# Utility of detecting fentanyl analogs during LC-MS/MS confirmation for positive fentanyl urine drug screens


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## Abstract

### Introduction:

Fentanyl immunoassays have considerable crossreactvitiy with fentanyl derivatives such as acetylfentanyl, acrylfentanyl, furanyfentanyl and the primary fentanyl metabolite norfentanyl. This poses substantial analytical challenges to clinical laboratories that confirm fentanyl by mass spectrometry and may lead to false negative confirmations in patients that have analogs or primarily metabolites in their urine. As a result, some laboratories have developed targeted liquid chromatography tandem mass spectrometry (LS-MS/MS) methods to detect analogs and/or metabolites. However, there is no data in the literature demonstrating the frequency of analog detection in urine drugs screens, and the potential false positive immunoassay rate as a result of analogs and metabolites that are undetected is not known. Of note, acetyl-, acryl- and furanyl-fentanyl analogs have been confirmed in our testing region, and they cross-react with the Ark Fentanyl I and II immunoassays.

### Methods

Results from 6,192 urine drugs screens positive for fentanyl by immunoassay [ARK I fentanyl assay and ARK II fentanyl immunoassays between January 1, 2020 to December 31, 2021 performed on a Roche cobas c502 chemistry analyzer with confirmatory LC-MS/MS (Waters Xevo TLD) at the Barnes Jewish Hospital Laboratory (St. Louis, MO) were retrospectively extracted from the laboratory information

system (Cerner). The confirmatory assay was clinically validated to detect fentanyl (0.3 ng/mL), norfentanyl (5 ng/mL), acetylfentanyl (1 ng/mL), acrylfentanyl (1 ng/mL), and furanylfentanyl (1 ng/mL). An internal standard mix was added to patient samples followed by vortexing, centrifugation, and separation of the supernatant over a 6 min linear gradient using a 2.1 × 150 mm 1.8 µm C18 column. Data were acquired in positive ion mode and resulted as positive if predefined criteria for relative retention time, product ion ratio, peak shape/resolution, and signal intensity were met.

**Results**

84.8% of the samples (n= 5247) confirmed positive for fentanyl, norfentanyl or a fentanyl analog, whereas 15.2% (n=944) did not confirm by LC-MS/MS. Of the 5247 positive results, fentanyl alone was present in 1,279 (24.4%) specimens while norfentanyl alone was present in 235 (4.48%) specimens. Both fentanyl and norfentanyl were present in 3968 (75.6%) specimens. 239 (4.55%) specimens confirmed positive for acetylfentanyl, all of which (100%) had fentanyl and/or norfentanyl concurrently detected. Among 61 (1.16%) specimens which were positive for acrylfentanyl, fentanyl and/or norfentanyl were concurrently detected in 60 specimens, and 1 had neither fentanyl nor norfentanyl detected. Furanylfentanyl was undetected in all specimens.

**Conclusions:**

The fentanyl analogs acetylfentanyl, acrylfentanyl, and furnaylfentanyl are infrequently detected in our patient population. Furthermore, the assessed analogs are almost exclusively detected in the presence of fentanyl and/or norfentanyl by LC-MS/MS confirmatory assays. This implies that fentanyl analogs may not be a common source of false positive results by fentanyl immunoassays.

## Topic Areas

Chemistry

# Analytical and Clinical Evaluation of the Automated Elecsys IL-6 Assay on the Roche cobas e602 Analyzer.


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## Abstract

**Objective:** This validation study evaluates the automated Roche Elecsys IL-6 electrochemiluminescent immunoassay (ECLIA) that has been granted emergency use authorization (EUA) by the FDA. Since the onset of the SARS-CoV-2, healthcare facilities have been under stress to maximize resources by efficiently distributing them to the patients who need them the most. Interleukin-6 (IL-6) is a proinflammatory cytokine that is associated with many inflammatory diseases, including those induced by acute respiratory infections such as corona virus disease 2019 (COVID-19). Monitoring IL-6 levels in patients with inflammatory illnesses, such as COVID-19, may aid in risk assessment by identifying those patients at greater risk of more severe illness and additional intervention. At present, there are no FDA-approved IL-6 assays available.

**Methods:** The IL-6 ECLIA assay was evaluated for precision, linearity, interference (by hemoglobin, bilirubin, triglycerides, and biotin) and clinical performance was compared to V-PLEX Human IL-6 immunoassay (Meso Scale Discovery).

**Results:** The IL-6 ECLIA assay is precise (intra-assay <3% CV, inter-assay < 5% CV), exhibits linearity across a measurable range of 1.5–4790 pg/mL, and is tolerant of significant interferences (H < 2522, I <62, L<2101, biotin <30 ng/mL). Comparison with V-PLEX Human IL-6 immunoassay revealed a 295% bias in patient samples evaluated for IL-6 concentration (n=43, range=1.5–1891 pg/mL, y=2.95x +32.7, $r^2$ =0.84). Bland-Altman analysis revealed an absolute mean bias of 151 pg/mL (SD =266 pg/mL).

**Conclusion:** The clinical utility of the IL-6 quantitative assay is to evaluate the evolution of inflammatory response over time for risk assessment. The Roche IL-6 assay showed good analytical performance; however, the large systematic bias compared to another reference method precludes using various methods to monitor IL-6 response. The random-access nature of an automated IL-6 assay on the Roche platform makes the test readily available.

## Topic Areas

Chemistry

# Analysis of the Effects of High-Sensitivity Cardiac Troponin Confounding Factors on AMI Diagnosis


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## Abstract

High-sensitivity cardiac troponin (hs-cTn) plays an essential role in facilitating the diagnosis of acute myocardial infarction (AMI). However, there are several known confounding factors affecting hs-cTn concentrations, including sex, age and renal dysfunction. Women have significantly lower hs-cTn concentrations compared to men in presumably healthy populations and in patients presenting to the emergency department (ED). Increasing age leads to elevated troponin levels; and decreasing estimated glomerular filtration rate (eGFR) associates with higher troponin concentrations. How to interpret hs-cTn results in the presence of these confounding factors remains an open question. The aim of this study is to assess whether an integrated prediction model that incorporates hs-cTnT and its confounding factors performs better than hs-cTnT delta threshold alone and to delineate the effect of each confounding factor on the AMI diagnosis. This retrospective cohort study included 17,842 ED patients with serial hs-cTnT measured using a 0h/3h algorithm and eGFR calculated by CKD-EPI creatinine equation at a US medical center. The primary outcome was AMI diagnosis at discharge. Model selection was performed using the logistic regression models of AMI diagnosis with hs-cTnT absolute delta changes and different sets of covariates, which included age, sex, eGFR, and time delta. Model with the smallest Aikaike

Information Criteria (AIC) was chosen as the final model, and two-fold cross validation was performed to evaluate model goodness of fit. The area under curve (AUC) of the best fitted model was 0.95. Empirical receiving operating characteristics analysis derived the best probability cutoff of 0.03 for AMI prediction based on a 90% specificity benchmark. The diagnostic performance of this cutoff was compared to that of the hs-cTnT 0h/3h sex-specific delta thresholds we published earlier. The agreement between the prediction model cutoff and the sex-specific delta thresholds was 95.8% (Kappa coefficient 0.80). The agreement to true diagnosis was comparable with hs-cTnT sex-specific delta thresholds alone (90.3% agreement, Kappa coefficient 0.32) and with the prediction model cutoff (90.5% agreement, Kappa coefficient 0.33). To further delineate the effects of the confounders, mediation analysis and spearman correlation analysis showed that hs-cTnT delta change has a much stronger correlation with AMI diagnosis than its confounders. Among the confounders, sex is more predictive of AMI diagnosis (logistic coefficient 0.53) than age (logistic coefficient 0.02) and eGFR (logistic coefficient 0.02). While most of the effects of age and sex on AMI were mediated through delta troponin, the effect of eGFR on AMI diagnosis was almost entirely canceled by mediation through troponin. In summary, the confounding factors play a weaker role than hs-cTn delta change in AMI diagnosis. The integrated prediction model incorporating all confounding factors does not perform better than hs-cTnT delta threshold alone. Sex-specific hs-cTnT delta thresholds remain to yield the highest diagnostic accuracy.

## Topic Areas

Chemistry

# Increased Specimen Minimum Volume Reduces Turnaround Time and Hemolysis


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## Abstract

Quantity not sufficient (QNS) specimens with minimal blood volume for testing are common in clinical laboratories. However, there is no universal definition of minimum volume for a QNS specimen and little data is available addressing the impact of low volume specimens on turnaround time (TAT) and sample hemolysis. The objective of our study was to: (1) Evaluate the effect of sample volume on sample TAT and hemolysis and (2) Implement a QNS sample policy and monitor its effects on TAT and hemolysis.

Through retrospective analysis, we compared the TAT and hemolysis index from samples ≤1.0 mL to all specimens received and quantified the number of specimens with reduced blood volume. The median laboratory TAT for samples with ≤1.0 mL of blood was 61 min (Interquartile Range, IQR: 50-82), in contrast to 28 min (26-34) for all samples. The hemolysis index for samples ≤1.0 mL was 112 (65-253) and 15 (8-29) for all samples. We also observed that the largest proportion (33.9%) of QNS samples were from the Emergency Department (ED).

A new QNS policy requiring ≥1.5 mL of sample in a blood tube for laboratory analysis was implemented, with healthcare provider education as well as a lead-up period. Requirement of a minimum volume of 1.5 mL of blood resulted in the proportion of samples with TAT ≥60 minutes to decrease from 6.57% to 3.93% hospital-wide ($p < 0.0001$), and 10.4% to 4.24% in the ED ($p < 0.0001$). It also resulted in specimens cancelled due to hemolysis to decrease from 1.29% to 1.09% ($p = 0.008$) hospital-wide, and 4.24% to 3.38% in the ED ($p = 0.041$).

Our study demonstrates that implementation of a QNS policy of ≥1.5 mL and provider education provided a significant and durable reduction in TAT and specimen hemolysis.

