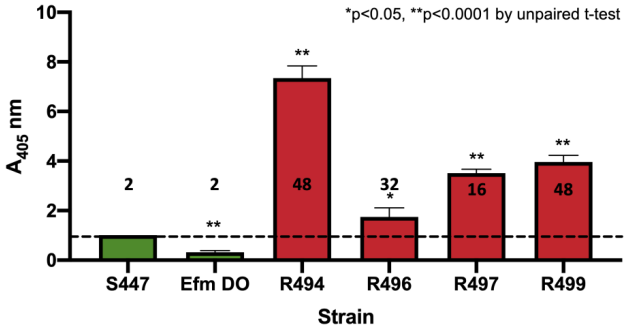
triggers the LiaFSR cell membrane stress response pathway, resulting in the extracellular release of the protein LiaX, a novel protein that functions as a regulator of the membrane response. We postulated that detection of extracellular LiaX correlates with DAP-NS in clinical strains of VR*Efm*.

Methods: We used 6 well-characterized VR*Efm* BSI isolates (2 DAP-S, 4 DAP-NS) as reference strains to optimize a whole-cell indirect enzyme-linked immunosorbent assay (ELISA) method for LiaX detection. We assessed limit of detection and reproducibility of the ELISA LiaX method. We then assessed 54 clinical VR*Efm* BSI isolates from pts with cancer for validation. We determined DAP MICs by broth microdilution (BMD). We collected clinical and microbiological details by chart review

Results: The 6 reference strains showed high reproducibility with low coefficient of variation. All DAP-NS reference strains had increased detection of LiaX ($p< 0.0001$) compared to DAP-S reference strains. Of the 54 isolates from pts, most pts (83.3%) had HM. The source of 62.9% of VRE BSIs was determined to be gastrointestinal. Six of the 54 isolates were DAP-NS by BMD MIC. The LiaX test and MIC had categorical agreement on 56% of isolates. Of the isolates with disagreement, 19 isolates were susceptible by MIC (median 2 µg/ml) but non-susceptible by LiaX ELISA, and 5 isolates were non-susceptible by MIC (6, 8, 8, 8, and 16 µg/ml, respectively) but susceptible by LiaX ELISA.

Whole-cell indirect LiaX ELISA A405nm of Efm reference strains shows ability to differentiate DAP susceptible MICs from DAP resistant MICs. DAP susceptible (MIC=2 µg/ml) Efm strains are shown in green and DAP resistant (MIC≥8 µg/ml) strains in red. DAP-S reference strains have no LiaFSR mutations. The dotted line indicates an example cutoff for DAP-S/R in this assay. *p<0.05, **p<0.0001 by unpaired t-test. Coefficieint of variance for each reference is <15%.



Conclusion: Detection of extracellular LiaX has important discrepancies with DAP MIC. Interestingly, LiaX may be a surrogate marker to detect strains with heightened DAP-mediated cell membrane response and potentially identify strains predisposed to DAP therapy failure. Further characterization of the discrepant isolates by genomic analyses and time-kill assays are warranted to fully validate the performance of LiaX ELISA.

Disclosures: **Cesar A. Arias, MD, MSc, PhD, FIDSA**, **Entasis Therapeutics** (Scientific Research Study Investigator)**MeMed** (Scientific Research Study Investigator)**Merck** (Grant/Research Support)

665. Lower Indeterminate Rates and Resolution by Retesting Using a Single Lithium-Heparin Tube Blood Collection Method for the QuantiFERON®-TB Gold Plus (QFT®-Plus)

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Session: P-25. Diagnostics: Bacteriology/mycobacteriology

Background: The QuantiFERON-TB Gold Plus (QFT-Plus) test is an assay for detecting a cell-mediated immune response to *M. tuberculosis* (MTB). The assay measures the *in vitro* quantitative IFN-γ responses to MTB or control antigens in an incubated blood sample. There are 2 options for QFT-Plus blood collection. One option is a lithium-heparin transport tube with sample aliquots subsequently transferred to 4 QFT-Plus Blood Collection Tubes (1-tube QFT-Plus); the 2nd option is to directly collect the blood sample in 4 QFT-Plus collection tubes (4-tube QFT-Plus). In this study, we compared the indeterminate (IND) rates by the 2 blood collection methods to assess which method was superior.

Methods: For both blood collection methods, QFT-Plus ELISA testing was performed at various Quest Diagnostics sites as specified in the assay's package insert. A retrospective data analysis of results for the above 2 blood processing methods was conducted. Also, we evaluated the rates of IND results in follow up blood collections. Statistical analyses were performed by the proportion test.

