ACLPS 2022

Young Investigator Oral Presentations

Chemistry Abstracts

Development of an Aid to Facilitate Clinical Adoption of Apolipoprotein B

Authors

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Abstract

Apolipoprotein B (apoB) has long been argued to be a better indicator of atherosclerotic cardiovascular disease (ASCVD) risk, and adequacy of lipid-lowering therapy than low-density lipoprotein cholesterol (LDL-C). Recent discordance studies have shown definitively that when levels of apoB and LDL-C do not agree, ASCVD risk tracks with apoB rather than LDL-C. Nevertheless, LDL-C remains the primary measurand used to guide treatment decision-making. A major practical barrier that inhibits more widespread clinical use of apoB is the lack of familiarity of general practitioners with apoB values. We aimed to develop a method to "translate" apoB results into equivalent LDL-C units so that guideline-recommended LDL-C thresholds and treatment goals that are familiar to clinicians can be adopted for making apoB-guided treatment decisions. LDL-C calculated from standard lipid panel data using the NIH Sampson equation, and apoB measured by immunoassay (both in mg/dL) were used to determine their respective percentile values in a large cohort of 15,153 patients tested by a commercial laboratory. Linear regression of LDL-C values against their percentile-equivalent apoB values (R^2=0.999) yielded an equation to readily convert apoB into corresponding LDL-C equivalent units: [LDL-C equivalents = (1.38 * apoB) – 29]. To examine the extent of discordance between LDL-C and apoB in this cohort, we created histograms of apoB (in LDL-C

equivalents) for patient subgroups with similar levels of LDL-C ranging from very low (55-70 mg/dL) to very high (175-190 mg/dL). For individuals with very low LDL-C, 40% had discordantly higher apoB, signaling that they had higher ASCVD risk than implied by LDL-C and might thus be under-treated. At the other extreme, 49% of those with very high LDL-C had discordantly lower apoB, suggesting a potentially lessened need for treatment. Overall in the 9 examined LDL-C subgroups, only a minority of patients (25 – 40%) had concordant levels of apoB, confirming that discordance between these alternate risk biomarkers is highly prevalent. Giving clinicians routine visibility to these discrepancies should help bring about more rapid and widespread adoption of apoB for refining treatment decisions to the benefit of patients.

Topic Areas

Chemistry

Analytical and Clinical Evaluation of the Automated Elecsys Tacrolimus Assay on the Roche cobas e602 Analyzer

Authors

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Abstract

Objective: This validation study evaluates whether the automated Roche Elecsys tacrolimus ECLIA has the required accuracy for implementation by comparison with the current UCMC laboratory developed test (LDT) for the quantitation of tacrolimus by liquid chromatography tandem mass spectrometry (LC-MS/MS). Tacrolimus is a first-choice immunosuppressant for solid organ transplantation due to a correlation with decreased cardiovascular risk and renal complications. Importantly, post-transplant tacrolimus target ranges are very narrow and specific. Different target ranges for the stages of kidney transplantation are within ±2 ng/mL: 8–10 ng/mL months 0–3, 6–8 ng/mL months 3–12, 4–6 ng/mL months 12 and onward.

Methods: The tacrolimus ECLIA assay was evaluated for precision, linearity, interference (by hemoglobin, bilirubin, triglycerides, and biotin) and clinical performance was compared to LC-MS/MS quantitation. Additionally post-extraction stability studies of patient samples (3.07–28.57 ng/mL) were performed at room temperature (21–23°C) and cold storage (4°C).

Results: The tacrolimus ECLIA assay is precise, exhibits a measuring range of 0.75–30 ng/mL, and is tolerant of significant interferences (plasma indices: H <2306, I <55, L <1427, and biotin <1200 ng/mL). Comparison with LC-MS/MS quantitation revealed a 21% bias in patient samples evaluated for tacrolimus concentration (n=43, range=2.2–24.4 ng/mL, y=1.21x +0.52, r^2 =0.97). Bland-Altman analysis revealed an absolute mean bias of 2.5 ng/mL (SD = 1.5 ng/mL). Post-extraction studies at room temperature, exposed to air, confirmed that samples were stable for up to 30 minutes, after which concentrations of tacrolimus were increased by

greater than 10% of the initial measurement. Samples that were capped within 15 minutes, which allowed for an initial measurement, and stored at -4°C were stable for up to 24 hours.

Conclusion: The relatively high bias observed by the tacrolimus ECLIA assay compared with the LC-MS/MS reference presents major challenges to the implementation of the tacrolimus ECLIA assay routinely. Due to the very narrow concentration ranges targeted (within ±2 ng/mL) across the stages of the transplantation process and recovery, the absolute mean bias (2.3 ng/mL) observed between the two methods led to the conclusion that the two methods gave rise to discordant clinical interpretations. In addition, post-extraction stability studies presented practical challenges to adjusting the automated laboratory workflow in order to minimize sample evaporation to prevent falsely elevated results.

Topic Areas

Chemistry

A novel and robust approach to defining population-based reference ranges for a serum free light chain assay in a clinical laboratory

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Abstract

Serum protein electrophoresis and immunofixation are used to detect the presence of monoclonal heavy and light immunoglobulin chains for the diagnosis of plasma cell neoplasms (PCN). In conjunction, the Freelite® assay has been used to detect excess free kappa and lambda light chains produced by PCN, where the kappa:lambda free light chain ratio (KLR) serves as a surrogate marker of clonality and disease. The manufacturer-defined KLR reference range of 0.26-1.65 is used in internationally recognized criteria for PCN diagnosis and monitoring, and KLR results affect clinical decision making, disease categorization, and response assessment. Our laboratory has verified the manufacturer-defined Freelite® KLR reference range three times using healthy control sera since assay implementation in 2006. Despite this, high percentages of abnormal KLR have consistently been observed among immunofixation-defined monoclonal-negative specimens. We therefore retrospectively interrogated 48,012 serum free light chain clinical results reported at our institution between 2010-2020 by extracting results from our

laboratory information system and analyzing population distributions using the R programming language. For all specimens, the distribution of KLR values was shifted towards the towards the upper limit of the manufacturer-defined reference range, indicating that it poorly describes both our monoclonal-negative and monoclonal-positive patient populations. Furthermore, >80% of monoclonal-negative specimens exhibited KLR above the median of the manufacturer-defined reference range (0.26-1.65), while 24% (1,226/5,057) unexpectedly exhibited KLR >1.65. We found that this phenomenon has been present consistently since 2010 and is not affected by patient sex or age, impaired renal function, lot-to-lot variation, or assay drift. We therefore sought to establish an institution-appropriate KLR reference range by defining a reference population from within our existing dataset. We leveraged International Classification of Diseases codes, estimated glomerular function data, and serum/urine immunofixation results to exclude specimens from individuals with PCN- or renal-related diagnoses, impaired renal function, and prior, current, or subsequent monoclonal components. The resultant 1,536 monoclonal-negative reference specimens were then classified according to the manufacturer-defined KLR reference range, where 13% remained abnormally elevated, consistent with the published false positive rate in healthy volunteers. Lastly, we determined a new KLR reference range of 0.66-2.21 using the central 95% of KLR values from these reference specimens. When applied to our dataset, this institution-specific KLR reference range improved the accurate classification of specimens containing monoclonal free light chains by immunofixation, while reducing the false-positive rate in monoclonal-negative specimens. Together, our findings support the use of historical KLR and immunofixation data from routine clinical testing to derive institution-appropriate KLR reference ranges, which can then be used for continuous quality improvement. Implementation of this more-accurate KLR reference range should improve subsequent test utilization by non-specialist providers, reduce concern for/over-diagnosis of kappa-involved disease, and improve the detection and monitoring of lambda-involved disease.

Topic Areas

Chemistry

Development of an Automated Complement C5 Functional Assay for Personalized Monitoring of Patients on C5 Inhibitors

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Abstract

Background: Humanized anti-C5 monoclonal antibody therapeutics has been heralded as a breakthrough treatment for Paroxysmal Nocturnal Hemoglobinuria and atypical Hemolytic Uremic Syndrome. Therapeutic drug monitoring is currently not widely available but assessing complement blockage maybe useful to monitor and optimize Eculizumab or Ravlizumab treatment efficacy. Recent studies have suggested using C5 functional assays as a specific target to assess complement blockage. The aim of this study was to assess the analytical performance of an automated C5 functional assay we developed by modifying the complement CH50 assay used in our laboratory. **Methods:** Patient sera mixed in different ratios (20-90%) with commercially available C5-deficient (C5d) serum (Quidel corp., SD) were tested for lytic activity using the CH50 liposome assay (Optilite, Binding site, Birmingham, UK). Normal lysis ensures intact C5 functional activity exclusively from the patient serum. A ratio that led to normal lysis in healthy sera and in patient sera defective in complement factors other than C5 was considered optimal. This ratio was adopted for preliminary reference intervals (n=34 normal donors) using bi-

weight quantile method estimation at a 90% confidence interval. Accuracy was assessed by recovery studies. Specimens (n=2) were created with varying levels of C5 functional activity using Eculizumab (Biovision, CA). Recovery was calculated (measured/expected) by adding back 100 ug/mL of purified C5 (Quidel corp., SD) and comparing the CH50 values to the original results. To evaluate specificity, patient specimens (n=22) defective in complement components other than C5 (C2, C3, C4) were mixed with C5d and lytic activity measured. **Results:** A ratio of 75% C5d to 25% patient serum was used to establish a reference interval of 32.3 – 46.7 U/mL. The two accuracy samples had initial CH50 values of 37.9 and 41.7 U/mL and 14.2 and 20.6 U/mL after Eculizumab depletion, respectively. Adding C5 back to the Eculizumab-depleted samples yielded values of 36.5 and 39.9 U/mL and recoveries of 96%. The non-C5 complement-deficient specificity samples had low CH50 values ranging from 11.8 to 28.8 U/mL. After mixing with C5d, 21/22 samples demonstrated low-normal or normal lytic activity (range: 28.4 -41.6 U/mL). **Conclusion:** A ratio of 75% C5d to 25% patient serum was optimal for maintaining CH50 activity in normal specimens and recovering activity in specimens with complement deficiencies other than C5. The C5-depleted specimens did not regain normal CH50 activity after mixing with C5d serum. Adding C5 to these specimens regained normal CH50 activity, confirming that C5 was non-functional and the C5 inhibitor was not interfering with the assay. Specimens' defective in complement components other than C5 recovered lytic activity when mixed with C5d serum in 95.5% of the specimens, confirming the analytical specificity of the assay. These data demonstrate an acceptable analytical performance for the C5 functional assay developed.

Topic Areas

Chemistry

Benzodiazepine Analysis by an Improved LC-MS/MS Method Demonstrates Usage Patterns in Washington State

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Abstract

Background

Benzodiazepines are a commonly prescribed class of pharmaceuticals that have abuse potential. The prevalence of several novel, usually non-prescribed, benzodiazepines has been trending up in the United State in recent years. In this work, we validated an improved LC-MS/MS method using an upgraded instrument for the detection of common/routine benzodiazepines, as well as some designer/novel ones. We also retrospectively investigated previously accumulated data to identify the prevalence and trends of individual benzodiazepine usage in Washington State, in addition to summarizing results from the newly validated method and elucidating observations that demonstrate usage patterns.

Material

Ten common/routine benzodiazepines were measured quantitatively, while seven designer/novel compounds, including two common “Z drugs”, were measured qualitatively as the “Quality Assurance (QA) panel”. All samples, controls, and standards underwent hydrolysis by beta-glucuronidase, followed by precipitation and centrifugation. The assay was developed and validated simultaneously on two identical Waters Acquity UPLC/Xevo TQXS LC-MS/MS systems. Assay performance

was evaluated following CLSI C62-A guidelines with the results correlated to a previous version of the assay performed on two Waters Quattro Micro (QM) LC-MS/MS systems. Accumulated patient screening and confirmatory data from 2017-2021, amounting to 10,506 total cases, were analyzed in RMarkdown accessed through RStudio.

Results

The run time per sample was reduced to 15-min from 30-min by the new TQXS method. The coefficients of variation for intra- and inter-day precision were within 10%. Correlation between the prior and current assay was linear, with an average R^2 of 0.99. No matrix effect or carryover was observed. The lower limits of analytical range were extended from 0.1 to 0.05 µg/mL. Unsurprisingly, clonazepam was found to be the most prescribed benzodiazepine medication in recent years, followed by alprazolam. Interestingly, 9% of all cases among the analyzed data in 2021 involved at least one positive result in the QA panel, with etizolam being the most common unprescribed benzodiazepine, indicating that this drug should be included in benzodiazepine assessments. Flualprazolam, another non-FDA-approved benzodiazepine, was detected in a total of 12 cases since its inclusion in the QA panel.

Conclusion

This newly validated method allows for a more efficient, sensitive, and robust assay for the quantitative identification of select common and qualitative detection of designer/novel benzodiazepines using LC–MS/MS. Clonazepam and alprazolam continue to be the most prescribed benzodiazepines, while the usages of unprescribed non-FDA-approved etizolam and flualprazolam are increasing in Washington state.

Topic Areas

Chemistry

The Clinical Significance of Light Chain Predominant Multiple Myeloma

Authors

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Abstract

Multiple Myeloma (MM) is a neoplastic clonal proliferation of terminally differentiated B- lymphocytes. It accounts for 1.6% of all cancers and about 10-15% of all hematological malignancies in the United States. Monoclonal immunoglobulins provide an indication of tumor burden in patients with plasma cell neoplasm. Recent development has shown that a proportion of intact immunoglobulin producing multiple myeloma secrete much higher amounts of free light chains than usual; i.e. light chain predominant multiple myeloma (LCPMM). About 18% of multiple myeloma secreting intact immunoglobulins secrete higher amount of free light compared to other intact immunoglobulin secreting myelomas and are associated with significantly poorer clinical outcomes. In this study, a retrospective investigation comparing the laboratory and clinical findings in conventional MM and LCPMM was undertaken. Three hundred and sixteen patients with conventional MM and seventy-one patients with LCPMM were studied. The results were remarkable for marked increase in death rate in the LCPMM group compared to the conventional MM group, 35.2% and 13.6% respectively; P-value .00001. The occurrence of dialysis was also markedly increased in the LCPMM group compared with the conventional MM; 18.3% vs. 3.5% P-value .00001. A markedly lower eGFR was observed in patients with LCPMM group compared to patients with conventional MM with an average value of 46.3 versus 69.4 ml respectively P value .0000003. In conclusion, light chain predominant plasma cell myeloma confers a significant disease burden, poor clinical outcomes and shorter survival. Further prospective trial will be helpful in understanding the pathogenesis, the course, and management of the disease. An assay for serum free monoclonal light chains has been described and could serve as a marker for minimal residual

disease in such patients. We aim to further study the clinical impact of light chain predominant plasma cell myeloma (LCPMM) to increase the current knowledge of the disease and investigate therapeutic interventions.

