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 Ef jm.

Methods: We used 6 well-characterized VR Ef jm 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 Ef jm 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 BSI 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 not susceptible by LiaX ELISA, and 5 isolates were non-susceptible by MIC (6, 8, 8, 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-NS reference strains have no LiaX MICS mutations. The dotted line indicates an example cutoff for DAP-S/R in this assay. *p<0.05, **p<0.001 by unpaired t-test. Coefficient of variance for each reference is <15%.

Conclusion: Detection of extracellular LiaX has important discrepancies with DAP-S/R. Gratifyingly, 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.

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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) Test

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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.

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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 (mcDNA-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 mcDNA-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, 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 (77% slow decay and 2/6 rapid decay; P = 0.02). Slow decay persisted beyond resolution of fever and in 3/7 of Charles Gawad, MD, PhD.

Figure 1. Pathogen DNA Concentration by mcDNA-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 mcDNA-seq DNA decay correlated with persistent fever or bacteremia. Post-BSI mcDNA-seq monitoring should be investigated with the goal of decreasing inappropriate antibiotic therapy and preventing treatment failure.

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667. Multicenter Assessment of Enterobacteriaceae, 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 Enterobacteriaceae, Salmonella spp. and P. aeruginosa on a MicroScan Dried Gram-negative MIC (MSDGN) Panel was evaluated with updated US FDA/CLSI levofloxacin breakpoints.

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MIC results were compared to results obtained with frozen broth microdilution panels prepared according to CLSI methodology.

Methods: A total of 839 Enterobacterales, Salmonella spp, and P. aeruginosa clinical isolates were tested at three clinical sites in efficacy and challenge combined. MSDGN panels were evaluated with turbidity and Prompt® methods of inoculation. MIC values obtained from the MSDGN panels were compared to MICs utilizing a CLSI broth microdilution reference panel. To assess reproducibility, a subset of 15 organisms were tested on MSDGN panels at each site. MSDGN panels were incubated at 35 ± 1°C and read on the WalkAway System, the autoSCAN-4 instrument, and visually. Read times for the MSDGN panels were at 16-20 hours. Frozen reference panels were prepared and read according to CLSI methodology. FDA and CLSI breakpoints (µg/mL) used for interpretation of MIC results were: Enterobacterales ≤ 0.5 S, 1 I, ≥ 2 R; Salmonella spp. ≤ 0.12 S, 0.25-1 I, ≥ 2 R; P. aeruginosa ≤ 1 S, 2 I, ≥ 4 R.

Results: Essential and categorical agreement were calculated compared to frozen reference panel results. Results for isolates tested during efficacy and challenge with Prompt inoculation and manual read are as follows:

| Read Method | Essential Agreement (CA) % | Categorical Agreement (CA) % | Very Major Error (VMJ) % | Major Error (MAJ) % |
|-------------|-----------------------------|-------------------------------|--------------------------|----------------------|
| Enterobacterales | 96.2 (638/663) | 96.7 (641/663) | 1.5 (2/133) | 0.0 (0/151) |
| Salmonella spp. | 100 (83/83) | 98.8 (82/83) | 0.0 (0/19) | 0.0 (0/38) |
| P. aeruginosa | 94.6 (88/93) | 93.6 (87/93) | 0.0 (0/34) | 1.9 (1/64) |

Conclusion: Levofloxacin MIC results for Enterobacterales, Salmonella spp, and P. aeruginosa obtained with the MSDGN panel correlate well with MICs obtained using frozen reference panels using updated FDA/CLSI interpretive criteria in this multicenter study.

669. Rapid Molecular Testing of Sputum for Identification of Pulmonary Tuberculosis: Impact on Duration of Respiratory Isolation
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Session: P-25. Diagnostics: Bacteriology/mycobacteriology

Background: Current guidelines recommend molecular testing directly on sputum of patients with suspected pulmonary tuberculosis (TB) to facilitate discontinuation of airborne infection isolation (AII). Henry Ford Hospital (HFH), a tertiary care center in Detroit, MI serves a population at high-risk for TB. Molecular testing of sputum using GeneXpert/MTB/RIF (Xpert; Cepheid) (GXTB) was implemented at HFH on March 2019. Providers were permitted to remove patients from AII using 3 negative acid-fast bacillus smears (AFB) or 2 negative GXTB results. We evaluated the impact of GXTB on duration of AII over a 2-year period: Pre-implementation (January 2018 to February 2019) and post-implementation (March 2019 to February 2020).

Methods: Retrospective data was abstracted for all patients placed in AII during the study period. Demographic data, TB risk factors, duration of AII, length of hospital stay (LOS), accuracy and turn-around-times (TAT) of AFB and GXTB were compared in the pre- and post-implementation periods. Categorical variables were studied using chi-square testing, and continuous variables were studied using T-test or Mann-Whitney U test as appropriate.

Results: During the study period, 269 patients with suspected TB were placed in AII: 137 pre-implementation and 132 post-implementation. Clinical characteristics and TB risk factors were generally comparable in both groups (Table 1). Abnormal chest X-ray was more frequent in patients in the post-implementation phase. All cases of culture positive TB were detected by AFB and GXTB. TAT of AFB results before and after implementation were similar and ranged from 20-24 hours (Table 2). In the post-implementation period, TAT of GXTB compared to AFB was 6.35 vs 21.28 hours (p < 0.0001). Duration of AII was shortened by almost 24 hours (70.2 vs 93.7, p = 0.0446). There was also an increase in the percent of admissions shorter than 2 days between the AP and SOC cohorts (12.5% vs 5.2%, p = 0.024) [Figure 1].