## Topic Areas

Chemistry

# Impact of switching from the 2009 to the 2021 CKD-EPI Equation for eGFR


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## Abstract

Recent national and international guidelines have recommended implementing the 2021 CKD-EPI equation for estimating glomerular filtration rate (eGFR). Relative to the 2009 CKD-EPI equation, the 2021 equation eliminates race-based modifiers in the calculation of eGFR based on a remodeling of the original CKD-EPI dataset. Further, updated guidelines now recommend integrating Cystatin C as a confirmatory test in all patients with an eGFR between 45-59 mL/min/1.73m$^2$. Here we assess the impact of switching from the 2009 CKD-EPI creatinine-based equation to the 2021 CKD-EPI creatinine-based equation on both non-black and black populations and assess the feasibility of implementing a reflex for Cystatin C on all patients with an eGFR between 45-59 mL/min/1.73m$^2$. The laboratory information system was queried retrospectively from 11/01/2021 to 12/31/2021 for age, sex, self-identified ethnicity, and serum creatinine concentrations from basic/comprehensive metabolic panels and renal panels. Slope and linearity of the reported eGFR with the 2009 and 2021 equations were compared using Deming regression and Fisher's exact test was used to assess the proportion of eGFR results <30 ml/min/1.73m$^2$ and between 45-59 ml/min/1.73m$^2$. Of the 64,657 serum creatinine values, 37,589 were from self-identifying non-black patients and 27,068 were from self-identifying black patients. Deming regression revealed a slope of 0.997 (95% CI; 0.997-0.998) and intercept of 2.69 (2.65-2.74) for the non-black population and a slope of 0.863 (0.862-0.864) and intercept of 2.451 (2.39-2.51) for the black population. The number of non-black individuals with an eGFR <30 mL/min/1.73m$^2$ decreased 5622 to 5060 ($p<0.001$) and black patients increased from 4018 to 4387 ($p<0.001$). The number of non-black individuals with an eGFR in

the range of 45-59 ml/min/1.73m$^2$
increased marginally from 4,410 (11.7%) to 4,525(12.0%, p=0.19) and the number of black individuals increased from 2,501 (9.2%) to 3,299 (12.2%, p<0.001). If a Cystatin C reflex test was performed on all patients with an eGFR between 45-59 mL/min/1.73m$^2$, 2325 assays would have been performed on out-patients and 5499 on in-patients. These results, in the context of recently updated guidelines imply that the changing from the 2009 CKD-EPI equation to the 2021 CKD-EPI equation is likely to increase the proportion of black individuals diagnosed with moderate to severe and severe loss of kidney function more so than the non-black populations. Implementation of a Cystatin C reflex on all patients with an eGFR between 45-59 mL/min/1.73m$^2$ is likely to lead to considerable overuse and should be limited to out-patients and physician ordered tests.

## Topic Areas

Chemistry

# Utilization of system-wide urine drug testing to safely confront opioid misuse.


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## Abstract

Abuse and addiction of opioids leading to overdose deaths has increased significantly in Michigan since 2014. Moreover, a recent study showed that a majority (92%) of clinicians at Henry Ford Health System (HFHS) had reported prescribing narcotics in a way that was out of their comfort zone. To tackle this menace, we needed to equip our physicians with the appropriate tools to monitor patient compliance. In 2017, our laboratory implemented a reflexive urine drug testing (UDT) strategy called the directed pain panel (DPP). This panel included testing for natural/semi-synthetic /synthetic opiates, benzodiazepines, and cocaine metabolites. In 2019, the DPP was enhanced by adding buprenorphine, fentanyl, and tramadol. It is beneficial to order the DPP as opposed to UDSC (Urine drug screen) because UDSC does not detect the synthetic narcotics methadone, fentanyl, tramadol, and buprenorphine. DPP also has better sensitivity for detection of

oxycodone. The primary aim of the current study was to evaluate UDT orders for chronic pain patients and to focus our efforts on improving the lab testing available to our providers to manage patients safely and effectively. Therefore, we evaluated the utilization of our testing strategy by doing a survey among chronic pain physicians in addition to retrospectively analyzing total orders for DPP (chronic pain panel, directed, urine) and UDSC (drug screen, urine) for chronic pain patients at five locations in Michigan over a one-year period (8/2020-8/2021). We collaborated with key providers and accessed their test ordering history to assess compliance with ordering the preferred UDT panel. The DPP represented 68% of all UDTs ordered for this population, however there were still a significant number of UDSC orders (32%). When order location was evaluated, the majority of UDSC orders originated from two locations (80%). When provider ordering patterns were evaluated, most providers (65%) ordered the DPP > 50% of the time. In addition, 70% of all UDSC orders came from just two providers. This data showed that HFHS pain management clinicians favored ordering the DPP, however a small minority of ordering providers may benefit from targeted education regarding the advantages of the DPP. Hence, we educated the physicians who were ordering UDSC. They were willing to modify their practice to order DPP, instead of UDSC. This may pave the way for better patient compliance as well as reduced overdose and abuse of opioids.

## Topic Areas

Chemistry

# Assessment of LDL-C Calculation Using the Newly Adopted NIH LDL-C Equation in Pediatric Population


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## Abstract

### *Background*

The Friedewald equation for calculating low-density lipoprotein cholesterol (LDL-C) has been widely adopted for clinical use since its introduction in 1972. One of the well-recognized shortcomings of this equation is its overgeneralization of the physiological ratio between masses of triglyceride (TG) and LDL-C, leading to erroneous estimations of LDL-C at low levels and in patients with abnormally high TG levels. Over the years, many attempts have been made to address this issue with the development of more sophisticated equations. In 2020, Sampson and colleagues developed a new equation using lipid samples from patients tested at the National Institutes of Health (NIH) Clinical Center. This new NIH equation was proposed to allow for a more accurate estimation of LDL-C at low levels compared to the Friedewald equation. In this study, a retrospective assessment of LDL-C calculations was done using the new NIH equation to investigate its benefits over the Friedewald equation in pediatric patients.

### *Methods*

Patient data was extracted from the lab information system, EPIC Beaker, which included results for total cholesterol, HDL cholesterol, non-HDL cholesterol, and TG between calendar year 2019 and 2021. A total of 14,356 results from 8719 unique patients aged <1 month to 21 years, median age 14 years, were analyzed in RMarkdown accessed through RStudio. Calculations of LDL cholesterol levels for each result set were conducted independently using the Friedewald and NIH equations.

***Results***

Concordance in LDL-C estimation was 95% (13636 out of 14356) using the Friedewald versus NIH equations in this cohort patient data, 15% of all “Low” results determined by the Friedewald equation have been re-classified as “Normal” using the NIH equation, and 2% of “Normal” were re-classified as “High”. A small fraction of the dataset, 2.27% (326 out of 14355), had elevated TG between 400 and 800 mg/dL, and 0.07% (106 out of 14355) had TG >800 mg/dL. Of the samples with TG between 400 and 800 mg/dL, 44% (145 out of 326) resulted in a Friedewald LDL-C estimation of less than 70 mg/dL, which had been classified as “Low” based on the population reference range. Using the newly adopted NIH equation for LDL-C, 41% (59 out of 145) of said fraction were re-classified from “low” to “Normal”.

***Conclusion***

When compared with the Friedewald equation, the newly adopted NIH equation allowed for a uniformly higher estimation of LDL-C levels in pediatric patients with TG levels between 400 and 800 mg/dL. This is consistent with other published reports that it has a higher degree of accuracy and is less likely to overestimate patient risk from false classification into a higher risk category (i.e., low LDL). We expect this to better facilitate medication management for patients’ receiving statin therapy post heart transplantation.

## Topic Areas

Chemistry

# Validation of Neutrophil gelatinase associated lipocalin (NGAL), a urinary biomarker for Acute Kidney Injury and the positive interference from high leukocyturia samples


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## Abstract

Acute kidney injury (AKI) is a sudden and serious kidney damage condition that affects more than 1.2 million people every year. KIDNEY DISEASE IMPROVING GLOBAL OUTCOMES (KDIGO) guidelines currently suggest measuring the increase of serum creatinine as a diagnostic marker for AKI. However, the elevation of serum creatinine levels can take up to seven days or when >50% kidney function is lost. Neutrophil gelatinase-associated lipocalin (NGAL) has been identified as an early biomarker for tubular injury, which can be detected in the urine of AKI patients in as early as three hours. In the current study, we evaluated the performance of BioPorto's (Hellerup, Denmark) NGAL assay, which uses particle enhanced turbidimetry immunoassay (PETIA) technology on the Roche (Indianapolis, USA) cobas c502 chemistry analyzer. Linearity was determined using linearity material from BioPorto and also verified by serial dilutions of a high NGAL patient sample. The limit of quantitation was determined by running urine samples with values of 20 ng/mL, 25 ng/mL, and 35 ng/mL, respectively in duplicate for 10 days. Within-run precisions were determined by running urine two samples containing 100 ng/mL and 400 ng/mL NGAL 20 times and between–run precisions were determined by running the same samples 2 times a day for 10 days. To determine the accuracy of the NGAL method on cobas 502, 40 samples were used to compare results with those measured on Siemens Atellica using the BioPorto's NGAL assay. Reference interval was verified in 40 urine samples collected from healthy donors. A urine dipstick test was performed to confirm the normal urine samples did not contain

leukocyte esterase activity. Since NGAL is also present in neutrophils, we evaluated the effect of leukocytes in urine with the NGAL assay. Results showed the sample with 75 leukocytes/µL had an NGAL of 68 ng/mL and the sample with 500 leukocytes/µL has an NGAL of 222 ng/mL. The linearity range was determined to be 0 - 3000 ng/mL with a limit of quantification of 26 ng/mL. The within-run precision mean and CV% are 121 ng/mL, 4.3% for Level 1 and 401 ng/mL, 1.8% for Level 2. Between-run precisions mean and CV% are 106 ng/mL, 4.8% for Level 1 and 399 ng/mL, 2.6% Level 2. Method comparison had a slope of 0.90 and an intercept of 32.9. The reference interval was determined to be <50 ng/mL and Leukocytes in urine did cause false-positive NGAL results.

## Topic Areas

Chemistry

# Low haptoglobin in pregnancy: physiological or intravascular hemolysis?


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## Abstract

A woman in her second trimester of pregnancy (24 weeks of gestation), diagnosed with B-cell acute lymphoblastic leukemia, received induction chemotherapy. On day six of treatment, she required transfusion with two red cell (RBC) units for anemia. Less than 12 hours later, laboratory results included elevated total bilirubin, 4.3 mg/dL [reference interval <=1.2 mg/dL], direct bilirubin, 0.3 mg/dL [reference interval <=0.3 mg/dL], haptoglobin below detection limit, <10 mg/dL [reference interval: 30 - 200 mg/dL], and normal lactate dehydrogenase (LD), 186 U/L [reference interval: 122 - 241 U/L]. These tests were ordered without any clinical suspicion for intravascular hemolysis, and upon receiving the results, the primary team consulted transfusion medicine due to concern for an acute hemolytic transfusion reaction (AHTR). After finishing the corresponding workup, the patient did not meet the Center of Disease Control (CDC) criteria for AHTR. The patient was discharged and on follow-up visits her haptoglobin increased to 15 mg/dL (25 weeks 3 days of gestation). Later, during her third trimester (27 weeks 3 day of gestation), haptoglobin increased to 142 mg/dL; LD always remained normal. Hemolysis is not associated with normal LD. Instead, some literature suggest that pregnant patients may have lower haptoglobin levels than reported in the non-pregnant population, with a nadir occurring in the second trimester. It is possible that low haptoglobin in this population may be due to the combination of hemodilution and a high estrogen state. But no literature is available that provide

trimester-specific reference intervals for haptoglobin, which may lead to misinterpretation.