Topic Areas

Chemistry

Deceptively simple: Can urine samples from NexScreen cup be re-used for confirmatory testing?

Authors

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Abstract

Background: The NexScreen urine drug screen cup (SKU: HCDOAV-8145EF1KT, NexScreen LLC, CO, USA) is a Clinical Laboratory Improvement Act (CLIA)-waived test used at our affiliated mental health institute. Specimens testing positive are sent for Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) confirmation. The manufacturer instructs patients to urinate directly into the NexScreen cup. Doing so raised concern about whether this urine can be re-used for confirmatory testing. Modifying the test by collecting urine in another container before pouring it into the NexScreen cup would reclassify the test as high complexity. This would require further regulatory oversight and negate the convenience of a CLIA-waived test. Requesting the patient to urinate into a second container is not always feasible, as patients may be unable or unwilling to provide another sample. A straightforward solution would be to take specimens from the NexScreen container and use them for confirmatory testing. However, the assay components of the NexScreen drug test strips may contaminate specimens and generate false positives with highly sensitive LC-MS/MS assays. In addition, false negatives on confirmatory testing could occur if NexScreen test strips or the plastic cup adsorb drug compounds from patient urine. The manufacturer provides no guidance in this regard. **Objective:** The goal of this study was to investigate if re-using urine from NexScreen cups for LC-MS/MS confirmatory testing was appropriate. **Methods and Results:** We pooled 25 NexScreen-negative patient specimens and confirmed by LC-MS/MS that no NexScreen-detectable compounds were present in this pool. To evaluate drug conjugate leaching into the specimens, 30 mL blank urine was poured into 10 NexScreen cups, followed by incubation for 1

hour at room temperature, which is the maximum time recommended by the manufacturer for reading NexScreen results. All 10 cups tested negative for all drugs. In addition, none of the negative samples tested positive on LC-MS/MS for any drug compounds targeted, proving that the test strips in the NexScreen cups do not interfere with LC-MS/MS testing. Next, to assess drug adsorption by the NexScreen drug cup, a representative analyte from each of the 13 drug classes was chosen and spiked at concentrations slightly above the published NexScreen positivity cutoffs. Then, 30 mL of spiked urine was placed into 10 NexScreen cups, incubated for 1 hour, and aliquots were sent for LC-MS/MS confirmation. Interestingly, 11 of the 13 analytes achieved at least 95% recovery, while buprenorphine and THC-COOH had 94% and 87% recoveries, respectively, indicating some minor adsorption. However, all analytes, including buprenorphine and THC-COOH, recovered well above the cutoffs for LC-MS/MS assays, suggesting that there is no significant adsorption to alter confirmatory results. **Conclusion:** Use of an aliquot from a NexScreen cup for confirmatory testing is appropriate within the manufacturer-recommended readability window of 1-hour post-collection.

Topic Areas

Chemistry

Evaluation of Bio-Rad D100 for HbA1c testing of dried blood spot samples

Authors

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Abstract

Hemoglobin A1c (HbA1c) testing is critical for diabetes diagnosis and monitoring. Although many studies have demonstrated the feasibility of measuring HbA1c from dried blood spots (DBS) using immunoassays, enzymatic assays, and certain high-performance liquid chromatography (HPLC) assays, there is limited evidence supporting the use of the Bio-Rad D100 HPLC method. We evaluated the feasibility of testing HbA1c from DBS using Bio-Rad D100 to support the assessment of glycemic control for the growing field of telehealth.

Deidentified residual EDTA whole blood samples were processed, and HbA1c was measured on the Bio-Rad D100 on day zero. Concurrently, DBS were prepared for each sample by spotting 50 µL of blood onto a Whatman® 903 filter paper. The samples were dried overnight and stored at room temperature for two days. On day 2, a DBS disc with a diameter of 3.2 mm was eluted with 4.5 mL of Bio-Rad diluent for 20 minutes. The supernatants of the DBS samples were then processed on the platform. HbA1c values from the DBS samples were compared to the EDTA whole blood results.

Compared to the EDTA samples, the HbA1c concentrations of the corresponding DBS samples were elevated, especially for samples with original results less than 7.0%. Furthermore, the chromatograms of the DBS samples were significantly altered; we observed baseline elevation and the appearance of unknown minor peaks. This issue was investigated. First, blank DBS samples were created by directly spotting filter paper with diluent or adding diluent into an empty EDTA tube and then spotting the filter paper with the mixture of diluent and EDTA. The blank

DBS samples were tested, and the lack of peaks suggested that the interference was not from the diluent, EDTA, or the filter paper. DBS samples were also prepared with sodium heparin whole blood, finger-stick capillary blood, and multiple brands of filter papers and tested using a different HPLC method. However, the interference was still present in those samples, indicating the interference is most likely due to the time-dependent degradation of the samples themselves. Of note, the alternate HPLC method showed less chromatogram alteration. To prevent possible adduct formation, DBS samples were eluted in the presence of various concentrations of cysteine (Jeppsson *et al.* 1986). However, the pretreatment did not eliminate the chromatogram changes. Finally, a multiple regression model was created using the results of day two A1c, A0, and F peaks, and a correction factor was generated. The corrected HbA1c concentrations were well within +/-6% of the initial HbA1c concentrations. In conclusion, the Bio-Rad D100 HPLC method appears particularly susceptible to stability changes in DBS samples, and further analysis is needed before employing a correction factor.

Topic Areas

Chemistry

Implementation and Assessment of a SmartZone Alert to Notify Clinicians of Critical Hyperbilirubinemia in Preterm Infants Less Than 35 Weeks Gestation

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Abstract

Background:

Neonatal jaundice, also known as hyperbilirubinemia in term and preterm infants, is treated with phototherapy when bilirubin results exceed gestational age- and age-specific medical decision levels (MDL) to prevent kernicterus and bilirubin-induced neurological damage. During phototherapy, unconjugated bilirubin is converted to water-soluble isomers that are excreted in the urine. Presently, the electronic medical record (EMR) at our hospital cannot use gestational age to stratify reference ranges thereby the associated flags and alerts would not be triggered, leading to delays in reviewing bilirubin results and placing phototherapy orders. The aim of this project is to replace the current manual assessment process for phototherapy with a newly designed alert to notify clinicians of elevated bilirubin results for preterm infants (<35 weeks gestation) and to minimize prolonged delays in placing phototherapy orders. We hypothesized that the SmartZone alert will encourage consistent and timely consideration of phototherapy.

Method:

A SmartZone alert, built with Cerner command language and Discern Expert rules, evaluates total or neonatal bilirubin results for patients in the Newborn and Infant Critical Care Unit (NICCU), calculates the patient's age using the recorded gestational age and birth time, and utilizes the following rules: 1) bilirubin result must exceed the pre-defined gestational age-specific MDLs, and 2) calculated gestational age is <35 weeks. Additionally, the SmartZone alert will not be triggered if there is an active phototherapy order. Once triggered, the alert will be visible for four hours to all caregivers who view the patient's EMR. We compared the duration of time between verified bilirubin results and phototherapy orders before and after the implementation of the SmartZone alert.

Result:

The SmartZone alert was implemented on 01/11/2022. Between 1/1/2021 - 1/11/2022, 95 preterm infants in the NICCU had neonatal and total bilirubin results,

in which 19 met the criteria for the alert to be triggered, and 17 had phototherapy orders. Further analysis revealed that 12 orders were placed <24 hours, four orders were placed 24 - 72 hours, and one order was placed >72 hours after bilirubin results were verified. Between 1/12/2022 – 2/13/2022, 13 newly admitted preterm infants in the NICCU had neonatal and total bilirubin results, in which one triggered the alert, and phototherapy was ordered within 15 hours.

Conclusion:

A SmartZone alert was implemented to encourage consistent and timely consideration of phototherapy for preterm infants <35 weeks with critical hyperbilirubinemia. The alert is functioning as intended. This alert will be continuously monitored to further optimize its functionality and utilization.

Topic Areas

Chemistry

Method validation of an Inductively Coupled Plasma Mass Spectrometry (ICP-MS) assay for the analysis of Magnesium, Copper and Zinc in RBC

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Abstract

Background: Laboratory measurements of trace elements such as magnesium (Mg), copper (Cu) and zinc (Zn) in red blood cells (RBCs) are used to assess nutritional status and diagnose metal toxicity. The purpose of this study was to develop/validate an ICP-MS method for the quantification of these elements in RBCs.

Methods: An aliquot of packed RBCs was diluted in alkaline diluent solution containing scandium (^{45}Sc) and gallium (^{71}Ga), as internal standards, 0.1% Triton X-100, 0.1% EDTA, and 1% ammonium hydroxide (NH4OH). The diluted specimen was aspirated onto an ICP-MS for quantitative analysis of ^{25}Mg, ^{63}Cu and ^{66}Zn. The method was validated for accuracy, precision, method comparison, linearity, analytical sensitivity, and carryover. Retrospective data was analyzed and non-parametric reference intervals (RIs) were calculated.

Results: Accuracy and linearity were within the expected range of ≤±15% for all analytes. Within-run, between-run, and total imprecisions were ≤15% CV. All other validation experiments met acceptance criteria. Established RIs for Mg, Cu and Zn in RBCs are 3.6-7.5 mg/dL, 59.0-91.0 mcg/dL and 794.0-1470.0 mcg/dL, respectively. Retrospective analysis of patient data for Mg RBC displayed no statistically

significant difference in mean concentrations between adults (>18+ yr) and children (0-18 yr) ($p > 0.05$); however, there was a significant difference in Mg concentrations between males and females ($p < 0.001$). The mean and SD for Mg RBC concentration for females and males were 5.38 + 0.68 mg/dL (n=8451) and 5.42 + 0.68 mg/dL (n=3954) respectively. The 95% RI was 4.2-6.9 mg/dL; >99% of patient results were within the established RI. Cu RBC mean concentrations were not statistically significant between males and females ($p > 0.05$) and age differences were not evaluated due to the low number of pediatric patients (n=37). The mean and SD for Cu concentrations were 72.2 + 11.7 mcg/dL (n=423). The overall 95% RI was 54.3 - 96.3 mcg/dL; 88.9% of patient results were within the established RI. For Zn RBC, there was a statistically significant difference in mean concentrations between children and adults ($p < 0.0001$) and no statistically significant difference between sex ($p > 0.05$). The mean and SD for Zn concentrations in children were 1103.7 + 225.5 mcg/dL (n=188). The 95% RI for this subgroup was 707.3– 1547.6 mcg/dL; 87.8% of pediatric patients were within the established RI. In adults, the mean and SD for Zn concentrations were 1311.7 + 196.2 mcg/dL (n=1579). The 95% RI was 932.5 – 1718.3 mcg/dL; 83.3% of patient results were within the established RI.

Conclusions: This method was validated and met criteria for clinical use to quantify three elements using ICP-MS for biological monitoring. Retrospective data analysis of patient results demonstrated that the method was suitable to assess nutritional deficiency and toxicity.

Topic Areas

Chemistry

Evaluating the utility of a two-assay serological algorithm for Hepatitis C screening

Authors

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Abstract

With the advent of novel therapies, infection with Hepatitis C virus (HCV) has become curable in those able to access and afford treatment, and the prevalence of chronic HCV has slowly, yet consistently declined. Per CDC guidelines, screening for chronic HCV is based on the detection of circulating anti-HCV antibodies. Using a single immunoassay to achieve this runs the risk of false positive results, especially in a low-prevalence population such as Iowa (~0.7%). Furthermore, previous studies have shown that false positives are more common in specimens with low reactivity, leading institutions such as ours to mitigate these by testing such specimens by a second immunoassay. Our two-assay algorithm for HCV screening is performed by first analyzing samples using the Elecsys Anti-HCV II assay (Roche) which assigns a “cutoff-index” (COI) based on signal generated by the patient sample compared to positive and negative controls. In our laboratory, specimens with COI > 20 are resulted as positive and those < 0.9 resulted as negative. For specimens that are positive according to manufacturer’s guidelines but have low reactivity (COI of 0.9 – 20.0), we tested by a second immunoassay. This was performed using the ARCHITECT anti-HCV assay (Abbott) which similarly generates cutoff values that are reported as positive (>1), negative (<0.8) or indeterminate (0.8-0.99) according to manufacturer’s guidelines. To evaluate the diagnostic utility of this approach we retrospectively analyzed the outcome of 59,908 screening tests from 47,706 individual patients over a 5-year period. Of these, only 180 samples (0.3%) from 139 unique patients were initially indeterminate by the Roche assay and required

second line serological testing. Final interpretations following a second immunoassay were either positive (9%), negative (87%), or indeterminate (4%). Further evaluation of the positive subset found that 59% of cases had either a definitive history of HCV infection or were subsequently confirmed positive by HCV PCR. In contrast, only 14% of indeterminate and 3% of negative samples had a history of HCV, and none were positive by PCR. Thus, the positive predictive value (PPV) of an indeterminate Roche result was 9%, which was significantly lower than the PPV using our two-assay approach (59%). We next investigated whether there were quantitative differences in the results of the initial serological testing (i.e., Roche) that could accurately predict the final interpretation. While we found statistically higher quantitative values in the group that ultimately resulted as positive (mean value of 13.4 vs. 5.5), there was significant overlap in the spread of individual values suggesting that prediction of positivity based on initial testing results would be unreliable. Taken together, these results emphasize the utility of our two-assay approach in maximizing the performance of HCV screening in a low prevalence population.

