tended to give lower values for albumin and higher values for α_1-globulins. Performance of the 2 systems was generally comparable for detection of monoclonal proteins, although the use of the CE system was associated with a higher rate of detection of small (<0.15 g/dL) monoclonal proteins. Caution should be exercised in reporting the presence of such small monoclonal proteins. The observed variation in the quantification of larger monoclonal proteins may have an impact on the categorization of patients with B-cell disorders (eg, myeloma) when such categorization is based on observation of defined concentrations of M protein in serum.

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Development of a Control Material for 15 *BCR*/*ABL* Point Mutations.

Jiankun Tong and Loren Joseph. Sponsor: Jonathan Miller. Department of Pathology, University of Chicago, Chicago, IL.

Point mutations in the kinase domain (KD) of *BCR*/*ABL* in imatinib-treated chronic myelogenous leukemia (CML) are a major cause of acquired resistance in CML patients. The detection of point mutations gives information for the clinicians to adjust therapy. The detection limit has been reported to be variable in a range of 1% to 50% depending on the method, most often sequencing. Validation of mutation detection assays for *BCR*/*ABL*, as for many genes, is limited by the availability of test samples for all but the most common mutations. New, relatively inexpensive DNA synthesis methods open the prospect of engineering complex controls for many disorders **(***Curr Protoc Mol Biol.* January 2008;Chapter 7:Unit 7.1).

The purposes of this study were to develop reference material for laboratories validating assays for detection of *BCR*/*ABL* point mutations and investigate the detection limit of different point mutations by sequencing.

A 789-base-pair DNA construct containing the most common 15 point mutations, flanked by T7 and T3 RNA polymerase promoters, was designed, synthesized, and cloned into a PUC-19 vector with Codon Devices. Primers for polymerase chain reaction (PCR) and sequencing are from a published protocol (*Clin Chem.* 2004;50:1205). A limited region was deleted to prevent contamination of the quantitative PCR *BCR*/*ABL* assay used in this laboratory.

The sequence was confirmed to show all 15 point mutations: M244V, L248V, G250E, Q252H, Y253H, E355K, D276G, F311L, T315I, F317L, M351T, E355G, F359C, L387M, and H396M. The sequence is in frame with the normal sequence using the indicated sequencing primers.

Control material for simultaneous detection of multiple *BCR/ABL* mutations has been successfully developed in plasmid form. It will not contaminate quantitative *BCR/ABL* real-time PCR assays based on the Europe Against Cancer Format (*Leukemia.* 2003;17:2318). The shortened amplicon size distinguishes it from the usual fusion transcript. The presence of RNA polymerase promoters should make possible the generation of RNA transcripts for testing of the initial stage in the mutation detection assay.

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A Comparative Evaluation of Calculated eGFR Values Obtained Using the Abbott i-Stat and Beckman Coulter Synchron Serum Creatinine Assays.

J. Ahmad Brown, Conrad Browning, Bonnie L. Wood, and Joshua A Bornhorst. Department of Pathology and Laboratory Medicine, UAMS, Little Rock, AR.*

Serum creatinine (Cr) value can be utilized to calculate the estimated glomerular filtration rate (eGFR) by the Modification of Diet in Renal Disease (MDRD) equation and is frequently used to identify patients with renal failure. A recently described disorder, nephrogenic systemic fibrosis, occurs in patients with severe renal impairment who have been exposed to gadolinium-based contrast agents (GBCAs) and is associated with debilitating and potentially fatal fibrosis. In 2007, the Food and Drug Administration issued a "black box" warning for all GBCAs, contraindicating the use of GBCAs in patients with eGFR values of less than 30 mL/min/1.73m^2. There is developing consensus that clinical precautions should be considered for patients with eGFR values of less than 60 mL/min/1.73m^2. Point-of-Care (POC) device measurement of Cr and subsequent eGFR calculation can promote expediency in busy radiology services. As small differences in measured Cr may lead to significant differences in eGFR, evaluation of the concordance between eGFR values derived from laboratory analyzer and POC device serum Cr measurements is prudent.

The data for 42 patients, aged 18 to 80 years, who had Beckman Synchron LX-20 and Abbott i-Stat serum Cr measurements performed within 24 hours of each other were retrieved from our electronic database. An eGFR was calculated using the original MDRD equation for both methods. Nearly equal numbers of male/female and white/black subjects were used.