Inspired by this case, we developed a quality improvement project to determine trimester-specific reference intervals for haptoglobin in pregnancy. Haptoglobin was tested on remnant serum samples (BD Vacutainer SST) collected from routine outpatient pregnancy patients (n=401; at least 80 samples per trimester) using Roche/Hitachi cobas c systems. Derived nonparametric reference intervals were 22-188 mg/dL for first trimester, 29-177 mg/dL for second trimester, 53-185 mg/dL for third trimester, and 30-185 mg/dL for the entire sample population. While overall reference intervals were similar, consistent with previous reports, we noticed a shift in haptoglobin distribution to the low end in the second trimester [29 ($CI_{95\%}$ 7-35) mg/dL; median 98 mg/dL] in comparison with first trimester [22 ($CI_{95\%}$ 15-47) mg/dL; median 113 mg/dL p = 0.34] and third trimester [29 ($CI_{95\%}$ 7-35); median 112 mg/dL p=0.34].In the case described, the first haptoglobin below limit of detection could have been due to the combination of pregnancy (second trimester), recent red cell transfusion of RBC units (28 days old) and hyperhydration with crystalloids as part of the chemotherapy plan. This case also serves as a reminder that laboratory tests should be ordered mindfully, to aid in confirming or ruling out clinically suspected syndromes/diseases that are unlikely.

## Topic Areas

Chemistry

# Interference on the Abbott i-STAT creatinine assay caused by hydroxyurea


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## Abstract

Serum creatinine is an important biomarker used for estimating glomerular filtration rate (eGFR). The present study was designed after a patient at our institution had a significantly elevated creatinine result taken from an i-STAT point of care test that utilizes electrochemical detection. The patient's creatinine from the i-STAT was 3.8 mg/dL and was not consistent with their clinical history. The patient's previous creatinine taken 2 days prior was 1.39 mg/dL and 1.27 mg/dL seven days prior. Both previous readings were measured with the Siemens Dimension Vista 1500 using a colorimetric enzymatic creatinine method. A subsequent creatinine measured on the Siemens Dimension Vista 1500 was 1.30 mg/dL. Upon chart review, the clinical team noted that the patient is taking 500 mg of hydroxyurea daily for treatment of a myeloproliferative disorder. Review of the i-STAT package insert revealed hydroxyurea as a known interfering substance and states for every 100 µmol/L hydroxyurea in the specimen, creatinine will be increased by approximately 1.85 mg/dL. Review of literature described the positive interference caused by hydroxyurea for the i-STAT creatinine and glucose assays but did not explain the mechanism of this positive interference. In order to characterize the mechanism of hydroxyurea interference, we investigated the situation further. A pool of plasma was spiked with hydroxyurea. The spiked pool was serial diluted (x2, x4, x8, x16, x32, x64, x128) to observe the creatinine results in the presence of and absence of hydroxyurea. The dilution series was analyzed using the Vista enzymatic creatinine and the Abbott i-STAT enzymatic creatinine methods. The i-STAT creatinine results show increasing creatinine concentration from interference as the concentration of hydroxyurea increased. The dilution series suggests increasing positive interference with hydroxyurea. Positive creatinine interference with

hydroxyurea was not observed when analyzed on the Vista platform. Hydroxyurea is a widely used treatment for myeloproliferative disorders and also for managing sickle cell disease. Hydroxyurea is known to cause positive interference in the i-STAT enzymatic creatine method that uses electrochemical detection, with the degree of interference correlating with the concentration of hydroxyurea. The Vista colorimetric enzymatic creatinine method was not observed to have hydroxyurea interference. While both methods employ enzymatic reagent systems the final detection approach is important. Patients undergoing hydroxyurea treatment should avoid i-STAT measurement systems for creatinine.

## Topic Areas

Chemistry

# A retrospective study of the eGFR differences between the 2021 CKD-EPI Equation and the MDRD equation in Black and White Patients


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## Abstract

Objectives: In implementing the 2021 CKD-EPI without race equation in our institution, we sought to investigate the differences between the new CKD-EPI equation and the current MDRD equation to assist clinicians in transitioning to the new equation.

Methods: A retrospective study was performed in patients with eGFR<60 ml/min/1.73 m2 calculated by MDRD. Stata was used to analyze the eGFR calculated by the two equations and measure the differences according to respective categories.

Results: eGFRs calculated by the 2021 CKD-EPI without race equation were well correlated to eGFRs calculated by the MDRD. However, notable differences were observed. Compared to the MDRD, the CKD-EPI w/o race equation for Black males with a serum creatinine less than 1.5 yielded decreased eGFR results with mean negative differences of -4.49 (18-60 years old), -6.43 (60-80 years old), and -8.87 (>80 years old). For serum creatinine cohorts between 1.5 to 2.0 and greater than 2.0, the differences were -3.35, -5.46, -7.48 and -1.86, -3.40, -4.14, respectively. A

similar trend was noted in Black females. For those with a serum creatinine less than 1.5, the CKD-EPI equation yielded mean negative differences that increased with advancing age: -2.56, -4.74 and -7.27. For serum creatinine between 1.5 to 2.0 and greater than 2.0, the negative differences were -2.31, -3.73 and -5.28 and -1.30, -2.06 and -2.70, respectively. Therefore, in Black patients, the mean eGFR difference between the two equations increased as age increased and serum creatinine level decreased. In contrast, the CKD-EPI equation for White males with a serum creatinine less than 1.5 yielded increased eGFR results with mean positive difference of 7.22 (18-60 years old), 4.09 (60-80 years old), and 1.26 (>80 years old). For serum creatinine cohorts between 1.5 to 2.0 and greater than 2.0, the differences were 5.15, 2.54 and 0.18 and 2.01, 0.69 and -0.15, respectively. Similarly, White females had mean positive differences of 8.09 (18-60 years old), 4.61 (60-80 years old) and 2.00 (>80 years old). For cohorts with serum creatinine between 1.5 to 2.0 and greater than 2.0, the positive differences were 4.21, 2.24, 0.51 and 2.02, 0.82, 0.10. Therefore, in White patients, the difference of eGFR between the two equations increased as age decreased and creatinine level decreased.

Conclusions: Although the 2021 CKD-EPI equation strongly correlated with the MDRD, distinct differences exist. In our study, Black males and females exhibited a negative difference of around -5.0 and -4.0, respectively. The difference in eGFR was greater in those of older age and serum creatinine less than 1.5. Meanwhile, White males and females exhibited a positive difference of around 2.5 and 2.7. The difference in eGFR was greater in those of younger age and serum creatinine less than 1.5.

## Topic Areas

Chemistry

# Machine Learning Models Can Accurately Detect Crystalloid Contamination of Basic Metabolic Panels


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## Abstract

A common cause of erroneous laboratory results is preanalytical contamination of samples with intravenous crystalloids. Rules-based approaches, such as delta checks or feasibility cut-offs, can detect these errors, but rely on prior laboratory results and lack sensitivity. In this study, we aimed to improve the detection of crystalloid contamination using two machine learning workflows for the detection of contamination by the four most common crystalloids; normal saline (NS), lactated Ringer's (LR), and their dextrose-containing counterparts (D5NS and D5LR). First, we employed a semi-supervised approach using manifold approximation and contrastive learning. To do this, we aggregated five years' worth of basic metabolic panel results (n = 67 million), then simulated contaminated samples by mixing random subsets of samples in silico with the crystalloid solutions at different ratios. The dataset was partitioned into a 70:30 split between training and testing sets with five-fold cross-validation. An autoencoder was trained to create an embedding of the data by minimizing the triplet loss. This embedding was then projected onto a two-dimensional manifold. When the four crystalloid solutions and the independent test set were mapped to the manifold, decision boundaries were drawn based on the threshold that maximized the F1 statistic, and labels were

assigned. This method detected the 10% in silico mixtures with an area under the receiver operating characteristic curve (AUC) of 0.97, 0.94, 0.99, and 0.99 for NS, LR, D5NS, and D5LR respectively. Next, we evaluated a fully-supervised approach by training and validating Random Forest and XGBoost models using the same training and testing sets as above. Both models performed well, with XGBoost slightly outperforming Random Forest. The XGBoost model demonstrated AUCs of 0.992, 0.990, 0.998, and 0.996 for the NS, LR, D5NS, and D5LR mixtures. This high-level performance was maintained even as the proportion of mixed samples in the dataset was decreased from 50% to 1% to more accurately reflect real-world contamination rates. For NS and LR, the variables with the highest relative importance were calcium and chloride. For their D5 counterparts, glucose and calcium were most important. Creatinine and blood urea nitrogen were the least important variables in both approaches. Overall, the fully-supervised learning approach outperformed that of the contrastive embeddings for the specific task of identifying contamination from pre-defined crystalloids. One advantage of the semi-supervised approach is that it could be adapted to identify outliers that are not pre-defined. However, considering the relative ease of translating tree-based models into practice, we conclude that our XGBoost model offers the most promising solution and plan to prospectively validate this model for real-time detection of sample contamination.

## Topic Areas

Chemistry

# The Pandemic as a Catalyst for Digital Pathology Adoption as an LDT


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## Abstract

Whole-slide images (WSI) are the basis for the application of artificial intelligence/machine learning and other informatics methods to histological diagnosis and will further blur the line separating anatomic and clinical pathology. FDA classified WSI systems for primary diagnosis as class III (highest risk) medical devices until 2017. This discouraged anatomic pathology laboratories at risk-averse domestic institutions like mine from investing in these digital pathology (DP) platforms. In 2017, FDA downgraded WSI to class II (moderate risk) when they de-novo approved a system marketed by Philips. We were not interested in that system at my institution, but the downgrade caused us to reset our perception of the risk of validating a RUO system for primary diagnosis. Cost remained a barrier. In April 2020, FDA issued temporary guidance stating they would not enforce premarket approval of WSI systems to facilitate pathologists working remotely during the SARS-CoV-2 pandemic. The guidance included a statement that “laboratories and hospitals consider performing a validation study.” In January 2021, FDA proposed making the temporary non-enforcement guidance permanent.