Topic Areas

Chemistry

On-Cell Stability for Therapeutic Drug Monitoring

Authors

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Dr. Kelly Doyle - University of Utah

Abstract

Introduction: A common problem in many clinical laboratories and outpatient clinics is processing specimens rapidly to comply with specimen stability criteria. Labs receive thousands of samples a day and may face challenges with staffing, equipment malfunction, or workflow, which can prevent immediate processing of samples. Currently, for therapeutic drug monitoring, the lab is required to centrifuge specimens and remove the serum/plasma from cells within 2-hours (CLSI-GP44-A4). Many of these drugs are monitored in the outpatient setting, which may require longer transportation times to get specimens from the collection location to the laboratory. This creates challenges for the lab in processing samples within 2-hours and may result in unnecessary rejection of otherwise acceptable specimens. Not all clinical sites have the staffing or space to process the specimens on site. Our objective was to determine if the on-cell stability of therapeutic drugs can be extended to beyond two hours. **Methods:** We performed spiking studies to determine the on-cell stability of phenytoin in whole blood that was collected from healthy donors in barrier-free red top tubes. Phenytoin was spiked into the whole blood at a final concentration of about 10 (therapeutic) or 40 (toxic) µg/mL. The spiked whole blood samples were mixed by inverting six times and then were left at ambient temperature for 30 minutes, 2-, 4-, 6-, or 12-hours, after which they were centrifuged, and the serum was removed for analysis of total phenytoin concentration on the Abbott Architect and free and total phenytoin on the Beckman AU (n=3 per time point and concentration). *In vivo* studies are ongoing for an extended list of therapeutic drugs, in which two extra red top tubes are collected

from patients being monitored for phenytoin, valproic acid, digoxin, vancomycin, lithium, or carbamazepine therapy. The two study tubes are kept at ambient temperature for 6- or 12-hours after collection before being centrifuged and separated from cells. The 6- and 12-hour drug concentrations were compared to the patient's standard of care tube that was processed within 2-hours, after clotting for 30 minutes. **Results:** Spiking studies demonstrated phenytoin was stable on cells for at least 12-hours at both 10 and 40 µg/mL. The percent total phenytoin remaining at 12-hours relative to the 30-minute control was 104 ± 2% at 10 µg/mL and 99 ± 16% at 40 µg/mL. Furthermore, the fraction of free phenytoin did not change over the 12 hours (10 µg/mL: 8 ± 1% free; 40 µg/mL: 10 ± 1% free). Our preliminary *in vivo* studies have shown no change in vancomycin or lithium concentration in samples processed 6- and 12-hours post-collection. **Conclusion:** We have demonstrated that phenytoin, vancomycin, and lithium collected in red-top tubes are stable on cells at ambient temperature for at least 12-hours.

Topic Areas

Chemistry

Compliance or Abuse? Detectable hydromorphone in patients taking oxycodone

Authors

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Dr. Ibrahim Choucair - Yale School of Medicine

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Abstract

A middle-aged woman with recent cervical diskectomy with spinal fusion at C3-C4 suffered a mechanical fall with bilateral upper extremity paresthesia requiring admission but no surgical intervention. Patient was seen by orthopedic service and was recommended to continue using Aspen collar and oxycodone for pain. Following discharge, a drug urine screen test was performed, and showed positive for oxycodone by immunoassay. Confirmatory urine test by liquid chromatography-tandem mass spectrometry (LC-MS/MS) showed detectable oxycodone, oxymorphone and hydromorphone.

In patients treated with opioid therapy for chronic pain, urine drug monitoring is an important tool to ensure that patients are adhering to the prescribed medication. Hydrocodone and hydromorphone are not metabolites of oxycodone. However, hydrocodone can be present in oxycodone preparations with an estimated impurity of <1%. Hydrocodone can be metabolized into hydromorphone, although, there is no literature reporting detectable hydromorphone in patients' urine with prescribed oxycodone only.

This case resulted in a quality improvement project where we evaluated the detection of hydrocodone and hydromorphone in positive oxycodone urine samples using LC-MS/MS. We analyzed 322 oxycodone positive urine samples. Twenty-one samples (6%) had detectable hydrocodone and/or hydromorphone below 100 ng/mL. Twelve out of the 21 were from patients taking no opioid medication other than oxycodone in the last week, with 33.3% showing detectable hydromorphone and 75% for hydrocodone below 100 ng/dL. The calculated

hydrocodone/oxycodone (HC/OC) and hydromorphone/oxycodone (HM/OC) ratios were on average 0.26% HC/OC = 0.26% (0.05-0.69% SD 0.002; HM/OC = 0.26% (0.18-0.37% SD 0.001)] each (<1%). Patients taking oxycodone and other opioids had ratios >1% [HC/OC = 168% (3-563% SD 2.53; HM/OC = 699% (1.09-6585% SD 15.96)] and had higher detected levels (>100 ng/mL).

Pain management can be very challenging; therefore, laboratory testing provides an objective assessment of drug exposure and adherence to treatment. Cut-off values for considering positive the detection of hydrocodone and hydromorphone may change the interpretation of the drug urine tests in patients with prescribed oxycodone. Using higher cutoff (100 ng/mL) and the ratios HC/OC and HM/OC (<1%) may be useful to determine that the detection of these components is due to impurity of oxycodone formulation.

Topic Areas

Chemistry

Evaluation of False Positive RPR Results and the Impact of SARS-CoV-2 Vaccination in a Clinical Population with a High Rate of Syphilis Utilizing the Traditional Screening Algorithm

Authors

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Dr. Sara Love - Hennepin County Medical Center

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Abstract

Background

Syphilis infection caused by the bacterium *Treponema pallidum* is a sexually transmitted infection leading to serious health complications if undiagnosed or untreated. Serologic diagnostic methods include a combined approach for detection of antibodies to non-treponemal (RPR) and treponemal antigens using a traditional or reverse algorithm. The BioPlex2200 Syphilis Total/RPR assay (BioRad Laboratories) is an automated method for simultaneous detection of treponemal/non-treponemal antibodies, but recently recalled due to potential RPR false reactivity following SARS-CoV-2 mRNA vaccination. The purpose of this study was to evaluate syphilis confirmation and false positive RPR rates over various intervals preceding and during the COVID-19 pandemic in a population with a high rate of syphilis, and a subset of pregnant females screened for syphilis prior to delivery, to assess the impact of mRNA vaccines on different RPR reactivity rates and methodologies.

Methods

The study cohort included all individuals screened for syphilis (n=48,288) and a subset of pregnant patients (n=2,944), where syphilis testing was ordered for routine clinical care utilizing the traditional algorithm. Five timepoints represented various phases and RPR methods utilized throughout the pandemic: 1) pre-COVID-

19 (automated RPR, July – December, 2019); 2) COVID-19, pre-mRNA vaccine (manual RPR, July – December, 2020); 3) COVID-19, mRNA vaccine (manual RPR, January – June, 2021); 4) COVID-19, mRNA vaccine/boosters (automated RPR, August – December, 2021); and 5) COVID-19, mRNA vaccine/boosters (RPR/*T.pallidium* sent out to reference laboratory, January – February, 2022). False positive RPR was defined as reactive RPR/nonreactive *T.pallidium*. Titers were determined for all reactive RPR specimens.

Results

Syphilis confirmation rates were between 4.5 – 7.9% for all patients and 0.15 – 0.41% for pregnant patients during the entire study period. False positive RPR rates increased in the screening (5% to 8.6%) and pregnant groups (4.4% to 7.5%) from 2019 to 2021. Comparing only timepoint 1 (2019) to timepoint 4 (end of 2021), both reporting automated RPR, overall reactivity rates increased from 8.2% to 14.1% in the screening cohort and 4.8% to 7.8% in the pregnant cohort. No significant differences were observed in manual RPR reactivity (5.2% vs. 6.3%) or false positive rates (1.3% vs. 1.2%) pre- and post-vaccination in the screening or pregnant population (reactivity rates: 0.68% vs. 0.29%; false positives: 0.27% vs. 0.15%). Over 90% of RPR false positives had titers less than 1:4. False positive RPRs decreased from 8.6% to 2.9% following implementation of RPR sendouts.

Conclusions

RPR false reactivity rates increased with SARS-CoV-2 vaccination but is specific to the BioPlex2200 RPR assay which has improved analytical sensitivity for anti-lipoidal antibodies compared to manual RPR methods, thus may be more susceptible to false positives post-vaccination. Increased RPR reactivity rates were clinically notable likely because the traditional syphilis screening algorithm is utilized at our institution.

Topic Areas

Chemistry

Detecting Exogenous Contamination of Clinical Blood Samples with Crystalloid Solutions: A Data Driven Approach to Derive and Validate Delta Checks

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Abstract

Pre-analytical error may occur when collecting blood samples from intravenous (IV) lines. Blood samples can be inadvertently diluted by exogenous solutions if an insufficient amount of blood is discarded prior to collection. Rules built in the laboratory information system or middleware can be used to alert laboratory staff to specimens that require co-investigation with the clinical team to determine if sample dilution is possible or likely. Currently, however, there is a lack of literature to guide laboratorians on how to define quality rules of this nature, particularly for detecting subtle, rather than gross contamination by commonly administered IV-fluids. Accordingly, the primary objective of this study is to derive sensitive and specific, multivariate delta checks to detect more subtle blood sample contamination by commonly used crystalloid solutions. To derive the rules, we began with *in vitro* experiments by spiking increasing volumes of major IV-fluids (normal saline (NS), lactated ringers (LR), and 5% dextrose (D5W)) into clinical blood samples that were collected from healthy donors (n=3). Crystalloid solutions were serially spiked into blood samples at 10% (solution:sample) increments for NS and LR from 10% up to 90%, and in 5% increments for D5W, up to 50%. Basic metabolic panel (BMP) analytes were measured and compared between neat and contrived samples. All testing was performed on Roche Cobas 8000 chemistry auto-analyzers.

Based on *in vitro* data, we derived multivariate delta checks using analytes in a BMP that would reflect 10-40% contamination by a given fluid. We then performed a retrospective data analysis on more than 28 thousand, serially collected BMP results to identify samples that were flagged by these rules. Chart review was then performed to identify EHR-based evidence of temporally associated administration of crystalloid solutions through a peripheral IV access line. Increasing sample dilution from the *in vitro* study showed significant changes in several BMP analytes across all fluid types. With respect to NS, the most significant changes relative to baseline were observed with potassium, calcium, and chloride, while for LR, the most significant changes relative to baseline were observed with glucose, bicarbonate, and calcium. For D5W, significant changes were observed with glucose, calcium, chloride and sodium. Accordingly, delta check rules were implemented using relative changes of the aforementioned analytes. On chart review, we found 80-100% of flagged samples came from patients with administration of that given fluid in the last 12-24 hours. The results from this study support delta checks that consider multiple analytes, and fluid-specific bias profiles, as this may provide a more sensitive and specific approach to detecting contamination of clinical blood samples by crystalloid solutions. Further evaluation using retrospective data analysis will be used to validate these rules sensitivity and specificity followed by prospective testing before implementation.

Topic Areas

Chemistry

Machine Learning Models Accurately Predict PTHrP Results But Fail To Predict Physician Behavior

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Abstract

Quantification of circulating parathyroid hormone-related peptide (PTHrP) aids in the diagnosis of humoral hypercalcemia of malignancy. However, this test is often ordered in settings of low pre-test probability or mistakenly ordered when parathyroid hormone (PTH) was desired. To improve utilization, all PTHrP orders at our institutions are reviewed by a laboratory medicine resident. If the order appears to be inappropriate, the ordering physician is contacted to ask for permission to cancel. In this work, we attempted to automate this labor-intensive review process by developing machine learning models to predict the orders the physician is willing to cancel. We collected 2171 PTHrP orders that were subjected to manual review over the past 10 years. We removed any repeats, leaving 1649 first-time orders. For each order, we assigned a class label of 'canceled' or 'completed' based upon the notes in the resident's documentation logs. For each order, we aggregated all data for the patient existing within the laboratory information system at the time of the first order (n = 40 million). Various strategies were applied to impute missing data, including leaving missingness as a feature, fill by medians, k-nearest neighbors, bagged trees, and linear regression. Class imbalances were adjusted using synthetic minority upsampling technique (SMOTE) and adaptive synthetic upsampling (ADASYN) for a final ratio of one:one. The dataset was partitioned into a 70:30 split between training and testing sets with five-fold cross-validation. Several machine learning algorithms were trained, including logistic regression, naive Bayes, random forest, and XGBoost. After training and cross-validation, the models were applied to the held-out test set, and

performance was evaluated using the area under the receiver operating characteristic curve (AUC). XGBoost was the best performer at predicting the provider's likelihood to cancel the test, but with an AUC of only 0.63. Surprised by this poor performance, we devised a second classification task of predicting the PTHrP results (normal vs abnormal, threshold = 4.2 pmol/L) for the subset of orders that were completed (n=1371). Using the same machine learning pipeline described above, we again observed that XGBoost was the best performer, but this time with an AUC of 0.89. The striking performance difference between the models trained on the two different targets suggests that the physician's willingness to assent to our intervention may be unrelated to existing laboratory data or underlying biology. Likely explanations may include that our intervention is reaching a provider who is not the primary medical decision-maker or the correct provider at the wrong time when they are too busy to revisit details of prior work. In either case, a reflex order set that defines specific criteria for performing PHTrP upfront may be a more effective way to improve utilization.

Topic Areas

Chemistry

ACLPS 2022

Young Investigator Oral Presentations
Hematology and Coagulation
Abstracts

Development of a 23-color flow cytometry panel for the characterization of myelomonocytic differentiation with applications for MRD testing.