Results: In 2019, the IND result rate for greater than an 1.8 million 1-tube QFT-Plus draws was less than 1% whereas, the IND result rate for 0.3 million 4-tube draws was 4% This difference was significant. The overall MTB positive rate was 7% for the 1-tube method and 6% for the 4-tube method. Within a one-month interval following an initial blood collection event, 464 patients with an original IND result had a 2nd blood sample collected and tested. Only 35% of the 2nd blood collection events produced an IND result, with 52% of the 2nd sample results reporting as negative and 13% were positive.

Conclusion: This study found that the 1-tube QFT-Plus collection method reduces the IND rates by 4-fold compared to the observed rate in the 4-tube process. Additionally, two thirds of patients with an initial IND result resolved to either a positive or a negative result when retested within 1 month.

Disclosures: **All Authors**: No reported disclosures

666. Microbial Cell-Free DNA Sequencing for Evaluation of Response to Antibiotic Therapy in Patients with Relapsed or Refractory Leukemia

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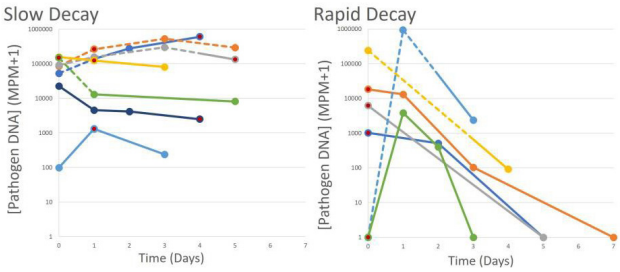
Session: P-25. Diagnostics: Bacteriology/mycobacteriology

Background: In patients with bloodstream infection (BSI), true eradication of infection takes longer than blood culture clearance. Therefore, optimal treatment duration, especially in immunocompromised hosts, is unknown. A sensitive test of microbiological response to treatment could improve care by indicating a time for safe antibiotic discontinuation. Microbial cell-free DNA sequencing (mcfDNA-seq) is a sensitive predictor of BSI, and we hypothesize that it might also be useful to measure response to treatment.

Methods: Eligible participants were < 25 years of age being treated for leukemia. Remnant plasma samples were collected as part of a prospective study (PREDSEQ), and underwent mcfDNA-seq by Karius Inc. in a CLIA/CAP-accredited laboratory. Pathogen DNA was reported in molecules per microliter (MPM). Testing was batched and blinded. Available samples from Day 1 through Day 7 after onset of bacterial BSI were included. We evaluated decay of the BSI pathogen DNA after initiation of effective antibiotic therapy, from the peak to last available sample, and compared episodes with slow (< 0.5 $\log_{10}$ MPM/day) vs. rapid DNA decay.

Results: There were 13 evaluable BSI episodes in 9 participants; 7 had slow DNA decay. Persistence of bacteremia or fever ≥1 day after initiation of effective antibiotics occurred in 9/13 episodes (7/7 slow decay and 2/6 rapid decay; $P = 0.02$). Slow decay persisted beyond resolution of bacteremia and fever in 3/7 of these cases.

Figure 1. Pathogen DNA Concentration by mcfDNA-seq During Antibiotic Treatment of Bacteremia; Dashed line, blood culture positive; Red circle, last fever



Conclusion: In this small convenience sample of patients with leukemia, slow mcfDNA-seq DNA decay correlated with persistent fever or bacteremia. Post-BSI mcfDNA-seq monitoring should be investigated with the goal of decreasing inappropriate antibiotic therapy and preventing treatment failure.

Disclosures: Joshua Wolf, MBBS, PhD, FRACP, Karius inc (Grant/Research Support) Asim A. Ahmed, MD, Karius (Employee) Desiree D. Hollemon, MSN, MPH, Karius inc (Employee) Charles Gawad, MD PhD, Karius inc (Grant/Research Support)

667. Multicenter Assessment of Enterobacterales, Salmonella spp. and Pseudomonas aeruginosa Using Updated CLSI Levofloxacin Breakpoints on MicroScan Dried Gram Negative MIC Panels

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Session: P-25. Diagnostics: Bacteriology/mycobacteriology

Background: Data from a multicenter clinical study with *Enterobacterales*, *Salmonella* spp. and *P. aeruginosa* on a MicroScan Dried Gram-negative MIC (MSDGN) Panel was evaluated with updated US FDA/CLSI levofloxacin breakpoints.