So, in a little more than three years, WSI for primary diagnosis had gone from class III to exempted from pre-market approval! This nicely aligned the approval framework for WSI with the approval framework for our conventional optical microscopes, which are statutorily exempted from approval, and further reset our perception of risk. In April 2021, FDA withdrew the proposal to make non-enforcement permanent, but the temporary non-enforcement guidance is still in effect at the time of writing. Amid all this FDA activity, the College of American Pathologists updated and reissued their consensus guidelines for validating WSI systems for diagnostic purposes in March 2021. The narrative mentions the FDA's recent approval of a few WSI systems and anticipates more, but the expert panel recommendations do not include any related to the approval status of systems. The reissue of this document reminded us that, as clinical laboratorians, we are capable of safely validating WSI as a laboratory-developed test and are supported in doing so by consensus guidelines from one of our leading professional organizations. In early 2021 we committed to funding a DP initiative to make WSI part of our routine histological process for 10% of our anatomic pathology cases. The initial capital investment is $1.5M. When realized, the microscope slides for designated pathology services will be transported directly from the cover slipper to a slide scanner and electronically distributed to pathologists using a clinical-grade image management system that we share with our radiology department. We made the decision to fund this in the context of the regulatory (decreased perception of risk), sociological (demand for remote telepathology), and technological (availability of scalable WSI systems) changes that occurred during the pandemic.

## Topic Areas

Informatics

# Measuring clinical lead testing coverage to support a population health initiative


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## Abstract

Despite national efforts to reduce lead exposure, some children are still exposed to lead, leading to damage to the nervous system, delayed development, and learning and behavioral problems. CDC has recommended targeting high-risk groups for screening. In addition, Texas Department of State Health Services reported a concerning 17% lead testing coverage of 0-to-5-year-olds in in Harris County (where Houston is located). With the eventual goal of improving lead-related clinical care in a large pediatric hospital network, we asked the following questions: 1) What are the high-risk groups specific to our patient population? 2) For patients who are screened positive, are we adequately confirming the results? 3) For those with confirmed lead exposure, are we providing adequate follow-up care? To answer these questions, we analyzed lead testing and hemoglobin testing data on all children less than 6 years of age from the Texas Children’s Hospital’s electronic health record between 2017 and 2021. Lead screening could be performed on capillary or venous blood, but for elevations on capillary blood we analyzed if the results were confirmed with a venous blood test, as recommended by CDC. We used hemoglobin testing as an indicator of follow-up care of lead-exposed children, because an anemia work-up is recommended for such children. We repeated analysis using two different lead level thresholds, because of recent change to the

CDC blood lead reference value from 5 mcg/dL to 3.5 mcg/dL in May 2021. We found 273 children (0.33% of children with capillary blood test) to have cBLL ≥ 3.5mcg/dL. There was a significant racial disparity in the rate of lead screening. For example, the screening rate of Black children was 14%, whereas that of White children were 23%. There was a geographic discrepancy between the areas with most elevated cBLL and testing volume. For example, the area where cBLL was most frequently elevated (3.7%) received less than a fifth of tests compared to the most well-tested area. Only 75% of children with a cBLL ≥ 5mcg/dL received a timely venous blood lead test for confirmation, and this dropped to 43% for children with a cBLL ≥ 3.5mcg/dL. Similarly, only 75% of children with a confirmed venous lead level ≥ 5 mcg/dL received a follow-up repeat vBLL test, and 55% received a follow-up hemoglobin measurement. There is room for improvement in population lead health, in terms of both equity and resource distribution. Improvement of lead-related clinical practice could benefit hundreds of children in the Houston metropolitan area.

## Topic Areas

Informatics

# RecutClub 2.0: Deployment of a Trainee-Led, Didactic-Centered Whole Slide Imaging Platform


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## Abstract

**Objectives.** The Houston Methodist Pathology training program encourages trainee participation in quality improvement (QI) projects for improving the didactics experience. One of these initiatives is RecutClub, a whole slide imaging (WSI) web-based platform for presenting surgical pathology unknown conference cases. With the evolving nature of pathology didactics in an ever-changing world where remote work plays an increasingly important role, several refinements were needed to ensure that the platform could continue to provide an unparalleled WSI experience for incoming trainees. Additionally, the burden of updating site content needed to be distributed from the site administrators to the end-users. **Methods.** RecutClub is operated through Google Cloud via a Google App Engine instance. It connects to Google Cloud Databases to store persistent tabular data and Google Cloud Storage for pyramidal JPEG files used to display whole slide images in a web-browser. In the next generation platform (RecutClub 2.0), the largest change was to migrate case uploading from a developer-controlled solution to a decentralized, trainee-driven web portal. To ensure a superior user experience, the entire site as well as the upload form was migrated to Bootstrap 4. An AJAX-native, asynchronous uploader was built to interface the existing Google Cloud platform to a new upload portal that allows authorized trainees to build cases from scratch. Additionally, a tagging solution was implemented to allow for server-side persistent storage of trainee-selected cases, such as “interesting” cases or cases for further review. This tagging complements the existing region of interest (ROI) and case search functionalities. **Results.** The deployment of RecutClub 2.0 involved multiple rounds of resident-led testing and feedback. Several regular testers offered ongoing user

feedback and suggestions for visual layout and usability. The response from the general trainee population after deployment was overwhelming positive, with many trainees commenting on the “improved” visual aesthetics as well as the convenience of the upload functionality. Administrator involvement in case-upload time decreased from 2 hours/month to 0 hours/month due to enabling trainee-driven uploading. **Conclusions.** The deployment of RecutClub 2.0 fulfilled the dual primary objectives of enabling straightforward trainee-led content updates, as well as decreasing administrator involvement in case-upload time. These and other changes ensure that RecutClub can continue to provide an unparalleled WSI experience for pathology trainees at Houston Methodist, while also providing fresh content in a landscape with several competing WSI platforms.

## Topic Areas

Informatics

# Making Complex Cancer Reports Easier Using Self-Built Interactive Templates


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## Abstract

Surgical pathology reports are becoming increasingly complex, especially with neoplastic cases. Pathologists need to verify all elements are addressed in the CAP synoptics, order proper reflex molecular testing or immunohistochemistry depending on care standards and institutional guidelines, and address key points in the report for clinicians. For example, breast cancer cases at our institution need repeat biomarkers for all cases status post neoadjuvant therapy, oncotype reflex testing for pT2N0, estrogen receptor positive tumors, and tumor bed size with residual tumor burden calculations for all breast cancer only after neoadjuvant chemotherapy. For residents and faculty alike, these expansive reports are challenging, especially for those new to the institution’s policies and conventions. Previous workflow included using Microsoft Word documents with previously signed out cases had significant risk for errors that stem from new updates in the CAP protocols, missing or different reflex testing from the previous case, and updated institutional guidelines. We set out to create a customized library of decision support and interactive templates using a software called PhraseExpander. We created PhraseExpander templates for all cancer resections. The templates included the associated synoptics required for accreditation by CAP, implemented automated staging using logic statements, and provided conditional reminders when reflex testing was indicated. Variable labels were utilized to help clarify notes in the CAP synoptic such as how to grade specific tumors and how many high-

power fields to count. The tools were distributed to all surgical pathology fellows, residents, and select faculty who expressed interest in participating. The templates were successfully implemented into the routine workflow for surgical pathology sign out. End-user feedback was overwhelmingly positive. First year and upper level residents appreciate the detailed descriptions on how to complete the report, most notably the reflex molecular testing reminders and many conditional aspects such as in our breast cancer reports. Fellows and attendings have also appreciated these aspects as well as the automated staging and reminders to do administrative tasks, such as adding the case to the Laboratory Information System tumor registry. Interactive templates with built in conditions and calculators are powerful tools that can be used for generating the increasingly complex reports in pathology. They provide pathologists real-time and non-interruptive clinical decision support, allow them to spend less time typing and reduce reliance on remembering the required reflex testing. The most notable disadvantage in coding these complex templates is the considerable build and maintenance time. We plan to expand use to flow cytometry reports, biopsy reports, and routine non-neoplastic cases while incorporating labels to assist in resident education.

## Topic Areas

Informatics

# Heart valve sequencing has greater yield than heart valve culture for identifying or confirming the etiologic agent of infective endocarditis


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## Abstract

Blood culture (BC), heart valve culture (VC), and heart valve sequencing (16S rRNA gene PCR and sequencing) (16S) are methods that may detect the etiologic agent of infective endocarditis (IE). Here we assessed the utility of 16S compared to BC and VC.

Valve specimens (n=261) from 83 patients submitted to the Microbiology laboratory for VC between 9/23/20 - 12/31/21 were retrospectively analyzed. 16S was performed on a portion of the same fresh tissue specimen that was submitted for bacterial VC. Results of BC (within 1 month prior to surgery), VC (n=126 bacterial, n=81 fungal, n=54 AFB valve cultures), and 16S were compared.

Forty-eight (58%) subjects were male and mean age was 52 yr (range 17-80 yr). Valves submitted for culture were from the left (n=62 patients; 75%), the right (n=17; 20%), or both (n=4; 5%) sides of the heart.

Bacterial VC had a higher yield (31% with reported growth) than fungal VC (7%) and AFB VC (0%). Organisms recovered in fungal VC (*Candida albicans*, *C. tropicalis*, *Burkholderia cepacia* complex) also grew in their respective bacterial VC.

Microorganisms were identified in specimens from 59/83 (71%) patients by at least 1 test method. BC was positive in 75%, 16S yielded bacterial DNA in 67%, and VC

was positive in 33% of patients tested (*P* <0.0001, 16S vs VC). The 3 test methods yielded concordant results (≥1 organism in common, or all negative) in 44% of patients with all methods performed. Concordance between BC and 16S was 86%; VC and 16S was 58%; and VC and BC was 56%. *S. aureus* was most frequently identified (n=21, 36%), followed by viridans group streptococci (n=15, 25%), *E. faecalis* (n=6, 10%), *Candida* spp. (n=4, 7%), *S. epidermidis* (n=4, 7%), and *S. agalactiae* (n=3, 5%). 16S was the only method that identified *Bartonella* spp. (n=2), and the only method which identified *S. mitis* group (n=3) and *S. agalactiae* (n=1) in some patients. 16S detected additional microorganisms not recovered in culture, including *Finegoldia magna* and *Morococcus/Neisseria* sp.

16S performed as well as BC and better than VC, demonstrating utility in identifying or confirming the etiologic agent of IE from a valve specimen, including in the setting of no growth cultures. 16S offered the greatest diagnostic benefit in cases where blood cultures were negative prior to surgery, identifying an etiologic agent in 6% of cases. Fungal and AFB VC had low return; for low volume valve specimens, bacterial VC and 16S should be prioritized.

## Topic Areas

Microbiology

# Evaluation of NG-Test CARBA 5 lateral flow assay with IMP-27-producing Enterobacterales


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## Abstract

Our objective was to evaluate an isolate of *Morganella morganii* that was reported by an outside facility as being positive for NDM and IMP carbapenemases in a geographic region with low prevalence of both resistance determinants.