Authors

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Abstract

It is well agreed that measurable residual disease (MRD) testing in acute leukemia patients following induction chemotherapy has profound prognostic implications. Flow cytometry is well suited to be the mainstay for MRD testing due to the heterogenous immunophenotypic and molecular natures of leukemia. Current flow cytometry MRD testing involves examination of more than 15 antigens, requiring multiple tubes to be run for each patient as current clinical flow cytometers are limited to examining 12 parameters per tube. Because multiple tubes are required, hematopathologists must infer antigen expression between tubes, which can be difficult. As MRD testing has such prognostic implications and defines treatment stratifications and outcomes, MRD testing requires very high sensitivity. While several groups have improved sensitivity by increasing the number of cells analyzed, we propose that increasing the antigenic targets in a single immunophenotyping assay will improve sensitivity by enhancing our ability to differentiate normal from abnormal hematopoietic cells. To test this, we utilized spectral flow cytometry, which allows for targeting more parameters with the same number of lasers for a single instrument. We developed a 23-color flow cytometry panel which was designed to thoroughly examine myelomonocytic differentiation

and included multiple other antigenic targets which are known to be aberrantly expressed on leukemia cells. The panel also included a live-dead stain to exclude dead cells from analysis. All data was collected with a 3-laser Cytek Northern Lights spectral flow cytometer, which can analyze 10 million events in ~10 minutes. We tested this panel on more than 50 bone marrow aspirate samples, including normal samples, multiple myeloid leukemias, myelodysplastic samples, as well as more than 20 aspirate samples which also had MRD testing completed at a reference laboratory. Our results for MRD testing had excellent concordance with results from the gold-standard reference laboratory method. As our method only required one tube to be run per sample, we were routinely able to analyze at least 10 million cells per sample. Using in silico modelling from our data, we determined the sensitivity of our assay to be an average of 0.005% (range 0.002 – 0.015%) depending on the specific immunophenotype, when analyzing 10 million cells. As we examined all marker expression in a single tube, we were able to model sensitivity as if only 10-parameters were collected per tube and inference was required between tubes for analysis as is the current standard practice. We found that our 23-color assay was significantly more sensitive ($p = 0.02$) compared to running multiple 10-color tubes and inferring between samples. Overall, higher parameter flow cytometric assays (>20 markers) allow for a more sensitive and robust MRD analysis than current gold-standard methods and should be explored for utilization in the clinical setting.

Topic Areas

Hematology and Coagulation

An Automated Pipeline for Cell Differentials on Whole-Slide Bone Marrow Aspirate Smears

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Abstract

Current pathologic diagnosis of benign and neoplastic bone marrow disorders relies in part on the microscopic analysis of bone marrow aspirate (BMA) smears and manual counting of nucleated cell populations to obtain a cell differential. This manual process has significant limitations, including the limited sample of cells analyzed by a conventional 500-cell differential compared to the thousands of nucleated cells present, as well as the inter-observer variability seen between differentials on single samples due to differences in cell selection and classification. To address these shortcomings, we developed an automated computational platform for obtaining cell differentials from scanned whole-slide BMAs at 40x magnification. This pipeline utilizes a sequential process of identifying BMA regions with high proportions of marrow nucleated cells that are ideal for cell counting, detecting individual cells within these optimal regions, and classifying cells into one of 11 types within the differential. Training of convolutional neural network models for region and cell classification, as well as a region-based convolutional neural network for cell detection, involved the generation of an annotated training data set containing 10,948 BMA regions, 28,914 cell boundaries, and 23,609 cell classifications from 73 BMA slides. Among 44 testing BMA slides, an average of 19,209 viable cells per slide were identified and used in automated cell differentials, with a range of 237 to 126,483 cells. In comparing these automated cell differential percentages with corresponding manual differentials, cell type-specific correlation coefficients ranged from 0.913 for blast cells to 0.365 for myelocytes, with an average coefficient of 0.654 among all 11 cell types. A statistically significant concordance was observed among slides with blast percentages less or greater than 20% ($p=1.0x10^{-5}$) and with plasma cell percentages less or greater than 10% ($p=5.9x10^{-6}$) between automated and manual differentials, suggesting potential diagnostic utility of this automated pipeline for malignancies such as acute myeloid leukemia and multiple myeloma. Additionally, by simulating the manual counting of 500 cells within localized areas of a BMA slide and iterating over all optimal slide locations, we quantified the inter-observer variability associated with limited

sample size in traditional BMA cell counting. Localized differentials exemplify an average variance ranging from 24.1% for erythroid precursors to 1.8% for basophils. Variance in localized differentials of up to 44.8% for blast cells and 36.9% for plasma cells was observed, demonstrating that sample classification based on diagnostic thresholds of cell populations is variable even between different areas within a single slide. Finally, pipeline outputs of region classification, cell detection, cell classification, and localized cell differentials can be visualized using whole-slide image analysis software. By improving cell sampling and reducing inter-observer variability, this automated pipeline has potential to improve the current standard of practice for utilizing BMA smears in the diagnosis of hematologic disorders.

Topic Areas

Hematology and Coagulation

Incidence of Heparin Induced Thrombocytopenia (HIT) in patients with severe COVID-19

Authors

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Abstract

Introduction: To prevent and treat thrombotic complications in patients hospitalized with severe COVID-19 infection, anticoagulation treatments primarily with heparin and low molecular weight heparin have been recommended. Heparin-induced thrombocytopenia (HIT) is a rare but conceivably fatal reaction to heparin that is characterized by a sudden drop in platelet count accompanied by new onset of thrombosis 4-10 days after heparin exposure. The purpose of this retrospective study was to investigate the prevalence of thrombocytopenia and HIT in hospitalized COVID-19 patients, as well as their association with mortality.

Methods: 3,672 plasma samples were collected from patients admitted to the first wave of COVID-19 in our institution at New York City (March to May 2020). All patients admitted with a platelet count of less than 150 k/ul were assigned to the thrombocytopenic group. In addition, two groups with similar demographics and normal platelet counts were randomly selected based on discharge outcome: alive vs. deceased (n= 88 per group). PF4 IgG Elisa and heparin neutralization were carried out in accordance with the manufacturer's instructions. A positive HIT result required an optical density (OD) greater than 0.4 and heparin neutralization greater than 50%. Statistical analysis was done in R studio (V.1.4.1717) to analyze demographics (age, gender, ethnicity), initial laboratory data, anticoagulation on admission, and thrombosis.

Results: Only 86 of the 3,672 (2.3%) patients admitted had thrombocytopenia. Only 1 of the 86 patients tested positive for HIT (1.1%). 4 cases of the non-survivors (4.5%) tested positive for HIT compared to none of the survivors in the two groups with normal platelet counts. One of these 4 cases had a history of thrombosis (DVT). Interestingly, the PF4 Elisa ODs in non-survivors were significantly higher than in survivors (0.09 vs. 0.06, p-value< 0.001). Although the platelet count did not differ significantly between the two groups, the mean platelet volume (MPV) on admission and its maximum peak during hospitalization were significantly higher in non-survivors than in survivors.

Conclusions: We only found HIT positive cases among non-survivors, implying that HIT is associated with COVID severity. The incidence of HIT in severe COVID-19 patients appears to be higher than the pre-COVID-19 historical rates of HIT in hospitalized patients (<1%). Although thrombocytopenia is relatively uncommon in COVID-19 patients, the MPV was significantly higher in non-survivors, suggesting that platelet activation and destruction may explain the higher rate of HIT in COVID-19.

Topic Areas

Hematology and Coagulation

A case report of type 2M Von Willebrand disease with unique mutations

Authors

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Abstract

Von Willebrand disease (vWD) is the most common inherited bleeding disorder. It results from quantitative or qualitative defects of the von Willebrand factor (vWF) protein, and is divided into three types, namely type 1, type 2(2A, 2B, 2M, and 2N), and type 3 vWD. Here we report a case of 55-year-old female who presented with recurrent gastrointestinal bleeding due to angiodysplasia. Her past medical history is significant for vWD (type unknown) with hemoptysis, epistaxis and menorrhagia requiring Humate-P replacement multiple times. She has one sister with mild vWD of unknown type and 4 other siblings with no bleeding history. Coagulation testing shows normal PT and aPTT. Von Willebrand panel shows vWF antigen of 33%, VWF ristocetin cofactor activity of 15%, and Factor VIII of 87%. The ratio (VWF: RCo/VWF: Ag) is low (0.45) and the multimer pattern is normal. This is consistent with type 2M vWD. VWD gene sequencing shows heterozygous variants of c.4241T>G (p.Val1414Gly) and c.5227G>A (p.Val1743Met). Of note, one variant (c.4241T>G/p.Val1414Gly) has been reported in two individuals with type 2A but not in type 2M vWD. This may truly represent different types of vWD caused by same mutation or could be due to variation in testing and/or result interpretation among laboratories. The other variant c.5227G>A(p.Val1743Met) in A3 domain has never been reported with uncertain significance. Further investigation including vWF panel testing and genetic analysis in family members would help characterize those mutations.

Topic Areas

Hematology and Coagulation

ACLPS 2022

Young Investigator Oral Presentations

Informatics Abstracts

More Than Just Heart Disease: Predicting the Presence of Lipoprotein X using Lipid Panel Results

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Abstract

Cholestatic liver diseases, such as primary sclerosing cholangitis and primary biliary cirrhosis, can lead to serum accumulation of lipoprotein X (LpX). LpX is a multilamellar particle high in cholesterol but lacking structural apolipoproteins A1 or B. The absence of ApoB results in no negative feedback on cholesterol biosynthesis and prevents LpX clearance from the liver. While clinical signs and symptoms typically precede laboratory findings, it is possible that in medically complex patients the identification of LpX could be the first observation of cholestatic liver disease. Traditional laboratory methods are insufficient to identify LpX as it is of similar density to low-density lipoprotein (LDL). LpX contains a high concentration of cholesterol which is erroneously reported as LDL-C by routine clinical methods. As LpX is a rare complication of liver disease, clinicians may

presume the elevation is a coincidental familial hypercholesterolemia rather than a sequela of liver disease. Currently, lipoprotein gel electrophoresis is the only laboratory method to identify LpX. In this method only performed in specialty lipid laboratories, LpX is readily identified by its unique reverse electrophoretic mobility relative to other lipoproteins. The *objective of this study* was to characterize lipid panels from LpX-positive samples and develop a suitable mechanism to identify LpX-containing samples with good clinical validity. From 21,377 clinical electrophoresis results reported between Nov 2011 to Nov 2021, LpX was identified in 157 serum samples. Overall, patients with LpX were younger (median 44y vs. 55y, $p<0.0001$) with significantly higher total cholesterol (812mg/dL vs 190mg/dL, $p<0.0001$) and lower high density lipoprotein-cholesterol (HDL-C; 3mg/dL vs 45mg/dL, $p<0.0001$). Data were randomly split (70/30) into training (n=14,964) and testing (n=6,413) cohorts. Receiver operator characteristic curve analysis identified optimal thresholds of 22.5 mg/dL HDL-C (AUC = 0.94) and 378.5 mg/dL total cholesterol (AUC = 0.91), as well as a nonHDL-C/HDL-C ratio of 9.2 (AUC = 0.995). Applying these cutoffs to the testing cohort achieved a sensitivity/specificity of 98%/81% for HDL-C, 96%/87% for total cholesterol, and 98%/94% for nonHDL-C/HDL-C ratio. A multivariate model combining these three parameters showed a sensitivity/specificity of 97%/85%, respectively. In conclusion, low HDL-C, elevated total cholesterol and a ratio of nonHDL-C/HDL-C >9.2 are associated with the presence of LpX. The ratio of nonHDL-C/HDL-C is the most sensitive and specific predictor of LpX. If confirmed in other cohorts, laboratories could include a reporting comment on lipid panels with a nonHDL-C/HDL-C ratio >9.2 cautioning a high suspicion for the presence of LpX and recommending confirmatory testing. It may also be prudent to caution that LDL-C results may not be accurate. *In conclusion*, identifying patients with high suspicion of LpX based on abnormal lipid panel results may aide in clinical diagnosis, even when an assay to detect LpX is not readily available.

Topic Areas

Informatics

Automated Gating and Interpretation of Clinical Flow Cytometry Data: A Computational Approach using Artificial Intelligence and Deep Learning

Authors

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Abstract

Flow cytometry (FCM) allows pathologists to accurately immunophenotype hematopoietic cells by detecting the expression of surface proteins with fluorochrome-conjugated antibodies. Accordingly, FCM plays a pivotal role in the diagnostic workup of many hematologic malignancies. However, post-analytic processing and the interpretation of FCM data are primarily manual processes that impact the consistency and limit the throughput of the method. The post-analytic processing workflow is colloquially referred to as ‘gating’ and involves the identification and characterization of immune cell populations by hand through successions of biaxial plots. The gated FCM data is then used by the pathologist to make a clinical interpretation and to then determine potential diagnoses from the patterns and cell frequencies seen in bivariate plots of the data. Since gating and interpretation are manually performed, post-analytic analyses of FCM data are not only laborious from a workflow perspective but remain subjective and prone to variability based on the experience and skill of both the medical laboratory scientist and pathologist. **The objective of this study was to develop a computational pipeline that leverages machine learning-based (ML) solutions to automate gating and clinical interpretation of FCM data to increase the throughput and improve the repeatability of FCM analysis.** Raw FCS files from clinical samples being evaluated for the presence of T-cell lymphoproliferative disease were exported from the on-instrument database. Automated gating was performed

using open-source, supervised-ML packages for flow cytometry data (flowCore and flowDensity; R). These packages implement all processing steps that would typically be done manually (e.g., applying compensation, quality control (QC), and gating). Plots of interest were then generated from the gated data and classified as normal or abnormal using the clinical interpretations that were applied during the normal clinical workflow. These binary labels were used to train an ML-based classifier (VGG-19; Python). To evaluate pipeline performance, we collected FCS files from 1,188 samples that were analyzed by our flow cytometry lab. The automated gating pipeline was used to gate for CD4+CD3+ cells and to create bivariate plots for CD7/CD26 expression. 1150 (96.8%) passed QC and were all gated correctly by visual inspection. Of the 38 (3.2%) samples that failed QC, 13 (1.1%) were lymphopenic and were gated correctly by visual inspection, and the remaining 25 (2.1%) were gated manually. Using the CD7/CD26 plots, the classifier demonstrated promising predictive performance and achieved a precision of 0.85 and recall of 0.83 (weighted average across classes). Our findings represent a novel effort to automate both the gating and interpretation of FCM data using artificial intelligence. These results suggest that ML-based tools have potential utility in aiding the processing and interpretation of FCM data and can augment the efficiency and consistency of workflows in the clinical flow cytometry laboratory.