Deming regression analysis yielded a 5% positive proportional bias for i-Stat Cr measurements (R^2 = 0.99) vs the Beckman Synchron (Cr range, 0.6-10.2 mg/dL). This translated to a negative proportional bias of 19% for all calculated eGFR values (range, 6-165 mL/min/1.73m^2) obtained using the i-Stat vs Synchron serum Cr measurements (R^2 = 0.96). However, when only the samples with calculated eGFR values of less than 60 mL/min/1.73m^2 (n = 26) were considered, good correlation between the calculated eGFR values was observed [i-Stat eGFR = (0.993*Synchron eGFR) + 0.8; R^2 = 0.93].

Cr measurements by i-Stat resulted in minimal underestimation of eGFR values of less than 60 mL/min/1.73m^2, making it unlikely that individuals with severely impaired renal function will go undetected. The use of i-Stat appears to be a reasonable alternative method for risk stratification of patients by eGFR prior to GBCA administration.

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Investigation of Vernix Caseosa as an Alternative Specimen Type for Detection of Prenatal Drug Exposure.

Julia C. Drees,[1] Katherine Drake,[1] Vanessa Munoz,[1] J. Colin Partridge,[2] Erin N. Shea,[1] Judy A. Stone,[1] and Alan H.B. Wu.[1] Departments of [1]Laboratory Medicine and [2]Pediatrics, University of California San Francisco.*

Drug exposure at any point during pregnancy has the potential to negatively impact the health of the fetus. However, methods to determine if a neonate was exposed to drugs of abuse in utero are limited in detection window or feasibility. At our institution, current practice is to order toxicology screens using urine, despite the difficulty of collecting an adequate sample from neonates and the short window of detection (7-10 days). Drug screens using meconium can detect maternal drug exposure during the entire second and third trimester, but meconium is a difficult sample to work with and existing tests are expensive and time-consuming. Vernix caseosa is a white substance found coating the skin of neonates and is thought to represent a long window of exposure in the uterus, similar to meconium. Vernix is easy to collect by swabbing a neonate with gauze, and the matrix is less challenging to work with than meconium. Our objective was to determine the potential of vernix as a specimen type to detect prenatal exposure to drugs of abuse.

Standards of 3 drugs of interest (morphine, methamphetamine, and cocaine metabolite) were spiked into solutions of drug-free vernix and methanol. A simple liquid/liquid extraction method followed by immunoassay detection at a cutoff of 300 ng/mL (1,000 ng/mL for methamphetamine) was developed and validated. Institutional review board approval was obtained. Vernix and urine were collected from neonates of mothers suspected of drug use during pregnancy from whom informed consent was obtained.

For phase 1 of this study, 20 neonates were tested. Two neonates had both urine and vernix positive for cocaine metabolite. One of these neonates was also urine-positive but vernix-negative for opiates. This discordance is consistent with an acute exposure, and, indeed, morphine was given to the mother during labor. One neonate was urine-negative but vernix-positive for opiates, which would be consistent with a remote exposure. The remaining 17 specimens were negative for all 3 drugs in both urine and vernix.

Vernix has several advantages that make it a promising material for use in determining prenatal drug exposure. Initial results suggest that it performs comparably to our current practice of urine screening and may increase the window of detection compared with urine. Liquid/liquid extraction for immunoassay screening, with LC/MS/MS confirmation of positives, may be feasible with a 24-hour turnaround time. Testing of more patients with comparisons with meconium is in progress. Vernix testing could improve patient care by allowing physicians to implement treatment of the drug-exposed neonate to mitigate potentially dangerous withdrawal symptoms.

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Efficacy of Rituximab Treatment for Acquired Thrombotic Thrombocytopenic Purpura (TTP) Resistant to Plasmapheresis Treatment: A Pediatric Case Report.

Bharathi Vennapusa, Kendall P. Crookston, and Prasad Mathew. University of New Mexico, Albuquerque.*

Our objective was evaluation of the efficacy of rituximab in a child with acquired TTP that was resistant to plasmapheresis.

TTP is caused by deficiency of von Willebrand factor cleaving protease (ADAMTS13). Deficiency can be congenital or acquired. Acquired deficiency is due to autoantibodies directed against ADAMTS13. Plasmapheresis is the mainstay of management for TTP. Rituximab, a monoclonal anti-CD20 antibody, has been used

successfully in resistant or chronic relapsing forms in adults, but little has been reported in pediatric age group.