MALDI-TOF MS was used to confirm the isolate's identification. Antimicrobial susceptibility testing (AST) was performed by Kirby-Bauer disk diffusion according to CLSI (M100, 31$^{st}$ ed). The isolate was tested for carbapenemase production by the modified carbapenem inactivation method (mCIM) with and without EDTA (eCIM), and for carbapenemases by Xpert Carba-R and NG-Test CARBA 5. The isolate was sequenced with Illumina NovaSeq.

The *M. morganii* isolate tested susceptible to ceftazidime, ceftriaxone, cefepime, aztreonam, and ertapenem, and tested intermediate to meropenem and imipenem. By mCIM testing, the isolate was positive for carbapenemase production, and by eCIM testing the isolate was positive for metallo-β-lactamase production. On Xpert Carba-R the isolate tested negative for carbapenemase genes (including KPC, OXA-48-like, VIM, IMP, and NDM). Whole genome sequencing

revealed the *M. morganii* isolate contained IMP-27 (but not NDM or other carbapenemases).

The NG-Test CARBA 5 lateral flow assay (LFA) is the original test from which IMP and NDM were detected. When the *M. morganii* isolate was tested using NG-Test CARBA 5 in our laboratory, it tested positive for IMP carbapenemase only. Other isolates of *M. morganii* were tested, including a KPC+ isolate and carbapenemase-negative isolates, which all generated concordant LFA results according to their carbapenemase profile. When we increased (overloaded) the bacterial test inoculum of the *M. morganii* isolate to determine if we could recreate the finding of NDM positivity, a band for NDM was detected. Overloaded test inoculum from other isolates did not universally generate a “positive” result for NDM.

A dual IMP+/NDM+ *M. morganii* is an unusual result that should prompt additional investigation, especially if the AST profile is not consistent with an NDM-producing strain. IMP-27 is not detected by Xpert Carba-R but is variably detected by NG-Test CARBA 5. The microorganism inoculum used for the NG-Test CARBA 5 assay must be carefully controlled for accurate results.

## Topic Areas

Microbiology

# Evaluation of Two Methods for Detection and Differentiation of Carbapenemase-Producing Enterobacterales Directly From Positive Blood Culture Broths


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## Abstract

Bloodstream infections caused by carbapenemase-producing Enterobacterales (CPE) are a significant health threat because CPE are resistant to first-line antimicrobial therapies, leading to delays in effective therapy. Carbapenemases hydrolyze β-lactams and are divided into those enzymes that are serine-dependent (SEs) (KPC, OXA-48-type), and metallo-β-lactamases (MBLs) (IMP, NDM, VIM) that require zinc ions for catalysis. MBLs are inhibited by the chelating agent EDTA, while SEs are not. Antimicrobial therapies differ for SEs and MBLs, and thus rapid detection and differentiation of CPE directly from positive blood culture (BC) broths is essential for optimal patient outcomes. The aim of this study was to evaluate 2 phenotypic methods for detecting and differentiating CPE directly from positive BCs, the first using 4 drops of BC broth inoculated into tryptic soy broth (TSB) and the second using 1 mL of BC broth. 64 Enterobacterales isolates (non-CPE, *n* = 19; CPE, *n* = 45 [MBLs, *n* = 20]) were spiked into BC vials and incubated. Once positive, 4 drops of BC broth were added to 2 tubes of TSB (2mL), one tube supplemented with EDTA and the other without EDTA (tube without EDTA termed “Blood-mCIM”; tube with EDTA termed “Blood-eCIM”). Additionally, 1 mL of BC broth was aliquoted into 2 tubes, again, one tube without EDTA and the other with EDTA (tube without EDTA termed “bc-CIM”; tube with EDTA termed “bc-eCIM”). A meropenem disk was added to each tube followed by incubation at 35°C for 4 h. Meropenem disks were

then applied to Mueller Hinton agar plates inoculated with a carbapenem-susceptible *Escherichia coli* isolate and zones read at 8 h and 18-24 h after disk application. Zones were interpreted using established Clinical and Laboratory Standards Institute criteria for testing bacterial isolates using the mCIM/eCIM. No zone in the absence of EDTA implies carbapenemase production, while a zone in the presence of EDTA suggests MBL production. Differentiation between enzymes (MBL vs. SE) was only performed if carbapenemase production was detected in the absence of EDTA by the Blood-mCIM or bc-CIM. Sensitivity and specificity of Blood-mCIM and bc-CIM for detection of CPE were >90% at 8 h and 18-24 h. The sensitivity and specificity of the Blood-eCIM for detecting MBL CPE were ≥90% and ≥80%, respectively, at both times. For bc-eCIM, sensitivity and specificity were 77.8% and 100%, respectively, at 8 h, and 95% and 100%, respectively, at 18-24 h. Blood-mCIM and bc-CIM accurately detected CPE at 8 h and 18-24 h. bc-eCIM performed better than the Blood-eCIM for differentiating MBL from SE carbapenemases but required 18-24 h to optimize test performance. In conclusion, these data demonstrate phenotypic detection and differentiation of CPE directly from positive BCs is possible using these simple, inexpensive methods.

## Topic Areas

Microbiology

# Evaluation of Ordering Practices, Microbial Yield, and Clinical Utility of Urinary Stone Cultures


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## Abstract

Urinary stones are a global problem. While it has been established that microorganisms can be associated with urinary stone formation, data on best practices for microbial culture and clinical interpretation of microbial culture of urinary stone specimens are limited. Our objective was to investigate the microorganisms present in urinary stone cultures (usc) and companion urine cultures. We conducted a retrospective study at a tertiry hospital examining cultures submitted between October 2018 and October 2021. We collected data from electronic medical records of patients who had at least at least one usc performed. As part of routine clinical workup, all urinary stone specimens submitted for culture were ground using a disposable tissue grinder and inoculated on a tryptic soy agar with 5% sheep blood agar plate (BAP), a chocolate agar plate (CHOC), and a MacConkey agar plate (MAC), streaked for isolation and incubated in 5% $CO_2$ at 35°C for up to three days. Urine cultures were performed using the Kiestra TLA system with 10 µL of inoculum onto each of a BAP and MAC, and plates were examined at 16 and 24 hours of incubation. For urine specimens submitted from a straight or “in and out” catheter, 10,000 CFU/mL was the threshold for workup. From clean-voided, midstream, ileostomy, nephrostomy, or indwelling/Foley catheter specimens, 100,000 CFU/mL was the threshold for

workup. The final clinical significance of cultures is determined by evaluating the culture result in light of the patient's clinical presentation. For usc and urine cultures, clinically insignificant growth was considered no growth or when no pathogens were recovered in culture. Urine cultures with more than 3 species over threshold were considered contaminated. A total of 1,049 usc were performed from 854 patients (55.3% female). The median patient age was 61 years (IQR 49-70). The most frequent microorganisms reported in usc cultures were Proteus mirabilis (N=98), Enterococcus faecalis (N=75), and Escherichia coli (N=73). No pathogen was identified from 57% of usc. 441 usc were performed in a context of urine culture (+/-2 days) in 336 (39.3%) patients. In addition, 138 patients (16.1%) had more than 1 usc, being the maximum as 11 usc performed in one patient. The most common urine/USC concordant result was no growth in 165 of 441 cultures (37.4%), followed by coagulase negative Staphylococcus. Cohen's kappa obtained from usc and urine culture results was 0.45, which is not the minimum acceptable agreement. A pathogen was identified from usc in 43% of cases. Usc and urine cultures rarely resulted in concordant results, but the most common concordant result was no growth in culture. Additional studies are warranted to evaluate the impact of usc results on patient care, including antimicrobial use and correlation of microbes isolated with stone chemical composition.

## Topic Areas

Microbiology

# LEPTOTRICHIA SPECIES BACTEREMIA IN HEMATOLOGICAL MALIGNANCIES


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## Abstract

*Leptotrichia* species are thin anaerobic gram-negative rods that inhabit multiple areas in the human body including the oral microbiota. Many of the reported infections that occur with *Leptotrichia* species occur in immunocompromised individuals classifying *Leptotrichia* species as opportunistic pathogens. Here we present four clinical cases of immunocompromised patients experiencing bacteremia caused by *Leptotrichia* species in the span of just a few months in one hospital system. Utilization of standard microbial identification methods of matrix-assisted laser-desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) initially yielded the same identification for all four *Leptotrichia* isolates as *Leptotrichia buccalis.* However, 16S rRNA sequencing confirmed the identification for only one of the four isolates as *L. buccalis,* while two of the four isolates were identified as *Leptotrichia trevisanii*. There were no commonalities among these patients to suggest a common bacterial acquisition. *Leptotrichia* bacteremia in patients with malignancies are generally associated with a dysbiosis of the patient's normal oral flora which is known to be very diverse between individuals. These four cases highlight the importance of considering opportunistic infection in immunocompromised patients with organisms that are considered members of the normal oral flora.


## Topic Areas

Microbiology

# Trends in Viral Respiratory Culture: Impacts of the SARS-CoV-2 Pandemic


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## Abstract

**Introduction:** Due to the increasing ease and availability of molecular assays, viral culture is rarely employed for the diagnosis of respiratory illnesses. Despite lower sensitivity than molecular techniques, viral culture may detect a wider array of viral pathogens at a lower cost relative to multiplex molecular panels. In this study, we examined the effects of the SARS-CoV-2 pandemic on viral respiratory culture ordering and trends in the rates of pathogen detection.

**Methods:** Viral respiratory culture results from Jan 1$^{st}$, 2017 to February 28$^{th}$, 2022 were analyzed for changes in the number of monthly orders, positivity rate, and incidence of individual pathogens. To determine changes in seasonal incidence and viral etiology, a comparison was made between winter (Dec-Feb) and summer (June-Aug) months as well as acute (Influenza A/B, RSV) and chronic (HSV, CMV) infections. Given SARS-CoV-2's classification as a BSL-3 pathogen, our viral culture assay was not designed to detect this virus. As a surrogate method to measure rates of SARS-CoV-2 in viral culture specimens, we performed NAAT testing with the ThermoFisher TaqPath COVID-19, Flu A/B, RSV assay on negative specimens.

