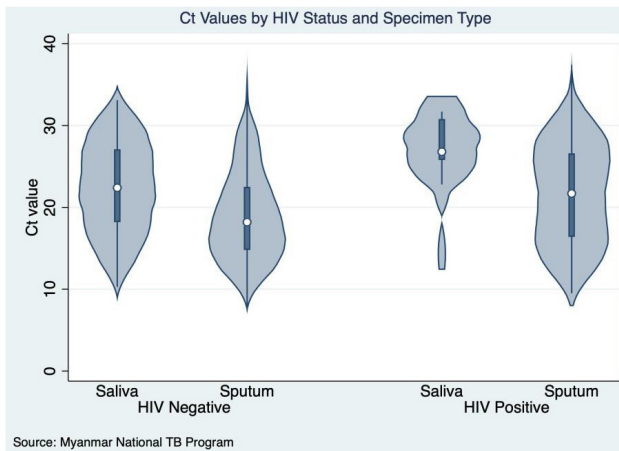


Figure 1. Ct Values by HIV Status and Specimen Type



Conclusion: Sputum samples have a significantly higher bacterial load on average compared to saliva samples independent of HIV status. Additionally, when looking at both saliva and sputum as sample types, HIV positive patients have significantly lower bacterial load than individuals who are HIV negative. Based on these results, sputum is the optimal sample type for Xpert TB detection, especially in people living with HIV.

Disclosures: All Authors: No reported disclosures

659. Evaluation of Four Chromogenic Agars for Urine Culture Including Time and Cost Savings Analysis

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Session: P-25. Diagnostics: Bacteriology/mycobacteriology

Background: With a volume of approximately 5000 urine culture specimens per month in our tertiary-care university center hospital's microbiology laboratory, we wanted to evaluate methods aiming to improve workflow and performance while reducing turnaround time and potentially overall cost.

Methods: 310 urine culture specimens as well as selected less frequent pathogens (*A. urinae* - 26 strains, *C. urealyticum* - 4 strains) were plated on four chromogenic agars in parallel with standard protocol MacConkey (MAC) and blood agar (BA). Chromogenic agars evaluated were: UriSelect™ 4 (Bio-Rad), CHROMID® CPS® Elite (bioMérieux), Brilliance™ UTI Clarity™ agar Biplate (Oxoid) and BD™ CHROMagar™ Orientation (BD). Primary outcome was overall growth performance for frequent pathogens and for gram positives, where chromogenic agars were previously reported to underperform. The number of additional tests needed and the appreciation of different media by laboratory personnel were also assessed. A sub-analysis measured the total time required to plate and to read 50 consecutive specimens comparatively for the 4 chromogenic agars and for MAC/BA.

Results: Global performance was 90% for UriSelect, 88% for ChromID, 89% for Chromagar and 81% for Brilliance compared to 84% for standard method. ChromID and Brilliance supported the growth of more *A. urinae* and *C. urealyticum* than the other 2 chromogenic agars. All mono-plate chromogenic agars were appreciated equally by technologists. In addition, for all chromogenic agars, working time was reduced by half as compared to MAC/BA. We estimated a time economy of approximately 80 hours per month in our laboratory, translating in a net annual economy.

Conclusion: All 4 chromogenic medias evaluated in our study had an acceptable performance, with specific strengths and weaknesses for each one. The choice of ChromID CPS Elite (bioMérieux) for our center was based on pre-established criteria including performance for more fastidious gram positives, best time and cost economy, and compatibility with current identification method and susceptibility testing platform. However, since the 4 chromogenic agars have been adequately verified in our laboratory, we consider that they could be interchangeable if needed.

Disclosures: All Authors: No reported disclosures

660. Evaluation of Qvella's FAST-Prep™ Liquid Colony™ for Early Antimicrobial Sensitivity Testing of Positive Blood Culture by Disk Diffusion Method

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Session: P-25. Diagnostics: Bacteriology/mycobacteriology

Background: Conventional antimicrobial susceptibility testing (AST) of microorganisms from positive blood cultures (PBC) can take ≥ 2 days. In order to improve the turnaround time for AST on a PBC, CLSI and EUCAST have made efforts to standardize procedures for disk diffusion (DD) direct from a PBC. Qvella Corporation

(Richmond Hill, ON, Canada) has recently developed FAST-Prep, an automated centrifugal sample preparation system that rapidly delivers a Liquid Colony consisting of a purified, concentrated, viable cell suspension directly from a PBC. This study was performed to investigate the feasibility of DD AST off of a PBC using a FAST-Prep Liquid Colony.