A 13-year-old girl was admitted with an initial diagnosis of idiopathic thrombocytopenic purpura (ITP) and was administered intravenous immunoglobulin (IVIG). On hospital day 3, she was diagnosed with TTP and plasmapheresis was initiated with discontinuation of IVIG. As the platelet count remained very low, volume exchange was increased from 1.3 to 2 on day 13. She was also started on corticosteroid therapy. Her platelet count improved initially, and she was discharged to receive plasmapheresis on an outpatient basis. The ADAMTS13 inhibitor level remained high at the time of discharge, and the platelet count started dropping with the tapering of plasmapheresis. In view of the high ADAMTS13 inhibitor level in spite of steroids and plasmapheresis, it was decided to administer rituximab. She received 3 doses of rituximab. Steroids and plasmapheresis were tapered and discontinued subsequently.

At the time of admission, the platelet count was 8,000/mm^3 and the lactate dehydrogenase (LDH) level was 3,061 U/L. Initial ADAMTS13 activity was less than 5% (normal, >67%) with an inhibitor level of more than 8.0 (normal, <0.4). ADAMTS13 activity remained low at less than 5% and the inhibitor level high at more than 6.0 at the time of discharge. B lymphocytes were 33% before rituximab treatment and reduced to 3% and less than 1% after the first and second doses, respectively. Activity of ADAMTS13 increased to 46% with a single dose of rituximab and was 86% after 3 doses. At 6 months of follow-up, ADAMTS13 activity remains high at 120% with B lymphocytes at 18%. The platelet count remained more than 200,000/mm^3 and LDH at around 500 U/L after rituximab treatment. She received a total of 38 plasmapheresis treatments.

Three doses of rituximab were effective in treating a pediatric patient with refractory TTP. The patient remains in remission after 6 months of follow-up. Because the inhibitor associated with TTP is an autoimmune phenomenon and rituximab has been proven to be effective in pediatric autoimmune disease, including ITP, additional studies are needed to evaluate its effectiveness in children with TTP.

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A Novel Approach for Monitoring Buprenorphine Treatment Compliance and Efficacy.

Kara L. Lynch, Jean S. Branch, Susan F. Gross, Judy A. Stone, and Alan H.B. Wu. Department of Laboratory Medicine, University of California San Francisco.*

Buprenorphine (BUP), a semisynthetic opiate analgesic, has been shown to be effective in the treatment of opiate addiction, as an alternative to methadone. BUP blocks opioid withdrawal by exhibiting partial agonist activity at the μ-opiate receptor. In the liver, BUP is metabolized by CYP 3A4 to norbuprenorphine (NBUP), followed by glucuronidation of BUP and NBUP. The purpose of this study was to determine the clinical utility and feasibility of monitoring treatment compliance and efficacy through the use of 2 BUP immunoassays that detect different BUP metabolites, followed by liquid chromatography–tandem mass spectrometry (LC-MS/MS) confirmation.

The BUP assays from Microgenics and Lin-Zhi International were validated on the Advia 1800 (Siemens). All urine screens for drugs of abuse ordered through the San Francisco General Hospital Clinical Laboratory were tested on both BUP assays for 31 days. Levels of BUP and its metabolites, for all positive samples, were confirmed using a targeted LC-MS/MS assay. The urine drug screen results, BUP assay results, and the patients' medical records were used to develop a method for determining compliance and efficacy. This method was then used to monitor a subset of compliant and noncompliant patients.

Characterization of the immunoassays showed that the Cedia assay (Microgenetics) detects BUP and BUP-glucuronide (a minor metabolite) and has significant cross-reactivity with opiates. The Lin-Zhi assay detects BUP and NBUP (a major metabolite) and has no opiate cross-reactivity. Since the assays differ in the metabolites they detect and their opiate cross-reactivity, we were able to establish defined criteria to determine compliance and efficacy, using both immunoassay values, the Lin-Zhi/Cedia ratio, and an opiate screen. Of the patients whose noncompliance was documented in their records (1 or more days without BUP use), 100% fit the established criteria for noncompliance. Among these patients, there was a positive correlation between the Lin-Zhi/Cedia ratio and the number of days without BUP use. This is due to the ability of the Lin-Zhi assay to detect NBUP whose half-life and elimination rate are longer than those of BUP, the parent drug. When determining efficacy, as measured by abstinence from opiates, in addition to BUP dosing compliance, we found that 67.1% of our patient population taking BUP were compliant and opiate-negative.