**Results:** Following the pandemic, testing volume decreased by 46.7% with the overall positivity rate decreasing from 6.67% to 4.85%. Among the 46 states for which more than ten orders were placed, monthly testing decreased in 38 states. Of the eight states with increased average monthly testing, the greatest increases were seen in Rhode Island, Nevada, and Montana. During the pre-pandemic timeframe, acute respiratory pathogens demonstrated a typical winter peak with low summer detection. Post-pandemic, there was an atypical increase of acute

respiratory pathogens, driven primarily by RSV. The positivity rate for chronic viral infections increased from 3.43% pre-pandemic to 4.09% post-pandemic. Following the pandemic, HSV has replaced influenza as the most commonly detected pathogen during winter months. Molecular studies of 229 negative viral culture specimens identified 36 (15.7%) samples positive for SARS-CoV-2, 9 (3.9%) for RSV, and none for influenza A or B. Median cycle threshold values for SARS-CoV-2 samples were 21.3 (range: 9.1-36.5).

**Conclusions:** Following the SARS-CoV-2 pandemic, the number of respiratory virus cultures ordered significantly decreased. There has also been a statistically significant decrease in the positivity rate driven by the absence of acute viral respiratory pathogens, including influenza A/B and RSV. We also observed an offseason increase of RSV during the summer months of 2021. Detection rates of chronic viral pathogens including CMV and HSV have remained relative stable. The presence of SARS-CoV-2 in negative specimens raises concerns for inappropriate test utilization. While less sensitive than molecular methods, viral culture has the potential to offer a lower cost alternative for monitoring a broad range of viral pathogens.

## Topic Areas

Microbiology

# Patient Outcomes in CMV and SARS-CoV-2 Coinfection


## Authors

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## Abstract

This study examines clinical outcomes in patients with cytomegalovirus (CMV) and SARS-CoV-2 coinfection. Between June and November 2020, previously immunocompetent patients with SARS-CoV-2 and CMV coinfection were identified at Houston Methodist Hospital as part of routine clinical correlation by a molecular pathologist. SARS-CoV-2 nasopharyngeal specimens were analyzed by real time reverse-transcriptase polymerase chain reaction (RT-PCR). All CMV tests were performed on plasma or bronchoalveolar lavage (BAL) specimens and analyzed by competitive polymerase chain reaction. 65 previously immunocompetent patients with CMV and SARS-CoV-2 coinfection were identified. Patient demographics include 41 male patients (63%) and 24 female patients (37%) ranging in age from 34 to 86 years (mean: 66.04, median 68). Documented pre-existing conditions include 27 patients with hypertension 41.5%), 19 patients with diabetes mellitus (29.2%), 9 patients with coronary artery disease (13.8%), and 3 patients with asthma (4.6%). Eight patients (12.3%) had no documented pre-existing conditions. The plasma CMV viral load ranged from <300 to 21,566 IU/mL. The CMV PCR results from bronchoalveolar lavage and bronchial wash specimens ranged from <300 to 59,127 IU/mL. CMV PCR was initially negative in 10 patients then positive on serial testing. 60 patients were critically ill requiring ventilator support (92.3%). 47 patients (72.3%) expired, 7 patients (10.8%) were transferred to a long term acute care facility, 3 patients (4.6%) were discharged to a rehabilitation facility, 3 patients (4.6%) were discharged home, and 1 patient (1.5%) remained in-patient at the time of analysis. The prevalence of CMV seropositivity and medical comorbidities increases with age. Reactivation of latent CMV is a known occurrence in critically ill patients that is associated with poor outcomes. The majority of the patients in our cohort were 50 years old, and all were severely to critically ill with a mortality rate of 72.3% These findings suggest CMV portends a worse prognosis in patients with

COVID-19. These findings also demonstrate the importance of clinical correlation in molecular testing.

## Topic Areas

Microbiology

# Limit of Detection for Rapid Antigen Testing of the SARS-CoV-2 Delta and Omicron Variants of Concern Using Live Virus Cultures


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## Abstract

The global SARS-CoV-2 pandemic continues with new divergent variant lineages which may impact the performance of available antigen diagnostic tests required for epidemic control. As of February 2022, the last two SARS-CoV-2 strains declared variants of concern (VoC) by the World Health Organization were Delta (strain B.1.617.2) and Omicron (strain B.1.1.529). Both variants contain several nonsynonymous mutations in the nucleocapsid, the SARS CoV-2 protein detected by antigen tests; potentially altering test analytical sensitivity. To assess this, we determined the limit of detection (LoD) for live virus preparations of Delta and Omicron variants compared with USA-WA1/2020 (WA1), the reference strain rain used for LoD studies described in the Instructions for Use (IFU) for all Food and Drug Administration (FDA) Emergency Use Authorization (EUA) - approved antigen tests. We examined the analytical sensitivity of three antigen tests widely used in the United States: the Abbott BinaxNow, AccessBio CareStart, and LumiraDx antigen tests. To do this, we quantified live virus by plaque forming units (PFU) and

calibrated RT-qPCR assays, serially diluted the virus in PBS to obtain a range of concentrations, applied 50 uL of the diluted viral samples to swabs, and then completed testing according to the IFU. For all three tests, we found that Omicron had a similar or lower 95% LoD threshold compared to WA1. Furthermore, the relationship of genome copies to PFU for Omicron and WA1 overlap, suggesting that the Omicron variant mutations do not change underlying diagnostic relationships between PCR and antigen tests. However, for Delta, the LoDs for all three tests were significantly higher compared to WA1. In addition, for any given quantity of PFU of Delta, the corresponding amount of genome copies was significantly lower than for WA1. These data suggest that Delta nucleocapsid mutations may alter antigen test analytical sensitivity and diagnostic parameters. Our results had findings that were not completely consistent with similar investigations, but these studies either fell short of the FDA's EUA requirement of 20 LoD replicates, included tests unavailable in the United States, and/or used gamma-irradiated or heat-killed virus that may artifactual affect test performance. In all, the SARS CoV-2 rapid antigen tests evaluated could effectively detect Delta and Omicron variants despite nucleocapsid mutations, even though we observed lower sensitivity for Delta. As VoC continue to emerge, we should continue to monitor antigen test performance to ensure they remain important tools to reduce transmission of SARS-CoV-2

## Topic Areas

Molecular Diagnostics and Cytogenetics

# MECOM copy number alterations identify a new group of myeloid neoplasms with a poor prognosis


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## Abstract


### *MECOM* copy number alterations identify a new group of myeloid neoplasms with a poor prognosis

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Objective: Myeloid neoplasms (MNs) harboring inv(3)(q21q26)/t(3;3)(q21;q26) or *MECOM* gene rearrangements as identified by fluorescence in situ hybridization (FISH) are known to carry a poor prognosis. The objective of this study is to assess prognostic impact of MNs with copy number alterations (CNA) in *MECOM* as compared to MNs with inv(3)/t(3;3) or *MECOM* rearrangements. We present those initial comparative results and secondarily assess whether genetic mutations that confer a worse clinical outcome are also associated with MNs with *MECOM* CNA.

Methods: A retrospective review of electronic pathology records was performed at Beth Israel Deaconess Medical Center. All MNs from 2016 – current with *MECOM* FISH (Abbott Molecular, Abbott Park, IL) confirmed analyses were extracted. Other additional items extracted included karyotype and next-generation sequencing (NGS) results of a 65 gene myeloid panel (SmartGenomics Myeloid Profile, PathGroup, Nashville, TN). Kaplan-Meier and ANOVA were performed using GraphPad Prism (San Diego, CA).

Results: A total of 307 cases were identified in which *MECOM* FISH was performed. Exclusion of cases lacking inv(3)/t(3;3), *MECOM* rearrangement, or *MECOM* CNA provided an initial study cohort of 20 cases. 5 cases had *MECOM* rearrangement with inv(3)/t(3;3), 7 had *MECOM* rearrangement without inv(3)/t(3;3), and 8 had *MECOM* CNA. 13 of 20 cases had a complex karyotype, 13 of 20 cases had a monosomal karyotype, 10 of 20 cases had both a complex and monosomal karyotype. Of the 20 cases in which *MECOM* FISH demonstrated inv(3)/t(3;3), *MECOM* rearrangement, or CNA, NGS was performed on 16 cases. Genes known to be associated with a significant negative overall survival (OS) include *ASXL1*, *EZH2*, *IDH2*, *KRAS*, *NRAS*, *RAD21*, *RUNX1*, *SRSF2*, *STAG2*, *TP53* with single and multiple mutations found in 9 of 20 cases. Kaplan-Meier analysis revealed no significant difference in OS among the three *MECOM* aberration groupings (Log-rank test; p=0.77). Single factor ANOVA analysis revealed enrichment for high risk gene mutations in *MECOM* CNA (p=0.04) and *TP53* mutations (p=0.04) when compared to MNs with *MECOM* rearrangements.

Summary: Our analysis of MNs with *MECOM* aberrations by FISH reveal that *MECOM* with CNA display similarly poor overall survival as MNs with inv(3)/t(3;3) and *MECOM* rearrangements. Additionally, the *MECOM* CNA group was enriched for high risk gene mutations and *TP53* as compared to inv(3)/t(3;3) and *MECOM* rearrangement groups. Overall, the initial results of this study reveal a new group of MNs harboring *MECOM* CNA that confer a poor prognosis and high risk mutational profile.

## Topic Areas

Molecular Diagnostics and Cytogenetics

# Clinical Utility of Next-Generation Sequencing Panel Testing in the Evaluation of Arteriovenous Malformations


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## Abstract

Arteriovenous malformations (AVMs) are vascular lesions in which an overgrowth of blood vessels of varying sizes develops with one or more direct connections between the arterial and venous circulation. AVMs are seen in sporadic and syndromic conditions that are caused by a variety of genomic alterations. Here, we performed a retrospective review of the cases sent for next-generation sequencing (NGS) analysis for diseases of somatic mosaicism (DoSM). Specimens from 54 patients with clinical indication of AVMs were submitted for the DoSM NGS panel between October 2013 and Dec 2021. DNA extraction and sequence analysis were performed on affected tissues, including fresh tissue, formalin-fixed paraffin-embedded tissue, fibroblast cultures, and buccal specimens. Single-nucleotide variants (SNVs) with variant allele fractions (VAFs) greater than 3% and small insertions and deletions (indels, <21 bp) were called using VarScan2, Genome Analysis Toolkit (GATK), and Pindel. Variants with VAFs between 1-3% were manually reviewed based on the sequencing data quality and the known clinical

significance. 38/54 (69.1%) patients were females and 16/54 (30.9%) were males. Ages ranged from 1 month to 73 years (median age 17 years). Most of the patients were indicated with sporadic AVM (81%), although additional clinical indications were noticed, including unspecified congenital vascular malformation (9.1%), capillary malformation (5.5%), arteriovenous fistula, and AVM associated with capillary and lymphatic malformations (1.8% each). The biopsied lesion was most often located in the head (43.6%), followed by the limbs (30.9%), and unspecified areas of the body in the remaining 25.5%. Among the 54 cases, 37 (68.5%) cases had pathogenic and/or likely pathogenic (P/LP) variants identified, 2 cases (3.7%) had variants of unknown significance, and the remaining 15 cases (27.8%) had negative results. MAP2K1 variants were found in 12 samples, followed by KRAS (8), TEK (7), PTEN (5), BRAF (4), TSC2 (2), HRAS (2), RASA1 (2), and PDGFRB (1). Of note, four cases had two P/LP variants. Among the 37 positive cases, 32 cases had somatic alterations; the remaining 5 cases had at least one germline P/LP variant, including PTEN (4) and RASA1 (1). In summary, the diagnostic yield of cases with clinical indication of AVMs was 68.5% using our in-house DoSM NGS panel. This study demonstrates the clinical utility of the DoSM NGS panel testing in the evaluation of a cohort with clinical presentations of AVMs.