Methods: Contrived PBC samples were prepared by spiking 6 species of Gram-positive and 4 species of Gram-negative bacteria (3-5 strains per species) into FA Plus bottles and incubating in the BACT/ALERT™ VIRTUO™ System (bioMérieux, Durham, NC). After positivity, 3 mL of PBC was added to the FAST-Prep cartridge. After 20 minutes of processing in the FAST-Prep instrument, the Liquid Colony was removed from the cartridge and a 0.5 McFarland sample was prepared for DD AST. In parallel, the DD AST from a PBC was performed using 4 drops of PBC (CLSI direct method). Both methods were compared to conventional colony-based DD AST. After 16-18 hours of incubation zone diameters and S/I/R interpretations were determined. Categorical agreement (CA) and errors for both DD AST methods were calculated. In addition, colony plate counting was performed on 0.5 McFarland suspensions of Liquid Colony and the plate colony to determine biomass recovery and sample purity.

Results: CA for a FAST-Prep DD AST for Gram-positive and Gram-negative bacteria was 95.6% and 98.6%, respectively, compared to CA for CLSI DD AST of 77.2% and 81.9%, respectively. Biomass in the Liquid Colony was 7.2x10⁸ and 1.2x10⁹ CFU for Gram-positive and Gram-negative bacteria, respectively. Cell concentration in the 0.5 McFarland suspension of the Liquid Colony was 3.7x10⁷ and 5.9x10⁷ CFU/mL for Gram-positive and Gram-negative bacteria, respectively, which was similar to the concentration for the reference colony suspension.

Conclusion: The results support the potential role of FAST-Prep in providing a Liquid Colony for use in rapid AST.

Disclosures: Susan M. Novak-Weekley, PhD, D(ABMM), Qvella (Employee, Shareholder) Aye Aye Khine, PhD, Qvella (Employee, Shareholder) Tino Alavie, PhD, Qvella (Employee) Namidha Fernandez, MS, Qvella (Employee) Laxman Pandey, MS, Qvella (Employee) Abdossamad Talebpour, PhD, Qvella (Employee, Shareholder)

661. Futility of Bacterial Bone Marrow Cultures: Experience over a 19 Year Period

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Session: P-25. Diagnostics: Bacteriology/mycobacteriology

Background: Bone marrow biopsies are often performed on patients with unclear diagnoses and cultures may be ordered for both routine bacterial, mycobacterial and fungal pathogens. They are performed in semi-sterile conditions and involve needle penetration through the skin, posing an increased risk of skin contamination. These cultures also require a substantial amount of laboratory personnel time.

Methods: Cultures collected from 2001-2020 were surveyed in the lab electronic record. We assessed the culture type (fungal, bacterial, mycobacterial), and the presence of pathogens and contaminants. An organism was deemed a contaminant if it was consistent with skin flora or listed as a contaminant in the report given to the physician. Organisms for which the role in bone marrow disease is unclear were included as possible pathogens. For questionable non-contaminant organisms, clinical significance was determined based on if patient was treated for the organism. For all bone marrow cultures, growth of the same organism within 1 month of the bone marrow specimen was surveyed to determine whether the organism would have been found by alternative methods.

Results: Of 483 bacterial bone marrow cultures, there were 110 (23%) positives, of which 76 (69%) were deemed contaminants. Twenty (18%) of the 76 contaminants grew in the routine bacterial culture. However, 49 (65%) contaminants grew in the AFB culture, of which 10 also grew in the bacterial culture. For the 34 non-contaminant organisms, 26 were determined to be clinically significant. Nineteen of the 26 had a matching culture (usually blood) growing the organism within 1 month. The majority of pathogens were mycobacteria (18 of the 34). Fungal organisms represented 5 cultures and 11 were bacterial. Of the 11 bacterial organisms, 1 was a *Helicobacter* species (grown in special media), and 4 had a matching positive blood culture. Only 4 (1% of 483) bacterial non-contaminants grew in the routine bacterial culture. Given an unknown number of true negatives, we can only conclude a positive predictive value (PPV) of 0.16 for routine bacterial cultures. Including AFB and fungal cultures, the PPV increased to 0.30.