Monitoring BUP treatment compliance and efficacy through the use of 2 BUP immunoassays and an opiate screen is feasible, reliable, and clinically useful.

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Evaluation of Afinion AS100 Hemoglobin A_{1c} Point-of-Care Testing Analyzer.

S.R. Hui, A. Charnot-Katsikas, and K.T. Yeo. Sponsor: Jonathan L. Miller. Department of Pathology, University of Chicago Medical Center, Chicago, IL.

The Diabetes Control and Complications Trial (DCCT) and United Kingdom Prospective Diabetes Study (UKPDS) have demonstrated that tight glycemic control as indicated by lower hemoglobin A_{1c} (A_{1c}) can dramatically improve long-term morbidity and mortality of diabetic patients. Therefore, it is critical that any A_{1c} method, including point-of-care testing (POCT) assays be traceable to the DCCT and UKPDS methods to justify using the recommendations of these studies for clinical care of diabetic patients.

This study examined the analytical performance of A_{1c} methodology on the Afinion AS100 analyzer and compared it with the Bio-Rad Variant II A_{1c} method. We also examined if the presence of hemoglobin variants significantly affects the bias between the Afinion and Bio-Rad assays.

The Bio-Rad Variant II method for A_{1c} is based on the cation-exchange methodology, while the Afinion AS100 uses boronate affinity in conjunction with reflectance to determine the percentage of A_{1c} in whole blood.

Between-run precision for the Afinion showed a coefficient of variation of 1.2% at 6.3% A_{1c} and 1.1% at 8.2% A_{1c}, respectively. The analyte measuring range was verified between 4.8% and 14.6% A_{1c}, with recoveries ranging from 94% to 117%. Comparison of the patient samples without hemoglobin variants over 11 days demonstrated a least squares regression equation of [Afinion A_{1c}] = 0.95 [Bio-Rad A_{1c}] – 0.03 (r^2 = 0.97; $S_{y/x}$ = 0.42; range, 5.3%-14.1%; n = 46). The bias between the Afinion and Variant II methods was not statistically different in the presence of hemoglobin variant (AS, AC, and F) as compared with nonvariant blood samples (P = .6346; Mann-Whitney test).

We find that the Afinion AS100 A_{1c} POC method demonstrated excellent precision with an upper measuring limit of 14.6% and showed good agreement with the Bio-Rad Variant II analyzer. Overall, the Afinion AS100 provides a quick and simple point-of-service test for clinicians to monitor A_{1c} in an outpatient setting; this finger-stick A_{1c} POCT is especially advantageous for the pediatric population.

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Is the New IgG Enzyme-Immunoassay for HIT a Solution for Our Diagnostic Dilemma?

R. Darrell Sanders,[1] Stephanie H. Carroll,[1] T. Aaron Jones,[1] David Arndt,[1] Jeffrey S. Dlott,[2] and Marisa B. Marques.[1] [1]UAB Hospital, Birmingham, AL; and [2]Quest Diagnostics Nichols Institute, Chantilly, VA.*

Heparin-induced thrombocytopenia (HIT) is a prothrombotic syndrome with potentially fatal consequences caused by platelet activating antibodies, mainly of the IgG class, against platelet factor 4 (PF4)-heparin complexes. Despite the availability of commercial assays, HIT remains a diagnostic challenge. Enzyme immunoassays (EIAs) to detect IgG/A/M antibodies are routinely used in clinical practice. Recently, the Food and Drug Administration cleared the use of an assay to detect IgG only.

We sought to determine if the newly available EIA (PF4 IgG, GTI Diagnostics) offered diagnostic advantages over an EIA that detects IgG/A/M antibodies (PF4 Enhanced, GTI Diagnostics). We retrospectively reviewed the records of patients with a positive IgG/A/M EIA between January 2007 and February 2008 and compared the clinical probability of HIT (4Ts score) with results of the PF4 IgG EIA and the serotonin release assay.