## Topic Areas

Molecular Diagnostics and Cytogenetics

# Molecular viability testing accelerates detection of Mycobacterium bovis BCG strain – assay development ahead of a human challenge trial


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## Abstract

Clinical trials of potential anti-tuberculosis drugs have recently involved human challenge by intradermal inoculation of *Mycobacterium bovis* Bacille Calmette-Guérin (BCG) strain. It is challenging to evaluate the presence of live BCG in the skin of inoculated volunteers and to assess the effectiveness of tested drugs in a timely manner because of the slow growth of BCG in cultured biopsies. Molecular viability testing (MVT) utilizes quantitative reverse transcription PCR (qRT-PCR) of pre-ribosomal RNA (pre-rRNA) present only in viable bacteria. This study preliminarily validated BCG MVT testing for use in future human clinical trials and included a mouse model study for an approved TB drug. BCG samples were generated by growth in nutrient-limited human serum with or without 0.5 µg/mL isoniazid (INH) for four days after which diluted samples were sub-cultured in 7H9 liquid media. For mouse studies, approximately 2 million CFU of BCG were intradermally injected into the ear of mouse (n=10). Five of 10 mice were treated with 1 mg INH/L water

four days later for four additional days. The inoculation site was excised, homogenized, diluted, and cultured in 7H9 media with aliquots harvested daily for the next five days. Aliquots were lysed and extracted for total nucleic acids using the easyMAG platform followed by amplification on QuanStudio 5 Real-Time PCR system. Pre-rRNA expression and normalized by number of rDNA was described as $2^{(|(CtRTPCR-CtPCR)|}$); dormant/dead BCG were expected to generate pre-rRNA:rDNA ratios of ~1. BCG cultured in nutrient-limited human serum generated pre-rRNA:rDNA ratios up to 75 after three days, indicating early replication; such growth was confirmed by increasing optical density ($OD_{600}$) measurable after four days. BCG cultured in INH yielded pre-rRNA:rDNA values ~1 with no change in $OD_{600}$ over three weeks of culture. In the mouse model, MVT testing of skin homogenates showed detectable BCG by RT-PCR and PCR but no increase in pre-rRNA on tissues tested immediately after being excised. However, after homogenates cultured in 7H9 media for one or more days, pre-rRNA:rDNA ratios rose 20-69-fold in untreated mice and 20-34-fold in INH-treated mice. One INH-treated mouse showed undetectable BCG pre-rRNA and DNA. This study demonstrated that BCG MTV can detect replication of live BCG in liquid culture much earlier than when turbidity-based measures or colonial growth are used. A pilot study in mice also showed recoverable, viable BCG in biopsies and refined the tissue collection and processing procedures. This pilot mouse study suggested that 1mg INH/L in water daily over a four-day period does not completely kill BCG. Increased INH in the feeding water and/or longer duration of treatment may be needed for future studies of BCG killing in mice. These findings have helped refine our anticipated use of BCG MVT for a future human challenge clinical trial.

## Topic Areas

Molecular Diagnostics and Cytogenetics

# Clearance of the Plasmodium 18S rRNA biomarker after treatment in controlled human malaria infections


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## Abstract

Controlled human malaria infection (CHMI) studies at non-endemic sites are increasingly used to evaluate promising anti-malaria drugs and vaccines prior to larger field studies in endemic regions. Historically, thick blood smears (TBS) have been used in CHMI studies to detect infection after challenge and to confirm the clearance of parasites after treatment. In the past several years, more sensitive assays for molecular biomarkers, such as *Plasmodium* 18S rRNA, have been qualified by the U.S. FDA for detection of infection after challenge and have supplanted TBS for this purpose. However, the FDA has not yet qualified such assays for confirming clearance of parasites after treatment. Over the past decade, we have extensively used 18S rRNA quantitative reverse transcription PCR (qRT-PCR) to test the blood of volunteers in numerous CHMI studies at our center before and after treatment. We compiled and analyzed post-treatment TBS and qRT-PCR data to prepare a package for regulatory review of this approach and to better understand infection trajectories after treatment. Our analysis included data from 7 CHMI studies, across which 142 participants developed malaria after challenge. For participants in earlier studies in which infection was defined by TBS positivity (corresponding to parasite densities at time of treatment typically between 5,000

and 200,000 parasites/mL, as estimated by qRT-PCR), we found that the biomarker cleared in an average of 3.48 ± 0.15 days. This was significantly longer than the mean time to negative TBS (1.81 ± 0.19 days). This finding highlights the greater sensitivity of qRT-PCR and the additional time required for parasite density to decrease from TBS-detectable levels (~10,000 parasites/mL) to the limit of detection of the biomarker assay (<20 parasites/mL). For participants in later studies in which infection was defined by qRT-PCR (corresponding to parasite densities at time of treatment typically between 20 and 40,000 parasites/mL), the biomarker cleared in an average of 3.34 ± 0.13 days, which was not significantly different from earlier studies. However, across all studies we found that time to biomarker negativity was significantly longer for participants with higher parasitemia at treatment ($p = 0.001$), indicating that initiating treatment earlier leads to faster clearance. Potentially concerning indicators post-treatment might include increasing biomarker concentration - either immediate or recrudescent - and new or recurrent malaria symptoms. Overall, the *Plasmodium* 18S rRNA can be relied upon to confirm the adequacy of treatment in CHMI studies.

## Topic Areas

Molecular Diagnostics and Cytogenetics

# Correlation of PDL1 Expression with Genomic Alterations in Non-Small Cell Lung Cancer: a 7-month retrospective analysis


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## Abstract

Background: PD1/PDL1 inhibitors have significantly improved overall survival in non-small cell lung cancer (NSCLC). PDL1 is now a standard biomarker along with molecular testing for therapy selection. Checkpoint therapy is given front-line for patients with high PDL1 and no actionable mutations or fusions, or after

progression with any PDL1 expression. Studies comparing PDL1 status in the context of actionable mutations or fusions in NSCLC are sparse.

Design: All patients undergoing molecular testing for a next-generation sequencing (NGS) lung cancer panel at our institution in a 7-month period were reviewed. This included an NGS DNA panel for actionable/driver genes: EGFR, ERBB2, BRAF, K/NRAS, PIK3CA, and IDH1/2; and a NGS RNA fusion panel for ALK, ROS1, RET, NTRK1/3, NRG1, and MET e14 skipping. PDL1 expression by IHC was scored for tumor proportion score (TPS). Statistical comparison was performed using Fisher's Exact test.

Results: 186 patients with NSCLC were included (126 adenocarcinoma, 33 squamous/adeno-squamous, 19 poorly differentiated, and 8 NSCLC-NOS); 162 of those had concurrent PDL1 IHC. 121 (65%) patients had a genomic alteration, most commonly RAS [28%], EGFR [24%], ALK [8%], MET [7%], and BRAF [5%]. Cases with genomic alterations were classified into those with and without RAS mutation/s. The prevalence of high PDL1 expression was greater in RAS-driven tumors: 47% vs 22% with non-RAS bearing genomic alterations (p=0.0173). There was no significant difference in the PDL1 expression between the genomically negative tumors and those with non-RAS bearing mutations. Recently, the FDA granted approval to sotorasib, a KRAS G12C inhibitor, in patients with locally advanced or metastatic NSCLC. However, there was no significant difference in the PDL1 expression between the tumors with RAS G12C mutation and those with other RAS mutations.

Conclusion: Our data indicate that close to half of RAS-driven NSCLC have high PDL1 expression, an important consideration for front-line therapy selection. The majority of genomically-actionable tumors have at least low-positive expression of PDL1, highlighting the potential importance of immune checkpoint therapy in the resistant-progression setting. In comparison, only about one third of genomically-negative cases have high PDL1 expression, and a significant percent are completely negative. When the actionable KRAS G12C mutation bearing cases were compared to those with other RAS mutations, no significant difference was noted in the PDL1 expression between these two subsets; further investigations and a larger cohort of

patients may be helpful in this case to determine whether PDL1 levels are affected by a specific RAS mutation.

## Topic Areas

Molecular Diagnostics and Cytogenetics

# A multimodal genetic testing approach to a diagnosis of Roberts-SC phocomelia syndrome, an ESCO2 spectrum disorder


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## Abstract

Diagnosis of a genetic syndrome has traditionally relied upon the identification of characteristic phenotypic findings, followed by targeted laboratory testing. Shared clinical manifestations between genetically distinct syndromes and variable expressivity of findings within a single syndrome can complicate the diagnosis. Multiple testing modalities may be necessary, even when the putative gene(s) is known. We describe the multi-pronged genetic testing applied to a patient with a suspected diagnosis of Roberts- SC phocomelia syndrome (RBS). RBS is an autosomal recessive developmental disorder (OMIM 268300) caused by pathogenic variants in the “establishment of sister chromatid cohesion N-acetyltransferase” (*ESCO2* ) gene mapped to chromosome 8p21.1. The *ESCO2* enzyme product is a conserved protein with acetyltransferase activity required for formation of the cohesin complex, which, among other activities, maintains sister chromatid association during S-phase of the cell cycle. Because of the defective cohesin complex, RBS joins other syndromes referred to as cohesinopathies. The patient, a newborn male, was small for gestational age, with hypertelorism, cleft lip and palate, shortened forearms, absence of the radius and thumbs, and bilateral clubbed feet. All of these findings are established characteristics of RBS. Targeted Next Generation Sequencing revealed a single variant in exon 10 of *ESCO2* (c.1654C>T, p.Arg552*) predicted to result in a loss of function. Chromosomal microarray was also performed and showed an estimated 15 Mb region of

homozygosity (ROH) extending from 8p11.21 to 8p21.2, which spans *ESCO2*. The finding of this ROH supported the interpretation of homozygosity for the variant, as did a family history of consanguinity. Additional cytogenetic analysis documented premature sister chromatid separation and repulsion of heterochromatin chromosomal regions characteristic of RBS. These chromosomal findings served as a functional assay supporting pathogenicity of the *ESCO2* variant. Interestingly, the only report of an identical variant within exon 10 of *ESCO2* was described for two brothers diagnosed with Juberg-Hayward syndrome. Juberg-Hayward was considered allelic to, but a distinct entity from RBS. In contrast, our findings support the conclusion that RBS and Juberg-Hayward are not distinct syndromes, but rather represent variability within an *ESCO2* syndrome spectrum. Such unification of diagnoses based on genomic findings has influenced numerous other clinical disorders. Further, our findings demonstrate the contributions of each of the genetic testing modalities, which may be applied, selectively or collectively, based on integration with clinical and family history data.