Topic Areas

Informatics

Effect of an Automated Hemoglobin A1c Reflex on Socioeconomic Disparities in Testing Frequency and Glycemic Control

Authors

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Abstract

The American Diabetes Association recommends semiannual hemoglobin A1c (A1c) testing for diabetic patients with stable glycemic control and quarterly testing for patients with a change in therapy or who do not meet A1c goals. To aid in achieving this goal our laboratory performs an automated, add-on A1c test for any diabetic and hospitalized patient with no A1c ordered in the previous 60 days. The primary aim of this study was to determine the impact of an automated reflex on A1c testing frequency and glycemic control. Our second aim was to determine socioeconomic differences of reflexed A1c testing. All A1c results performed by the BJH laboratory from 5/1/2016-1/22/22 and associated patient demographics were retrieved from the laboratory information system. Patient addresses were geocoded, and a CDC-validated social vulnerability index (SVI) was assigned to each patient based on their census tract of residence. Patients were then stratified in the bottom SVI half (most vulnerable) and the upper SVI half (least vulnerable). A total of 32,861 A1c's were performed on 17,699 patients, 6,341 of whom had >1 test. 2,952 (46.6%) patients had at least one reflexed A1c (add-on group). The median average time between A1c tests was 144.4 days in the add-on group and 209.0 days in the no add-on group ($p<0.001$). The average time between A1c tests for patients in the add-on group in the bottom SVI half was 143.8 days vs. 145.3 days in the top SVI half ($p=0.84$). In contrast, the median average time between A1c tests for patients in the no add-on group in the bottom SVI half was 215.5 days and was 199.5 days in the top SVI half ($p<0.05$). The number of patients with A1c in the uncontrolled range (A1c ≥9%) decreased by 11.5% in the add-on group compared to

1.7% in the no add-on group ($p<0.001$). In those with add-on testing, the proportion of patients in the bottom SVI with initial A1c ≥9% and a longitudinal decrease in A1c was 11.3% compared to 11.9% in the top SVI half ($p=0.39$). In the no add-ons group, the proportion of patients with A1c ≥9% increased by 3.3% in the bottom SVI and decreased by 12.1% in the top SVI ($p=0.01$). A socioeconomic disparity is observed in A1c testing frequency and rates of uncontrolled diabetes over time for patients without add-on testing, but not for patients with add-on testing. Automatic add-on testing for hospitalized diabetic patients may reduce socioeconomic disparities in A1c testing frequency and glycemic control.

Topic Areas

Informatics

Rapid Analysis and Integration of Patient Clinical Data with SARS-CoV-2 Genome Sequence Data

Authors

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Abstract

Objectives. The COVID-19 pandemic has evolved the importance of monitoring viral evolution and correlated clinical outcomes in directing patient care and health policy. Our institution sequences the viral genome from essentially all SARS-CoV-2 infected patients in our hospital system's diverse patient population, allowing for continuous monitoring of the virus composition in a major US metroplex. Our objective was to support the vision of near real-time assessment using bioinformatics and data management methods designed to enable rapid and accurate analyses. **Methods.** Our bioinformatics pipeline is designed to analyze genomic data with paired patient data as rapidly as possible. To always have the most up-to-date patient information available, all co-morbidity, therapeutic and outcomes data are extracted from the Electronic Medical Record (EMR) system every night. Essentially all positive SARS-CoV-2 samples in our healthcare system are sequenced with an Illumina NovaSeq 6000 instrument in batches of 768 genomes. Immediately after the batched sequencing data is complete, the pipeline assembles the genomes using a Singularity container provided by the Bacterial and Viral Bioinformatics Resource Center (BV-BRC). Lineage calls are made using the latest available version of Pangolin. The genomic data is merged with the patient data, and 36 different statistical analyses (such as age, ethnicity, co-morbidity,

admission rate, and mortality rate comparisons) are performed in 11 different patient cohorts (such as all Omicron vs Delta, vaccinated vs non-vaccinated, and monoclonal antibody recipients only). 30 interactive Tableau figures are automatically refreshed with the latest data, and summary reports are emailed to key stakeholders. The pipeline is orchestrated using the Snakemake workflow management system on an on-premise high-performance compute cluster (HPC). **Results.** The bioinformatics analysis for a batched run containing 768 genomes, including genome assembly, all patient cohort comparisons and dashboard updates, completes in four hours. **Conclusion.** Throughout the COVID-19 pandemic, but especially during the early weeks of each surge, our hospital leaders have been extremely interested in the analysis for each sequencing run. The use of a modular, container-based architecture orchestrated using a workflow manager on an on-premise HPC enables us to deliver results very quickly. The performance of this design allowed for dynamic decision making of our organization as new strains emerged in the Houston area.

Topic Areas

Informatics

Development of a generalizable UMAP-based approach for comparing clinical flow cytometry data with application to NPM1-mutated AML cohorts

Authors

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Abstract

Intro: AML with mutated NPM1 is associated with heterogeneous clinicopathologic features. We sought to study the association between phenotype, genetics, and clinical behavior using treatment-naïve bone marrow samples of NPM1-mutated AML. Prior phenotypic studies using flow cytometry data have primarily focused on the blast population in isolation or used less comprehensive analysis techniques, such as simple visual histogram assessment. We applied a dimensionality-reduction algorithm (UMAP) *t*o analyze retrospective clinical flow cytometry data of tumor and non-tumor cells in an initial small cohort of NPM1-mutated samples.

Methods: Custom software was developed using python, FlowKit, and umap-learn to create a dictionary of the various antibody panels, detect the antibody panels that were used in each raw data (FCS) file, and determine the flow cytometer channels that should be disregarded. Subsamples from each FCS file for a given antibody panel were combined and analyzed using UMAP to create an embedding that could then be applied to all FCS files of the given antibody panel. FCS files were subsequently prepared for analysis in FlowJo, including using UMAP coordinates. The initial pilot phase included analysis of the EuroFlow AML1 panel of 11 cases, which included 3 primary refractory cases, 3 early relapsed cases, and 5 cases which achieved clinical remission without relapse. FlowJo was used to gate and

examine the clusters identified by UMAP with respect to phenotypic parameters. These same UMAP gates were applied to all 11 cases for direct comparison.

Results: The blast count ranged from 50 to 88 in these cases. The blast phenotype was determined to be myeloid (n=3), monocytic (n=4), or other (CD34-/HLA-DR-)(n=4). Although standard CD45 by side scatter gating delineates four major cell types (lymphocytes, monocytes, granulocytes, blasts), gating using the UMAP algorithm with input data from eight phenotypic markers in conjunction with scattering parameters, produced at least 10 distinct UMAP clusters of variable cellular composition. Interestingly, applying those gates to side scatter (SSC-A) by CD45 histoplots revealed 5 distinct gates falling into the traditional “blast” region of the histoplot. The UMAP gates thus identified provided quantitative values for further statistical analysis.

Conclusion: We have developed a useful tool to automatically identify the antibody panels used to generate prior flow cytometry data, preprocess the data, and apply the UMAP algorithm for creating embeddings that can be applied to additional cases. Our preliminary analyses revealed significant phenotypic heterogeneity among a small cohort of NPM1-mutated cases. Ongoing work includes expansion of the cohort and number of antibody panels incorporated into the analyses to elucidate prognostic and predictive features of tumor and non-tumor populations in treatment naïve samples of NPM1-mutated AML.

Topic Areas

Informatics

ACLPS 2022

Young Investigator Oral Presentations
Laboratory Management Abstracts

Keep calm and invert on: Reducing blood recollection rates in the neonatal intensive care unit (NICU)

Authors

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Abstract

Background: Minimizing blood specimen recollection due to specimen integrity issues is a continuous quality improvement goal in the neonatal intensive care unit (NICU). Specimen integrity issues, such as clotting or hemolysis, are often the result of improper specimen collection processes and lead to blood loss requiring transfusion, additional painful procedures, and delays in patient care. Data collected from Mayo Clinic Hospital - Methodist Campus NICU at Mayo Clinic in Rochester, MN between January – July 2019 showed that clotted complete blood count (CBC) specimens accounted for one-third of rejected neonatal specimens followed by hemolyzed specimens for direct bilirubin (DBIL) testing. The aim of this project was to reduce the percent of CBC and DBIL tests canceled/specimens rejected due to specimen integrity issues in the NICU from 17% to 10% using the Plan-Do-Study-Act methodology.

Methods: A multidisciplinary quality improvement (QI) team was formed, inclusive of both NICU and laboratory representatives. Bedside direct observations (n=28) were performed by NICU nurses to help identify inconsistencies in the blood collection processes. A total of three Plan-Do-Study-Act (PDSA) cycles were conducted during the 2020-2021 time period. An assessment card was created and

revised with each PDSA cycle. The card was completed by the nurse(s) present during the blood collection and initially identified the lab tests ordered, collection tubes, collection type (venipuncture, venous, capillary), number of tube inversions required, staff present during collection, any delays during collection, and whether the draw was deemed successful. Additionally, staff education videos for nursing and laboratory phlebotomy staff were created to demonstrate correct collection tube inversion technique.

Results: Direct observation identified inconsistent practices in tube inversion during specimen collection. The first PDSA cycle (n=207 collections, April 1 – July 13, 2021) introduced a verbal inversion count and utilization of the assessment card. The following cycle (n=336 collections, July 14 – October 10, 2021) implemented an educational video and modified the assessment card to include start and end time of the draw. The third cycle (n=243 collections, October 11 – November 30, 2021) used a simplified assessment card including patient label, tests ordered, tube type and inversions. The combined rejection rates for CBC and DBIL tests were 4.3%, 3.8% and 2.8% for the 1st, 2nd and 3rd PDSA cycles, respectively.

Conclusions: Our QI initiative reduced the specimen rejection rate for clotted CBC and hemolyzed DBIL specimens from 17% to 2.8% in the NICU. Interventions focused on improving tube inversion techniques and promoting collaborative relationships between phlebotomists and nursing staff. The use of educational videos and completion of assessment cards during collections were effective at reducing specimen rejection rates.

Topic Areas

Laboratory Management

ACLPS 2022

Young Investigator Oral Presentations

Microbiology Abstracts

In vitro activity of plazomicin and conventional aminoglycosides against genetically characterized carbapenem-resistant Enterobacterales bloodstream isolates

Authors

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Abstract

Carbapenem-resistant Enterobacterales (CRE) are a major public health threat. Although aminoglycosides have often been used to treat CRE infections, CRE frequently have aminoglycoside modifying enzymes (AMEs) that confer resistance to conventional aminoglycosides. Plazomicin (PLZ) is a novel aminoglycoside that retains activity against the vast majority of AME-containing isolates but does not have activity against isolates with 16S ribosomal methyltransferases (RMTases).

More studies characterizing the *in vitro* activity of PLZ and conventional aminoglycosides against CRE isolates from the United States (US) and the prevalence of RMTases among these isolates are necessary. We compared the *in vitro* activity of PLZ to amikacin (AMK), gentamicin (GEN), and tobramycin (TOB) against 141 CRE bloodstream isolates from eight New York and New Jersey medical centers collected between 2016-2018. Antimicrobial susceptibility testing was performed by reference microbroth dilution. Clinical and Laboratory Standards Institute breakpoints were applied for conventional aminoglycosides and US Food and Drug Administration breakpoints (susceptible: ≤2 μg/mL; intermediate: 4 μg/mL; resistant: ≥8 μg/mL) were applied for PLZ. Isolates underwent whole-genome sequencing (WGS), and antimicrobial resistance determinants were characterized. The most common CRE bloodstream isolates in our dataset were *Klebsiella pneumoniae* (n=92), *Escherichia coli* (n=22), and *Enterobacter cloacae* complex (n=16). Carbapenemases were present in 110 (78%) isolates with KPC (n=92) being the most common. 132 (93.6%) isolates were susceptible to PLZ, two (1.4%) were intermediate, and seven (5.0%) were resistant. In contrast, 79.4%, 64.5%, and 21.3% were susceptible to AMK, GEN, and TOB, respectively. The PLZ minimum inhibitory concentration $(MIC)_{50}$ and MIC_{90} were ≤1 μg/mL and 2 μg/mL, respectively. Of the 110 isolates for which WGS AME data were available, 94 (85.4%) had at least one AME, and the median number of AMEs was 3 (interquartile range: 2-4) with 84 (76.4%) of isolates having multiple AMEs. Isolates with more AMEs tended to be resistant to GEN and TOB, but not to PLZ and AMK. Of the 110 isolates for which WGS RMTase data were available, only nine (8.2%) had at least one RMTase. However, five of the seven PLZ-resistant isolates had an RMTase, and all isolates with RMTases *rmtB*, *rmtF*, or *armA* were PLZ-resistant. PLZ has increased *in vitro* activity against CRE compared to conventional aminoglycosides, and its activity is unaffected by the presence of AMEs. However, PLZ-resistant CRE isolates that harbor RMTases have emerged in the New York/New Jersey area. The RMTases *rmtB*, *rmtF*, or *armA* were only found in isolates resistant to PLZ suggesting their importance in mediating resistance to this novel aminoglycoside. Thus, PLZ susceptibility testing is critical to guide the appropriate use of PLZ for CRE infections.