We identified 44 patients with frozen samples to perform the PF4 IgG and SRA tests for comparison. Although only 30 (68%) of 44 samples had a positive IgG result, a paired Student *t* test showed strong correlation between the results of the 2 EIAs ($P = 5 \times 10^{-7}$). Only 4 (9%) were SRA+, and all had ODs of more than 2.0 in the IgG/A/M (mean ± SD, 2.74 ± 0.30) and more than 1.0 in the IgG-only EIA (2.12 ± 0.77). For the SRA– patients, (91%), the mean IgG/A/M OD was 1.29 ± 0.77 and the mean IgG-only OD for the positive samples was 1.33 ± 0.71. While more patients with positive EIA results had a moderate 4Ts score, there were similar numbers of patients with low and high probabilities. All three 4Ts scores were represented among the patients with a positive SRA. A total of 17 patients had documented thrombotic events; however, only 2 had a positive SRA and 5 were negative by IgG EIA. Furthermore, the mean OD (IgG/A/M EIA) of the patients with thrombosis was not different from that of the whole group by the Student *t* test ($P = .78$).

Our data show that the IgG-specific EIA reduced the number of HIT-positive samples by 32% but did not detect 5 patients with thrombosis. Similarly, the SRA was negative in patients with high clinical probability scores and thrombosis. We conclude that HIT remains a clinicopathologic diagnosis and that there is still a need for an assay to better predict thrombotic risk in HIT.

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Aggressive B-Cell Neoplasm With Both Immature and Mature Features.

Ami Goradia and Dale Frank. Sponsor: Malek Kamoun. Department of Pathology and Laboratory Medicine, Hospital of the University of Pennsylvania, Philadelphia.

The patient, a 56-year-old woman, presented with neutropenic fever, lymphadenopathy, and splenomegaly with a rapidly increasing WBC count composed of monotonous intermediate-sized mononuclear cells with slightly condensed chromatin, rare nucleoli, and scant cytoplasm. Flow cytometry on peripheral blood demonstrated a surface κ-restricted, CD5(dim)+/CD10(dim)+/CD23–/CD19(dim)+/CD20(dim)+ small B-cell neoplasm. An enlarged axillary lymph node (5.5 cm) contained rare, scattered, preserved follicles with polarized germinal centers and mantles surrounded by a diffuse interfollicular expansion of small lymphocytes with round nuclei, clumped chromatin, and scant cytoplasm that were shown by immunostains to be CD5(dim)+/CD10(dim)+/CD43+/BCL2–/BCL6–/cyclin D1–/Zap70–/CD20(dim)+/CD79a+/p53+ small B cells with a Ki-67 rate of more than 50%. A concurrent bone marrow biopsy showed diffuse replacement of marrow space by the neoplasm. Karyotype and fluorescence in situ hybridization studies on peripheral blood showed a normal female karyotype (XX,46[19]) and a deletion of chromosome 13q (174/200 cells), respectively.

Given the available findings, a diagnosis of consistent with atypical CLL/PL was made. The patient responded well to prednisone and Rituxan and then achieved a clinical complete remission (peripheral counts normalized, and imaging showed reduction of splenomegaly and lymphadenopathy) on a regimen of fludarabine, Cytoxan, and Rituxan. However, 5 months later, the otherwise-well patient developed low-volume uterine bleeding. A uterine wall biopsy showed a diffuse monotonous lymphoid infiltrate composed of large cells with irregular nuclei, fine chromatin, inconspicuous nucleoli, and scant cytoplasm. Mitotic figures were prominent. Immunostains demonstrated that the neoplasm was composed of CD3–/CD5(dim)+/CD10+/CD43+/BCL6–/CD20(weak)+/CD79a+/PAX5+/p53+ large B cells with a Ki-67 rate of approximately 90%. The neoplastic cells were shown to be CD34– but strongly and diffusely terminal deoxynucleotidyl transferase (TdT)+. A TdT immunostain performed retrospectively on the lymph node excised at presentation showed focal weak nuclear expression. Additional molecular studies assessing IgH and TCR clonality at both time points are pending. Given the clinical response to CLL therapy, the initial findings of a small B-cell neoplasm with surface light chain restriction, and the shared immunophenotypic features with the current uterine lesion, a diagnosis of an aggressive B-cell neoplasm with both immature and mature features was rendered.