## Topic Areas

Molecular Diagnostics and Cytogenetics

# A strategy for detecting an anomalous batch of kappa serum free light chains measurements using nonparametric analysis of patient deltas


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## Abstract

Patient-based quality control (PBQC) involves monitoring for shifts in patient results to detect analytical problems. Established methods perform well, but only for assays with high volumes and approximately normal result distributions. Recent publications have described monitoring the average of patient deltas (AOD) as a way generalize PBQC methods more broadly. Here we propose a nonparametric analysis of patient deltas (NPAOD). To illustrate the concept we present a case of an analytical issue in the kappa serum free light chain assay (kappa sFLC) caused by a bad reagent pack and how it could have been detected using NPAOD. We were alerted to a possible issue by the ordering clinicians who noticed a series of unexpectedly high results. Significantly lower values were observed when the samples were repeated with a new reagent pack (median [IQR]: 5.3 [3.12-13.52] versus 2.52 [0.95-4.25], $p < 0.5$ e-7 by paired Wilcoxon test). To evaluate the use of PBQC in detecting this error, we analyzed one year of retrospective data comprising 7657 results from 3061 patients. The distribution of kappa sFLC values was positively skewed (mean = 14.5 g/dL, median = 2.2 g/dL, skew = 26.6). Both volumes and distributions varied significantly by day of the week following the scheduling of subspecialty clinics. These properties make the conventional approach of simple moving averages poorly suited for monitoring kappa sFLC at our institution. We computed delta values as the difference between the current result and the most recent previous result and produced delta values for 4596/7657 (60%) of the samples. The distribution of deltas was zero centered and symmetric (mean = 0.03

g/dL, median = 0.01 g/dL). Using the historical distribution as a reference, we found that the average of deltas was not significantly different for the results of the bad reagent pack (p = 0.78 by Student's T test). In contrast, a nonparametric approach yielded a significant difference whether by comparison of medians using a Wilcoxon test (p = 0.0004) or comparison of empirical distributions using a Kolmogorov-Smirnov test (p = 1e-7). The latter approach may offer several benefits. First, the Komogorov-Smirnov test has greater statistical power because it considers both the position and the shape of the distribution. Second, an algorithm based on comparison of empirical distributions may have fewer hyperparameters to tune. We continue this work with an in-silico characterization of detection and false alarm rates at different magnitudes of analytical bias. In conclusion, this case study provides initial evidence that a nonparametric analysis of patient deltas may help to extend PBQC to additional assays that have unfavorable distributions and volumes for the simple moving average algorithm.

## Topic Areas

Laboratory Management

# Establishing a combined Immunology/HLA/Coagulation Rotation for Pathology Residents


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## Abstract

Given the breath of subspecialties within Clinical Pathology (CP), creating a curriculum with adequate exposure to all areas is challenging. Immunology, coagulation, and HLA rotations have been cited repeatedly by pathology trainees at our institution as targets for improvement. To address these concerns, a combined immunology/ HLA/ coagulation rotation was implemented wherein trainees spend a month exploring testing methods and their interpretations in each of these three different specialized laboratory sections. Herein, we describe the experience of the first 7 trainees (approximately the size of one residency class in our institution) who

completed the newly implemented rotation. The rotation combines didactic lectures, assigned reading, time at the bench (to observe procedures), online modules, and case study discussions in each of the areas. The goal was to compare and contrast related concepts/techniques across lab sections. The month is divided so that residents spend the mornings of the first two weeks in HLA, the mornings of the third week in coagulation, and the mornings of the fourth week in immunology. Residents spend afternoons completing assignments and providing narrative interpretations for coagulation tests (such as the Lupus Anticoagulant/Antiphospholipid Antibody test) that they then review with attending physicians. Residents were assessed at the beginning and end of the rotation for their medical knowledge in each area. Additionally, they were provided the opportunity to share their expectations and evaluations of the rotation at its onset and completion, respectively. There was a significant increase in average scores when comparing pre- and post-rotation assessments (46% compared to 78%; $p < 0.01$) of medical knowledge. Overall, trainees ranked the rotation 4.25 in a Likert scale of 5 and favored the asynchronous eLearning modules (4.7) over discussions in signout (4.29), in person case review (4.43), and assigned reading (4.0). Residents were also asked to rank their comfort in generating reports within each section; they felt more confident with Coagulation profiles (2.86 in a Likert scale out of 3) compared to ANA patterns (2.29) or interpreting HLA results (1.86). Residents described the rotation as “very useful” and “challenging.” Learners cited signing out coagulation cases and exposure to the HLA lab as highlights of the rotation. Coordinating individual schedules across three lab sections was a persistent challenge for trainees. In conclusion, we implemented a new CP rotation combining Coagulation, HLA, and Immunology topics into one month-long rotation. Evaluation of trainees to-date demonstrated an increase in medical knowledge post-rotation compared to pre-rotation, and an overall satisfaction with the rotation based on their feedback. HLA learning modules were restructured in response to limited confidence in HLA report interpretation. Based on the success of the combined rotation, it will become a permanent component of the CP curriculum for all residents.

## Topic Areas

None of these topics really captures it

# Levels and in-unit stability of 11-nor-9-carboxy-Δ9-tetrahydrocannabinol (9-carboxy-THC) in donor platelets and whole blood: is enough present to have an impact on recipients?


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## Abstract

Objective: The safety of the United States donor blood supply has been questioned in the context of recreational marijuana legalization in Colorado and other states nationwide. It is unclear whether cannabis use by donors presents a danger to recipients, either through direct toxicity or socioeconomic ramifications. Our objectives are to both determine if the active metabolite of tetrahydrocannabinol (THC), 11-nor-9-carboxy-Δ9-tetrahydrocannabinol (9-carboxy-THC), is present in donated units in a dosage high enough to impact the recipient and to assess for in-unit metabolism or degradation.

Methods: We will quantify the amount of 9-carboxy-THC in platelet units donated by self-reported cannabis users through liquid chromatography with tandem mass spectrometry (LC-MS/MS) testing and correlate the assay data with donor survey responses. To gain insight into in-unit metabolite stability, we also will test whole blood units at several time points throughout storage, collected from participants who self-report taking a known oral dose of THC prior to donation.

Results: Institutional review board approval for a study evaluating marijuana at a children’s hospital presented a challenge, requiring the addition of an

organizational risk review and an update to the hospital transfusion consent for approval. Thus, the projected timeline of this study has been extended. Study recruitment flyers and an email screening survey have generated interest from volunteer donors. The email survey was sent to 9,208 donors who had previously donated at least once, from which we received 372 responses (4.04% response rate). Of the respondents, 49 individuals reported daily marijuana use, 33 reported marijuana usage 4-6 times per week, 32 reported usage 1-3 times/week, 30 reported usage 1-3 times/month, 52 reported rare usage, and 167 reported never using marijuana. Overall, out of the survey respondents, over 50% reported at least some marijuana usage, and 31% reported at least weekly marijuana usage. Although this number is small, our prior study found that many were repeat donors. Research visits are currently being scheduled for interested participants.

Conclusions: This study highlights the challenges of performing marijuana research at a children's hospital and illustrates why there is a lack of published data evaluating the effects of recreational marijuana legalization. Although completion of this project investigating this controversial topic has been delayed, persistent advocation for this area of interest is opening doors for future studies and will hopefully pave the way to fill our knowledge gaps. The results of this research study will inform decisions regarding donor eligibility and safety of the national blood supply as well as address associated socioeconomic implications.

## Topic Areas

Transfusion Medicine

# Hyperhemolysis in a patient with sickle cell disease and recent SARS-COV-2 infection, with complex auto- and allo-antibody work-up, successfully treated with tocilizumab


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## Abstract

Hyperhemolysis syndrome (HHS) is a severe delayed hemolytic transfusion reaction seen in sickle cell disease (SCD) patients, characterized by destruction of donor and recipient RBCs. It results in a drop in hemoglobin to below pretransfusion levels and frequently reticulocytopenia. The pathophysiology behind HHS is poorly understood. There are several proposed mechanisms for the bystander hemolysis, including macrophage activation, HLA antibodies, or complement-mediated destruction. Avoidance of additional transfusions and supportive care is currently the mainstay treatment for HHS, however, monoclonal antibodies such as eculizumab, rituximab and tocilizumab have resulted in good outcomes as well. We report a case of a man in his 30s with SCD with a recent hospitalization two weeks prior for COVID-19. His red cell antibody history included anti-Fy(a) and warm autoantibody. At that time he was given two units of pRBC and discharged with a hemoglobin of 10.2 g/dL. He returned to the hospital approximately 1.5 weeks later with a hemoglobin of 6.0 g/dL and symptoms concerning for acute chest syndrome. Pretransfusion testing now showed a new 4+ pan-agglutinin in both gel-based and tube-based testing. Alloadsorption identified an anti-N with broad thermal amplitude and a strong cold agglutinin. Three least incompatible units were

transfused to this patient over several days, with evidence of hemolysis. Further reference lab work revealed anti-Fy(a), anti-Fy(b), anti-Le(a), anti-Le(b), and an HTLA-like antibody with KN specificity. The patient's hemoglobin nadired at 4.4 g/dL and LDH and ferritin were increased. The patient was treated with a single dose of tocilizumab, a monoclonal antibody that blocks the interleukin-6 (IL-6) receptor and prevents macrophage response to that pro-inflammatory cytokine, resulting in stabilization of his hemoglobin and inflammatory markers and clinical improvement for discharge. Tocilizumab has been used to treat various autoimmune conditions including macrophage activation syndrome (MAS), cytokine release syndrome (CRS), and most recently, patients with severe COVID-19 pneumonia. Any predisposition of SARS-CoV-2 infection for HHS is at this point unproven, however, it is possible that inflammation related to COVID-19 could lower the threshold for HHS. There are clinical features of HHS that are similar to MAS/CRS and tocilizumab appears to be a promising treatment for patients with HHS. Transfusion services should have a low threshold for frequent repeat alloadsorption studies in sickle cell patients with RBC transfusion refractoriness.

## Topic Areas

Transfusion Medicine