Topic Areas

Microbiology

Evaluation of the performance and clinical impact of a rapid phenotypic susceptibility testing method directly from positive blood culture at a pediatric hospital

Authors

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Abstract

Bloodstream infection poses a significant medical emergency that necessitates timely administration of appropriate antibiotics. Rapid identification and antimicrobial susceptibility testing (AST) of organisms from positive blood culture can allow for faster time to optimal therapy (TTOT) and reduce the use of broad-spectrum agents. The Accelerate Pheno Blood Culture panel (Pheno) provides rapid phenotypic testing of select Gram-negative organisms directly from positive blood cultures. We evaluated the performance and the clinical impact of Pheno at our pediatric hospital**.** We retrospectively compared two patient cohorts with blood cultures positive for on-panel Gram-negative organisms: 82 cases tested by conventional AST methods, and 80 cases post-implementation of Pheno. Outcomes such as duration of therapy, TTOT, and length of stay (LOS) were calculated. In both cohorts, the most frequently isolated on-panel Gram-negative organism was *Escherichia coli* (71), followed by *Klebsiella* species (35) and *Pseudomonas aeruginosa* (23). We recovered fewer multidrug resistant organisms in our post-intervention cohort (20.7% vs 10%, p = 0.08). Susceptibility testing from Pheno yielded overall 91.5% categorical agreement with a broth microdilution-based reference method with 8.1% minor error, 1.2% major error, and 0.1% very major error rates. 74.2% (56/62) of the minor errors observed overcalled resistance, particularly with ampicillin-sulbactam, piperacillin-tazobactam, and ceftazidime. The median time from blood culture positivity to AST decreased from 20.0 hours to 9.7 hours (p <

0.001), leading to an overall decrease in TTOT from 36.0 hours to 25.0 hours ($p < 0.001$). There was no observed change in LOS or 30-day mortality. Median duration on meropenem decreased from 64.8 hours to 31.6 hours ($p = 0.04$). In instances of antibiotic escalation, time to escalation from initial Gram stain report improved from a range of 18.5 – 60.1 hours ($n = 9$) in the pre-intervention period to 12.0 – 21.0 hours ($n = 4$) in the post-intervention period. Of the four cases prompting escalation in the post-intervention period, two were multi-drug resistant *E. coli* and initial empiric therapy was insufficient in both cases. We conclude Pheno had accurate performance and implementation allowed for faster AST reporting, improved time to optimal therapy, and decreased duration on meropenem in children. Although Pheno did not significantly decrease LOS at our institution, it may have potential for stronger impact with further stewardship support or in settings with higher rates of multidrug resistance.

Topic Areas

Microbiology

Development of a Syphilis-Specific Molecular Assay

Authors

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Abstract

Syphilis is a sexually transmitted disease caused by the spirochete *Treponema pallidum* subspecies *pallidum*. Rates of infection have been rising for 20 years, with nearly 39,000 cases of primary and secondary syphilis reported in the United States in 2019. Early diagnosis is critical for preventing disease progression and transmission but remains challenging. Dark-field microscopy is laborious and unavailable in most clinical laboratories. Serology, the mainstay of diagnosis and monitoring, may be nonreactive in up to 47% of patients with early primary syphilis. Immunofluorescence and immunohistochemistry can be difficult to interpret due to cross-reactivity and nonspecific staining.

Given these limitations, nucleic acid amplification testing (NAAT) via polymerase chain reaction (PCR) is an attractive diagnostic tool. It offers the possibility of high sensitivity and specificity; detection across a wide variety of specimens; and applicability to early primary lesions, extragenital sites, and late-stage syphilis.

However, no FDA-approved *T. pallidum* NAATs exist, and few commercial laboratory-developed tests are available. Due to this lack, clinicians have turned to broad-range bacterial PCR targeting the 16S rRNA gene offered by our molecular microbiology reference laboratory, particularly for detection in formalin-fixed paraffin-embedded tissue. To understand usage patterns, we searched the laboratory information system, revealing 45 specimens (representing 40 unique patients) in which *T. pallidum* was detected by this assay. We identified an additional 4 specimens from 2 patients in whom syphilis was suspected. The results of gold-standard testing were available for 19 cases, yielding a positive percent agreement of ~73% and negative percent agreement of 100%. These findings both highlight the utility of a molecular assay and suggest an organism-specific assay could increase sensitivity.

To build on the promise of PCR-based *T. pallidum* diagnostics, we developed an assay targeting *tprCDFI*, a 400bp region conserved across four paralogous genes and unique to *T. pallidum*. We hypothesized that the multiple copies of *tprCDFI* will increase the assay's analytical sensitivity as compared with the single-copy *tp47* and two-copy 16S rRNA loci. Ten candidate primer pairs were evaluated bioinformatically for species-specificity and five were chosen for empiric testing. Two primer sets successfully detected both synthetic DNA target and gDNA extracted from two clinical isolates and two laboratory strains (Nichols, SS14) of *T. pallidum.* The analytes were spiked into 50ng of human gDNA to simulate patient matrix. Preliminary experiments showed a limit of detection of 40 or fewer genomes per reaction. Studies are under way to determine analytical sensitivity, specificity, and limit of detection and to compare the performance of the *tprCDFI* primers with those for 16S rRNA and *tp47* genes before proceeding to clinical validation in a variety of pluri- and paucicellular specimens.

Topic Areas

Microbiology

Detection of mecA-mediated methicillin resistance and evaluation of antimicrobial susceptibility characteristics of Staphylococcus saprophyticus isolates from geographically diverse locations

Authors

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Abstract

Staphylococcus saprophyticus is a Gram-positive bacteria that is commonly detected in urine cultures. Routine antimicrobial susceptibility testing (AST) for *S. saprophyticus* recovered from urine is not recommended by the Clinical & Laboratory Standards Institute because historically this species has been susceptible to drugs that concentrate in the urine. The objective of our study was to understand trends in antimicrobial resistance within a large cohort of *S. saprophyticus* and assess the use of oxacillin and cefoxitin as surrogate antibiotics for *mecA* prediction. We evaluated the antimicrobial resistance profiles of *S. saprophyticus* isolates (n=279) from 5 medical centers in North America and a globally diverse cohort. We performed antimicrobial susceptibility testing using

reference disk diffusion methodology on all isolates in our cohort against a panel of 12 antimicrobials. PCR was used to evaluate the presence of *mecA* and *mecC* in each isolate, the PBP2a SA Culture Colony Test and Oxoid PBP2´ Latex Agglutination Test to confirm production of PBP2A, and an uninduced cefinase assay to evaluate β-lactamase contribution towards resistance phenotypes. 5% (14/279) of isolates were positive for *mecA* and had cefinase activity, 63% (177/279) of isolates were negative for *mecA* but had cefinase activity, 4% (11/279) of isolates were positive for *mecA* but were cefinase negative, and 28% (77/279) of isolates were *mecA* and cefinase negative. 100% (279/279) of isolates were susceptible to delafloxacin, ciprofloxacin, rifampin, linezolid, and nitrofurantoin. 92% (258/279) were susceptible to doxycycline, 95% (264/279) were susceptible to trimethoprim-sulfamethoxazole, 53% (147/279) were susceptible to erythromycin, and 89% (248/279) were susceptible to clindamycin. 38% (106/279) showed both clindamycin susceptibility and erythromycin resistance with 19.8% (21/106) displaying inducible clindamycin resistance, via the D-test. Our results demonstrate oxacillin has unacceptably low categorical agreement between phenotypic resistance and *mecA* presence, between 16-33% depending on the interpretive criteria used, and thus it is not suitable for surrogate testing. Cefoxitin has promise as a surrogate for prediction of *mecA*-mediated methicillin resistance. Using a proposed *S. saprophyticus* species specific cutoff of 26mm/25mm for susceptible/resistant for cefoxitin, we demonstrate an optimal compromise between sensitivity and specificity of 89% and 88%, respectively, with 12% (3/25) very major errors. Our work supports CLSI guidelines that *S. saprophyticus* is routinely susceptible to antibiotics that concentrate in the urinary tract and highlight difficulties with testing β-lactam resistance.

Topic Areas

Microbiology

In vitro Activity of Eravacycline and Tigecycline Against Bloodstream Carbapenem-Resistant Enterobacterales Isolates

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Dr. Michael Satlin - Division of Infectious Diseases, Department of Medicine, Weill Cornell Medicine, New York

Abstract

Carbapenem-resistant Enterobacterales (CRE) are resistant to many antimicrobials and have become a worldwide public health threat. Treatment options for CRE infections are limited and there is an urgent need for new and effective anti-CRE therapies. Eravacycline (ERV) is a novel tetracycline derivative that has expanded activity against antimicrobial resistant gram-negative bacteria and has improved tolerability and pharmacokinetic parameters compared to tigecycline (TGC).

However, there are limited data comparing the *in vitro* activity of ERV to TGC against CRE bloodstream isolates. Thus, our aim was to compare the *in vitro* performance of ERV and TGC against 141 CRE bloodstream isolates from eight medical centers collected between 2016 and 2018. Antimicrobial susceptibility testing was performed by the reference broth microdilution method. Minimum inhibitory concentration (MIC) values were interpreted as susceptible (≤0.5 µg/mL) and non-susceptible (>0.5 µg/mL) for ERV, and susceptible (≤2 µg/mL), intermediate (4 µg/mL), and resistant (≥8 µg/mL) for TGC based on US Food and Drug Administration (FDA) breakpoints. The most common CRE bloodstream isolates included *Klebsiella pneumoniae* (n=92), *Escherichia coli* (n=22), and *Enterobacter cloacae* complex (n=16), *Klebsiella oxytoca* (n=5), *Serratia marcescens* (n=4), among others. Carbapenemases were present in 110 (78%) isolates and comprised KPC (n=92), OXA-48-like (n=8), NDM (n=6), NMC-A (n=1), SME-4 (n=1), VIM-1 (n=1), and NDM/OXA-48-like (n=1) enzymes. The MIC_{50} and MIC_{90} values for ERV were 1 µg/mL and 2 µg/mL, respectively, and the MIC_{50} and MIC_{90} values for TGC were 1 µg/mL and 4 µg/mL, respectively. ERV and TGC MIC values were identical in 53% of isolates and in 43% of isolates the ERV MIC was one dilution lower than the TGC MIC. Sixty-seven (48%) isolates were susceptible to ERV and 125 (89%) were susceptible to TGC. ERV and TGC showed similar MIC distributions against bloodstream CRE isolates. Nevertheless, a lower percentage of CRE isolates were considered ERV susceptible than TGC susceptible because of the 4-fold difference in the FDA susceptible breakpoints between these agents. In conclusion, these data suggest the breakpoints for these agents could be reevaluated to allow their optimal use.

Topic Areas

Microbiology

Performance of selective and chromogenic SSNoPRO and CT-SMAC agars for the isolation of Salmonella, Shigella spp., and E. coli serogroup O157 from stool cultures

Authors

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Abstract

Stool cultures are an integral part of the clinical microbiology laboratory and challenges laboratories may encounter include supply chain disruptions and manufacturing discontinuations that force changes in media. Choosing the optimal combination of plates is important for patient care and the laboratory workflow and budget. This study evaluated the performance of SS NoPRO and CT-SMAC compared to current media for the detection and isolation of major enteric pathogens including *Salmonella*, *Shigella* spp., and *E. coli* O157. In addition to routine media for bacterial pathogens (sheep blood, MacConkey, Hektoen Enteric (HE), CHROMagar O157), stool specimens were inoculated onto SS NoPRO and CT-SMAC plates. Black and teal colonies from SS NoPRO, suggestive of *Salmonella* and *Shigella* spp., respectively, and colorless colonies on CT-SMAC, suspicious for *E. coli* O157, were identified by MALDI-TOF MS. Results from the SS NoPRO and CT-SMAC agars were compared to routine stool culture results. Confirmed negative stool cultures were spiked with pathogens of interest at various concentrations to further evaluate the differential performance of the plates. Of 3968 routine stool cultures, 41 cases of *Salmonella*, *Shigella* spp., and *E. coli* O157 were identified. *Salmonella* and *Shigella* spp. were correctly identified by SS NoPRO in 31/32 and 2/5 specimens, respectively. Black colonies were observed earlier on SS NoPRO than HE in 6.3% of salmonellosis cases. The false positivity (FP) rate of the SS NoPRO was 2.5% and most commonly associated with *Citrobacter* spp., *Hafnia alvei*, *Morganella morganii*, and *E. coli*. HE had a FP rate of 3.4% and *Proteus* spp. accounted for 17% of false

positives. In comparison, *Proteus* spp. were completely suppressed on the SS NoPRO. Four cases of *E. coli* O157 were successfully identified with the CT-SMAC and confirmed by latex agglutination. The CT-SMAC displayed a FP rate of 7.9% and non-*E. coli* colorless colonies accounted for 61% of false positives. Two *Shigella* spp. isolates not detected on the SS NoPRO were recovered on CT-SMAC. Supply costs were estimated to be reduced by 68% with the use of CT-SMAC compared to CHROMagar O157 despite additional costs from identifying false positives. Stools spiked with *Salmonella* (n=16), *Shigella spp.* (n=45), and *E. coli* O157 (n=51) grew as expected on SS NoPRO or CT-SMAC, and on routine media at all dilutions. SS NoPRO performed comparably to HE and can potentially shorten turnaround-time for salmonellosis cases. For microbiology laboratories with low *E. coli* O157 positivity rates, CT-SMAC can decrease supply costs despite the higher proportion of false positives. Labor rates and identification methods that supplement selective and differential plates are major factors in the cost of stool cultures, but implementation of SS NoPRO and CT-SMAC agars may improve efficiency and decrease costs.

Topic Areas

Microbiology

ACLPS 2022

Young Investigator Oral Presentations
Molecular Diagnostics and Cytogenetics Abstracts

HLA-B evolutionary divergence is associated with poor outcomes after SARS-CoV-2 infection

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Abstract

OBJECTIVES: Human leukocyte antigens (HLA) are highly diverse transmembrane proteins that present viral peptides to T cells and launch pathogen-specific immune responses. We aim to investigate the correlation between HLA evolutionary divergence (HED), a surrogate for the capacity to present different peptides, and the outcomes of SARS-CoV-2 infection in a cohort from the St. Louis Metropolitan area. METHODS: We enrolled adult patients with SARS-CoV-2 infection confirmed by RT-PCR who were hospitalized at two tertiary hospitals in St. Louis between March and July 2020. Genomic DNA was extracted from peripheral blood and genotyped by next-generation sequencing (NGS). HLA alleles were assigned based on key-exon sequences (G group) and limited to the 2-field resolution. HED was calculated by Grantham distance, which considers the difference in composition, polarity, and molecular volume between each pair of amino acids from maternal and paternal HLA. The HED score was obtained for HLA class I (HLA-A, -B, and -C) genotypes using the HLAdivR package in R. Clinical data were collected retrospectively from electronic medical records. A poor outcome was defined as an admission to the intensive care unit (ICU), a need for mechanical ventilation, or death. A favorable outcome was defined as the absence of the above poor outcomes. RESULTS: A total

of 234 patients were enrolled in this study, 96 being females (41%). The median age and BMI were 66 years old and 28.30 kg/m^2, respectively. African Americans comprised 71.4% of the cohort. Only 19 patients (8.1%) presented with no comorbidity; the rest had one or more comorbidities, with cardiovascular diseases being the most common. A total of 137 (58.5%) patients had poor outcomes from SARS-CoV-2 infection, while 97 (41.5%) patients had a favorable outcome. We detected a significant association between higher HLA-B HED and favorable outcomes, with each 1-point increase in HLA-B HED associated with 8% increased probability for the composite endpoint (OR 1.08, 95% CI=1.01-1.16, P = 0.04). The HED scores calculated for HLA-A or HLA-C were not significantly different between patients with favorable or poor outcomes. In a multivariate logistic regression analysis, increased HLA-B HED score, younger age, and no comorbidity were independently associated with favorable outcomes (P = 0.02, P = 0.01, and P = 0.05, respectively). CONCLUSION: Our study shows a significant correlation between lower HLA-B HED scores and poor outcomes after SARS-CoV-2 infection. This finding suggests that maximizing the presentation of diverse SARS-CoV-2 peptides by HLA-B alleles may improve the clearance of SARS-CoV-2. Further studies are warranted to understand the functional and mechanistic implications of this finding.

Topic Areas

Molecular Diagnostics and Cytogenetics

Detection and quantification of EBV transcript in whole blood, plasma and paraffin embedded tissue samples from organ transplant recipients with concern of post-transplant lymphoproliferative disorder

Authors

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Abstract

Epstein-Bar virus (EBV) is a ubiquitous virus that infects up to 90% of adults worldwide. EBV is typically controlled by the immune system and does not cause significant mortality in most healthy patients. However, transplant patients can develop EBV reactivation and viremia due to immunosuppression, leading to the development of post-transplant lymphoproliferative disorder (PTLD), a complication with high mortality. Detection of EBV in whole blood and tissue is essential for the monitoring and diagnosis of EBV+ PTLD. Current protocols rely on a mix of PCR and RNA in situ hybridization (ISH) for diagnosis of EBV+ PTLD. However, to date no EBV DNA quantitative assay has been FDA approved for this purpose and thus, the current assays are all laboratory developed tests (LDT). Furthermore, no WHO EBV DNA standard is available, making the comparisons of EBV DNA quantitative results between laboratories challenging. Here, we develop and validate a droplet digital

PCR (ddPCR) assay for the quantification of EBV viral load in whole blood, plasma and formalin fixed paraffin embedded (FFPE) tissue for the monitoring and adjunct diagnosis of EBV+ PTLD. ddPCR is a novel PCR platform that provides absolute quantification of amplified targets by partitioning and separately analyzing each PCR reaction. The results are independent of a calibration curve and can significantly increase the precision of quantification. ddPCR therefore provides a potentially better testing option for EBV DNA detection in post-transplant patients. Assay validation experiments demonstrated a limit of detection of ~111 copies/ml (positive results in >=95% repeated measurements) and linearity over 4.5 $\log_{10}$. Interestingly, the ddPCR assay was consistently measuring 1 $\log_{10}$ lower EBV DNA concentration than the commercial assay. Measurement of paired whole blood and plasma samples demonstrated reduced EBV viral loads in plasma. However, when EBV DNA was normalized to a housekeeping gene *Abl1*, plasma samples demonstrated higher normalized viral loads. Lastly, we demonstrated ddPCR EBV quantification in FFPE tissues from lymph nodes from healthy controls and diagnostic EBV+ PTLD cases, with healthy controls showing a 2 $\log_{10}$ range of normalized EBV DNA, and PTLD cases demonstrating a 7 $\log_{10}$ range. Of note, some PTLD samples had similar normalized EBV viral loads to patients with healthy controls. Thus, there may be biological differences in disease pathogenesis between tissues with different quantities of EBV DNA present. Ongoing studies include correlating morphologic variants of EBV+ PTLD with normalized EBV DNA quantity and analyzing EBV RNA from FFPE to determine if active viral replication helps to distinguish healthy controls or PTLD morphologic variants. We strongly believe that the improved quantification and detection of EBV by ddPCR may lead to earlier detection of PTLD in organ transplant recipients and translate to better clinical outcomes.

Topic Areas

Molecular Diagnostics and Cytogenetics

ACLPS 2022

Young Investigator Oral Presentations
Student Awards Abstracts

The role of cohabitation on adaptive and innate immune cell profiles in the Health and Retirement Study

Authors

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Abstract

Immune cells distribution is shaped by numerous factors including environmental factors, age, and genetics. Cohabitation has been associated with similar microbiomes, possibly due to dietary patterns and exposure to similar pathogens but has not been studied in the context of adaptive and innate immune systems previously. We used immunophenotyping data of 2283 households with participants living in the same household and compared it to 2283 randomly generated pairs of participants from the Health and Retirement study. The adaptive immune cells (subsets of T-cells and B-cells), and innate immune cells (monocytes, natural killer cells, and neutrophils) were compressed to two coordinates using multidimensional scaling. The Euclidean distances between participants in the same household were compared to the distances between the random pairs of participants using two sample independent t-tests. The mean distances of the immune coordinate points for adaptive immune cells between participants in the same household were lower than the randomly paired participants (p-value < 0.0001) and the variability of intra-household distances was lower than the random pairs (IQR: 7.18 vs 8.99). For the innate immune cells, the mean distances between participants in the same household were slightly lower than the randomly paired participants (p-value = 0.03) but the variability of the intra-household distances was higher than the random pairs (IQR: 4.08 vs 3.65). Variability in the adaptive immune

system among participants living in the same household were substantially lower indicating the influence of shared environmental conditions in determining the adaptive immune profiles.

Topic Areas

None of these topics really captures it

Replacement Rates of Sodium in Severe Hyponatremia: Implications for Laboratory Monitoring and Notification

Authors

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Abstract

Background: Sodium replacement in severe hyponatremia needs to be carefully controlled to prevent development of osmotic demyelination syndrome, which is a potentially devastating neurological complication of a rapid rise in plasma [Na^+]. Clinical guidelines recommend that [Na^+] should not be allowed to rise by more than 12 mmol/L in the first 24 hours of treatment of severe hyponatremia and by 18 mmol/L in the first 48 hours. Methods: We examined medical records from hospitalized patients over 18 years of age with documented severe hyponatremia (defined as [Na^+] <120 mmol/L) who received sodium replacement therapy during their inpatient course. Data were collected in a single academic medical system. All [Na^+] results were recorded over 48 hours following a first observation of severe hyponatremia. A linear mixed-effects model was used to determine the rates of rise in [Na^+]. Outcomes were achievement of within-guideline rates of rise of [Na^+] at 24 and 48 hours. Results: Medical records were retrieved for 1,547 patients identified between 2010 and 2020. The mean (SD) age was 64.7 (16.5) years (range 18 to 98 years), and 58% were female. The mean lowest (SD) [Na^+] was 115.1 (3.9) mmol/L. Sodium replacement rates exceeded 12 mmol/L in 24 hours in 250 patients, exceeded 18 mmol/L in 48 hours in 160 patients, and exceeded both in 127 patients; 1,264 patients did not exceed guideline limits. Among patients who exceeded a rise in [Na^+] of 12 mmol/L in 24 hours, the rate of rise of [Na^+] was 0.81 mmol/L/hr vs. 0.24 mmol/L/hr in those who did not exceed a rise of 12 mmol/L in 24 hrs. Among patients who exceeded a rise in [Na^+] of 18 mmol/L in 48 hours, the rate of rise of [Na^+] was 0.54 mmol/L/hr vs. 0.22 mmol/L/hr in those who did not exceed a rise of 18 mmol/L in 48 hours. Conclusions: During management of severe

hyponatremia, the rate of rise of [Na^+] was 3.4 times greater in patients who exceeded the recommended guideline limit of 12 mmol/L in the first 24 hours than in those who did not exceed the guideline limit. The rate of rise in [Na^+] was 2.5 times greater in patients who exceeded the recommended guideline limit of 18 mmol/L in the first 48 hours than in those who did not exceed the guideline limit. These results suggest that sodium replacement rates should be closely monitored, and appropriate alerts made by the laboratory if results indicate overly rapid correction of hyponatremia is occurring.

Topic Areas

Chemistry

ACLPS 2022

Young Investigator Oral Presentations
Transfusion Medicine Abstracts

Mouse Model for Platelet Aggregation using Flow Cytometry

Authors

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Abstract

Platelets play crucial roles in hemostasis, bleeding, and thrombosis. Within the United States, an estimated 7,000 platelet units are transfused daily. The gold standard to measure platelet survival in humans is to determine post-transfusion recovery after an autologous transfusion of radiolabeled platelet units, however, the ability to detect platelets circulating after transfusion does not provide information on how well these platelets function in hemostasis. Clinically, platelet unit function is routinely measured using aggregometry, which requires large volumes of platelet concentrates and normal platelet counts. While the clinical demand for platelets continues to increase with advances in medical care, there remains a lack of *in vitro* measures to properly assess platelet function in animal models. Here we outline an *in vitro* method for characterizing platelet function using flow cytometry to assess platelet activation and aggregation simultaneously in a mouse model. Using two commercially available transgenic mouse lines, one with platelets expressing red fluorescent protein (RFP) and the other with platelets expressing green fluorescent protein (GFP), whole blood is collected from each transgenic mouse line by aseptic cardiac puncture, and leukocyte-reduced platelet rich plasma (LRPRP) is isolated after two consecutive centrifuge spins and leukocyte reduction. RFP- and GFP-labeled platelets can be visually separated by flow cytometry. Platelets are activated after incubation with 4 mM GPRP peptide followed by exposure to 0.5U/mL high activity bovine thrombin for 2 minutes. Platelets are then stained with anti-CD41a, labelling all platelets, and anti-CD62P, labelling activated platelets; these platelet populations can be analyzed by flow cytometry. When RFP- and GFP-LRPRP are combined prior to activation with thrombin, RFP- and GFP-labeled platelet aggregation can be measured as the number of cells doubly expressing RFP and GFP by flow cytometry. Thrombin

stimulation activated 93.31% of the total number of RFP- and GFP-platelets. Of the activated, CD62P-positive platelets, 11.03% expressed both RFP and GFP, indicating that these platelets were aggregated. Of the inactivated, CD62P-negative platelets, 0.81% expressed both RFP and GFP. These results suggest that thrombin exposure activates platelets and causes platelet aggregation, while <1% of inactivated platelets aggregate. Additionally, platelet aggregation is easily measured by flow without differentially labeling the two separate platelet populations. These data indicate that our model is a viable measure of platelet function *in vitro*. Our model of platelet activation and aggregation provides a baseline measure of *in vitro* platelet function in RFP and GFP mice. This *in vitro* method of assessing platelet function can be applied to various experimental populations and used to quantify the effects of experimental conditions on platelet function in mice. Using this model of platelet function, factors influencing platelet storage can be characterized and ultimately used to improve platelet transfusion therapies.

Topic Areas

Transfusion Medicine

Allocating Blood Products During a Time of Remarkable Shortage

Authors

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Abstract

The COVID-19 Pandemic adversely impacted the nation's blood supply such that blood suppliers could not fill standing orders. To address the dwindling blood supply, the American Red Cross (ARC) implemented daily thresholds, which distributed red blood cells (RBCs) based on clients' historical blood orders and the ARC's inventory. The threshold system left the blood bank to devise a process to judiciously allocate the reduced RBC supply across our institution. Herein, we describe an internal RBC utilization audit, the data from which we used to devise an algorithm to predict our ability to meet transfusion requirements for operating room (OR) procedures. An OR adjudication committee used our report to prioritize elective surgical cases taking places the following day. Thirty-one non-consecutive days of blood inventory release slips were reviewed across three months of recent transfusions. Weekdays were disproportionally investigated to predict the blood utilization for days with scheduled OR procedures. For all RBCs released, the blood type requested and the final disposition (transfused vs returned to blood bank) of the units were recorded. Average use was calculated as well as interquartile range

(IQR) to account for transfusion variability. Utilization data was then used to develop a worksheet-based tool to predict ability to meet RBC requirements. Overall, the hospital transfused an average of 80 RBC units each weekday (IQR: 64-92 units). Approximately 40% of all requested RBC units were released to the OR. In turn, the OR transfused only 39% of the RBCs released to them, which represented 20% of transfusions hospital-wide. The OR requested an average of 41 RBC units each weekday (IQR: 25-57 units). The OR transfused an average of 16 units of RBCs each weekday (IQR: 9-21 units). The outpatient cancer center infusion clinic used less than 15% of total RBCs. The biggest user of RBCs were inpatients, who were transfused 65% of RBCs during the week and 82% of transfusions during the weekend. These percentages and the average-to-third-quartile range were used to devise a blood allocation algorithm to inform the OR if the blood bank could support anticipated use. The data was also used to devise a blood allocation worksheet for the on-call transfusion medicine physicians to predict our ability to provide adequate blood for emergent, non-elective procedures that require transfusion support such as liver transplants and aortic repairs. The audit of RBC disposition informed blood inventory management practices during a time of remarkable shortage. Within the confines of the threshold system, the transfusion medicine service allocated the expected number of units based on historical request and transfusion data to predict whether inventory levels could support scheduled and nonscheduled OR procedures.

Topic Areas

Transfusion Medicine

ACLPS 2022

Young Investigator Oral Presentations

YIA Distinction Abstracts

Impact of Microbiome Diversity and Gut Colonization Density on Risk of Bacteremia with Fluoroquinolone-Resistant Enterobacterales in Colonized Hematopoietic Cell Transplant Recipients

Authors

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Abstract

We previously found that hematopoietic cell transplant (HCT) recipients colonized with fluoroquinolone-resistant Enterobacterales (FQRE) were at high risk of developing Gram-negative bloodstream infection (BSI) while receiving FQ

prophylaxis during neutropenia and the BSI was usually caused by the colonizing FQRE strain. The purpose of this study was to examine how FQRE colonization density and gut microbiome diversity influence the risk of gram-negative BSI in FQRE-colonized patients undergoing HCT. We analyzed frozen stool specimens collected during the week prior to transplant (baseline) and within one week after transplant (post-transplant) from patients undergoing HCT from 2016-2019 who were colonized with FQRE. All samples were collected prior to any BSI. We performed quantitative cultures for FQRE and 16S ribosomal RNA gene sequencing. We then compared the following between patients who did and did not develop FQRE BSI: 1) FQRE colonization density; 2) gut microbiome diversity; 3) relative abundance of Enterobacterales. We analyzed samples from 48 FQRE-colonized patients, of whom 15 (31%) developed FQRE BSI during neutropenia. We found no difference in FQRE colonization density between patients who did and did not develop FQRE BSI in the baseline (median colony-forming units [CFU]/g: $2.8x10^4$ in both FQRE BSI and non-FQRE BSI patients) or post-transplant samples (median CFU/g: $1.1x10^4$ in FQRE BSI patients vs. $1x10^3$ in non-FQRE BSI patients; p=0.5). There was also no difference in microbiome diversity between groups in the baseline (median Shannon diversity index [SDI]: 2.4 in FQRE BSI patients vs. 2.6 in non-FQRE BSI patients; p=0.4) or post-transplant samples (median SDI: 2.2 in both FQRE BSI patients and non-FQRE BSI patients). In contrast, patients who developed FQRE BSI had an increase in the median relative abundance of Enterobacterales from 3.8% (interquartile range [IQR]: 0.9%-18.7%) in their baseline sample to 40.6% (IQR: 8.8%-69.8%) in their post-transplant sample, whereas those who did not develop FQRE BSI had a decrease in the median relative abundance of Enterobacterales from 4.9% (IQR: 0%-22.7%) to 0.2% (IQR: 0%-21.7%; comparison of post-transplant relative abundance in FQRE BSI vs. no FQRE BSI: p=0.035). This study found that FQRE-colonized HCT recipients who develop FQRE BSI during neutropenia have a relative expansion of Enterobacterales preceding their BSI compared to colonized patients who do not develop FQRE BSI, but they do not have an absolute increase in the density of FQRE colonization or significant changes in gut microbial diversity.

Topic Areas

Microbiology

Detection and quantification of Plasmodium spp. and Babesia sp. intraerythrocytic parasites by flow cytometry.

Authors

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Abstract

Background: Clinical diagnosis of *Plasmodium* and *Babesia* infections is achieved by molecular methods or light microscopy, while quantification of parasite burden requires light microscopic (LM) examination. Recently, it has been demonstrated that *Plasmodium*-infected red blood cells can be identified and quantified from venous whole blood with less than one minute of analytic time using an automated flow cytometer (FLC). The objective of this study was to apply this novel FLC method to detect and quantify *Babesia* parasites in venous blood, with results compared to LM and PCR. **Methodology**: An automated hematology analyzer (XN-31; Sysmex, Inc.) was used to detect and quantify *Plasmodium falciparum* or *Babesia microti*-infected RBCs (iRBCs) from residual venous blood samples (n = 345; *Babesia* positive by LM or PCR = 180, *P. falciparum* positive by LM or PCR = 37, negative by LM or PCR = 115). Samples were processed onboard according to manufacturer's protocol and subjected to single cell analysis with light scatter and single-channel fluorescent parameters recorded for each. Cells with nucleic acid (e.g., white blood cells or iRBCs) were detected using a violet (405 nm) semiconductor laser. *P. falciparum* infected samples were analyzed using instrument software. As no instrument software exists for *Babesia*, a machine learning-based algorithm (ML) was developed to facilitate analysis. Samples with abnormal scattergrams (as reported

by instrument software), which can be associated with sample degradation or other analytic confounders, were excluded from downstream analysis (n = 154). **Results**: To validate the ML framework, *P. falciparum* infected samples analyzed by the ML model and instrument software were compared and showed excellent agreement by linear regression analysis (R^2 = 0.995, slope = 1.02). The ML model results correlated well with *P. falciparum* LM results in both qualitative (positive versus negative; AUC = 0.998, Sensitivity = 100%, specificity = 97%) and quantitative (percent parasitemia; R^2 = 0.96, slope = 1.1) comparisons. Next, the model was used to analyze *Babesia* infected samples. Relative to PCR, the FLC method analyzed by the ML model showed an AUC of 0.966, with sensitivity of 95.0% and specificity of 95.0%. When compared to LM, the FLC method displayed an AUC of 0.988 with a sensitivity of 100% and specificity of 96.7%; however, correlation of %iRBCs was poor for *Babesia* (R^2 = 0.569, Slope = 0.551). **Conclusion**: This investigation demonstrates that *Plasmodium* and *Babesia* parasites can be detected and quantified directly from venous blood samples in less than one minute, albeit only in a subset of cases without instrument-defined abnormalities. While promising, opportunities remain to improve the general applicability of the method (include or prevent abnormal scattergrams) and quantitative correlation for *Babesia* samples.

Topic Areas

Microbiology

Cost savings from a machine learning model to identify low prevalence SARS-CoV-2 samples for pooled PCR testing

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Abstract

Community-wide SARS-CoV-2 testing has been crucial to mitigating the spread of COVID-19. Pooling samples is an approach for laboratories to increase their capacity to perform Real-Time Polymerase Chain Reaction testing when analytical capacity is limited. However, pooling is only effective when prevalence rates are low. As the B.1.1.529 (Omicron) variant spread rapidly across the world in late 2021, positivity rates soared, limiting the use of pooling. Since March 2020, our laboratory has conducted one-third of SARS-CoV-2 testing in our state, with the majority of samples collected at open access community testing sites. Through the application of machine learning methods to patient metadata, we developed a high-throughput workflow to identify a subset of samples with low prevalence so that pooling could resume even in the setting of a high overall prevalence. For each sample, age, reason for testing (categorized as symptomatic, exposure, or asymptomatic), vaccination status, testing site, and patient zip code were used as input features for a Balanced Random Forest classifier (Python package imbalanced-learn 0.9.0). Each day, an updated model is trained on 4 days of sample data antecedent to the day before. Sample metadata is uploaded from the patient scheduling software (Solv Health, Oakland, CA, USA), and a prediction is generated based on the model trained overnight. A custom web application provided to specimen processing supports a workflow in which a barcoded specimen identifier is scanned to retrieve the prediction indicating whether a specimen should be pooled. From February 7th

to February 24th 2022, 70,714 community site samples were received by the lab. 19,475 samples were selected for 4:1 pooling. The positivity rate of the pooled samples was 3.6%, compared to the overall positivity rate of 12.0% for all samples received during this period. For pools that result as positive, each sample within the pool was individually retested. Based on this workflow, we estimated that the total reagent cost was $1,174,104. Were we to test each sample individually, the estimated reagent cost would be $1,399,278, resulting in a cost savings of $225,174 over 18 days. On the other hand, were we to naively pool all samples, regardless of the pretest probability, the estimated cost would have been $1,426,698, resulting in a savings of $252,594. A dynamically trained machine learning model was able to substantially reduce the pretest probability of samples selected for pooling. Furthermore, we introduced the model into a high throughput laboratory testing workflow, increasing analytical capacity during a surge in testing demand and reducing the cost of reagents.

Topic Areas

Informatics

Cross-sectional analysis of Cytomegalovirus seropositivity, T cell subsets and Epigenetic age acceleration in the Health and Retirement Study

Authors

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Abstract

Cytomegalovirus (CMV) infection is associated with several age-related chronic diseases. The mechanism behind this association is likely multifactorial but remains poorly defined. Epigenetic age, defined as a DNA methylation (DNAm) pattern related to age and health phenotypes, is increasingly being used as an indicator of biological age, and several different epigenetic clocks have been developed to capture epigenetic age. To date, there has only been one small study that performed a limited evaluation of the effect of CMV infection on epigenetic age. Hence, we used data from 3869 participants who were 56 years or older in the Health and Retirement Study (HRS), who had data on epigenetic age, CMV seroprevalence, and T cell subsets to evaluate the impact of CMV exposure on epigenetic age acceleration (difference between epigenetic age and chronological age). We used survey regression models adjusted for complex survey design parameters and other covariates such as age, sex, race/ethnicity, comorbidity, education, smoking, and % neutrophils. Sixty-three percent (N=2451) of study participants were CMV seropositive. We evaluated 13 (9 first generation and 4 second generation) epigenetic clocks for their association with CMV seropositivity. We found that participants with CMV seropositivity had more rapid epigenetic age acceleration in five (4 first generation and 1 second generation) of 13 clocks than

CMV seronegative participants after adjusting for covariates. The associations between CMV and epigenetic age acceleration were attenuated after adjustment for CD4+ and CD8+ effector T cell subsets. The adjustment for markers of chronic inflammation did not change the association between CMV and epigenetic clocks. Our study findings suggest that CMV seropositivity has a global effect on biological aging that likely extends beyond its effect on the immune system and that this effect is independent of chronic inflammation that frequently accompanies CMV infection. Further studies are needed to understand the mechanisms through which CMV infection affects epigenetic age beyond its effect on the immune system and inflammation.

Topic Areas

Basic science

Optical Microring Resonators for the Rapid Detection of Ebola Virus Disease

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Abstract

Ebola virus (EBOV) is a highly infectious pathogen, with a case mortality rates as high as 90%, and over 20 outbreaks since 1976. The symptoms of EBOV disease (EVD) lack specificity, as many symptoms are shared with other hemorrhagic diseases and endemic tropical diseases. Given the high mortality and significant symptom overlap, there is a critical need for sensitive, rapid diagnostics for EVD. Our study objective was to develop a highly sensitive assay for the detection of EVD.

We created a rapid and highly sensitive diagnostic assay for EVD through the combination of microring resonator sensors with soluble glycoprotein (sGP), a unique biomarker of EBOV infection that can appear before PCR-based methods

are positive. Microring resonator sensors are a class of optical devices in which light is confined to a small volume. This confinement leads to a drastic improvement in analytical sensitivity of the device. The kinetics of sGP production in vivo was tested in rhesus macaques challenged with EBOV and compared using quantitative ELISA and qRT-PCR. Micoring sensors were covalently functionalized with antibodies against EBOV sGP, Sudan Virus (SUDV) sGP, and a mouse immunoglobulin control. Our assay used a sandwich assay with pan-EBOV-SUDV antibodies and an enzymatic amplification step. For sensor readout, we utilized the Maverick Matchbox instrument from Genalyte.

In vivo studies demonstrated the presence of sGP preceded qRT-PCR positivity in infected rhesus macaques. Our assay was able to detect EBOV and SUDV sGP in under 40 minutes with a limit of detection of 1.72 ng/mL and 1.00 ng/mL, respectively. Furthermore, our assay was able to detect EVD from 29 out of 30 infected non-human primate serum samples.

Our results demonstrate the utility of a highly sensitivity diagnostic platform for detection of sGP for diagnosis of EVD.

Topic Areas

Basic science

Microvesicles in Stored Red Blood Cell Units Activate the Kallikrein-Kinin System Leading to Bradykinin Generation.

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Abstract

Background

Hypotension is a common adverse reaction of transfusion with variable pathophysiology. The risk of hypotension is increased when the patient is receiving an angiotensin converting enzyme (ACE)-inhibitor. Bradykinin is a potent vasodilator and may accumulate in the plasma of patients on an ACE-inhibitor. It has been reported that bradykinin can be generated in blood products during storage but may be quickly degraded before transfusion. We previously demonstrated that microvesicles (MVs) in stored red blood cell units (sRBC-MVs) can activate the contact system leading to thrombin generation.

Aim

This study aims at investigating if sRBC-MVs can also activate the kinin-kallikrein system, leading to high molecular weight kininogen (HK) cleavage and bradykinin generation.

Methods

sRBC-MVs were isolated from expired units of RBCs by centrifugation, washed twice, and re-suspended in Hepes-buffered saline in the presence of physiologic plasma concentrations of purified HK and/or prekallikrein (PK). Synthetic lipid vesicles (sLVs) made of 15% phosphatidylserine, 44% phosphatidylcholine and 41% phosphatidylethanolamine were used to assess the specificity of sRBC-MVs. The mix of sRBC-MVs and purified HK and/or PK was incubated at 37ºC for up to 2 hours. Activation of PK to kallikrein (PKa) was assessed using a fluorogenic substrate sensitive to PKa. HK cleavage and bradykinin generation were measured at timed intervals after 0.5, 1- or 2-hours of incubation. Cleavage of HK was assessed by an in-house sandwich ELISA measuring residual intact HK (HKi) and bradykinin was quantified with a commercial ELISA.

Results

A steady concentration of HKi and no bradykinin generation were observed when HK alone was incubated up to 2h with or without sRBC-MVs, indicating no direct

cleavage of HK by sRBC-MVs or spontaneous bradykinin generation. Addition of PK to HK and sRBC-MVs induced a gradual decrease of HKi from baseline (53% at 30min; 88% at 1h; 98% at 2h) and bradykinin accumulation over time (up to 20ng/ml in 2h). However, these effects were also observed at a smaller scale without sRBC-MVs (~10ng/ml at 2h), most likely due to contaminant factor XIIa or PKa in the PK reagent. Using a PK reagent with confirmed purity, kaolin (positive control) or sRBC-MVs induced bradykinin generation in the presence of HK + PK (mean +/-SD at 1h; kaolin: 11.6 +/-3.8ng/ml; RBC-MVs: 9.5 +/-5.4ng/ml), while sLVs did not. In parallel experiments on the same samples, kaolin and sRBC-MVs, but not sLVs induced PK activation to PKa (Mean RFU +/- SD; Kaolin: 10,486 +/-3,245 and RBC-MVs: 8,890 +/-5,402).

Conclusion

sRBC-MVs induced HK cleavage and bradykinin generation *in vitro* by a PK-dependent mechanism. This effect seems to be related to specific components of sRBC-MVs, since it was not reproduced by sLVs. The relevance of this pathway *in vivo* to patients receiving RBC transfusions needs further investigation.

Topic Areas

Basic science