Conclusion: Our findings indicate that routine bacterial bone marrow culture is unlikely to yield a novel result and is likely a poor use of lab resources.

Disclosures: All Authors: No reported disclosures

662. Identification of Clinically Relevant Microbes with the MasSpec Pen

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Session: P-25. Diagnostics: Bacteriology/mycobacteriology

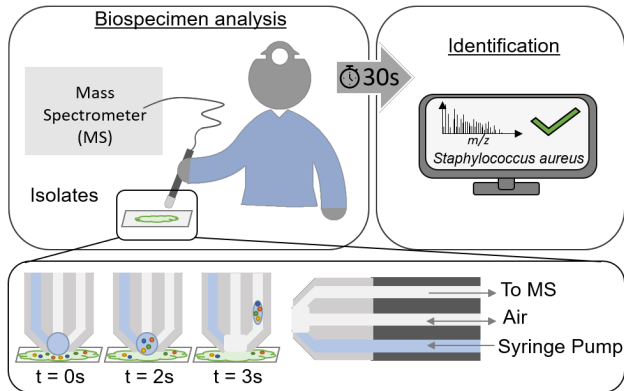
Background: In the age of antimicrobial resistance, rapid identification of infectious agents is critical for antimicrobial stewardship and effective therapy. To this end, ambient ionization mass spectrometry techniques have been applied for rapid identification of microbes directly from culture isolates. We have developed a handheld, mass spectrometry-based device, the MasSpec Pen, that permits direct molecular analysis of

a biological sample in seconds (Scheme 1). Here, we employ the MasSpec Pen to identify clinically relevant microbes directly from culture isolates.

Methods: *Staphylococcus aureus*, *Staphylococcus epidermidis*, Group A and B *Streptococcus*, *Kingella kingae* (*K.k.*), and *Pseudomonas aeruginosa* (*P.a.*) were cultured on 5% sheep's blood nutrient agar at 37 °C overnight. Colonies were transferred to a glass slide where they were analyzed directly with the MasSpec Pen coupled to a Q Exactive mass spectrometer (Thermo Scientific) in negative ion mode. For MasSpec Pen analysis, a 10 µL droplet of water was held in contact with the sample surface for 3 seconds and then aspirated to the mass spectrometer for analysis.

Data was normalized and the molecular features resulting from the analysis solvent and nutrient medium were removed. The least absolute shrinkage and selection operator (lasso) statistical method was used to build classification models for prediction of bacterial identity. Model performance was evaluated by leave-one-out cross-validation and a validation set of samples.

Scheme 1: MasSpec Pen workflow



Results: Various small molecules were detected including metabolites and glycerophospholipid species. The mass spectral profiles for each species exhibited qualitative differences among them (Figure 1). Additionally, several quorum-sensing molecules were observed in *P.a.* including hydroxy-heptyl-quinoline (*m/z* 242.155).

Lasso statistical classifiers were created to differentiate organisms at the level of Gram type, genus, and species with each model comprised of a sparse set of molecular features. Accuracies of 90% or greater were achieved for all lasso models and as high as 98% for the differentiation of *Staphylococcus* (*Staph.*) and *Streptococcus* (*Strep.*).

Figure 1: Molecular profiles of species analyzed

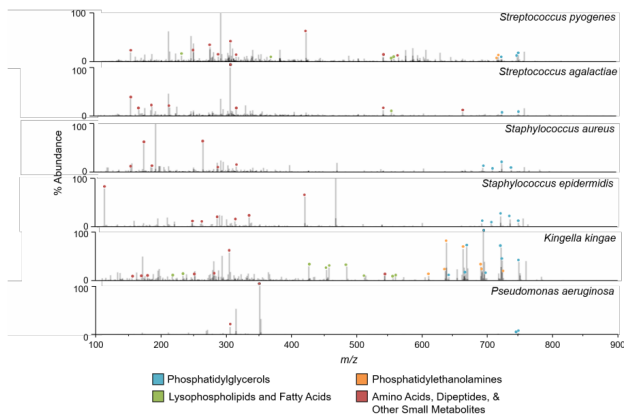
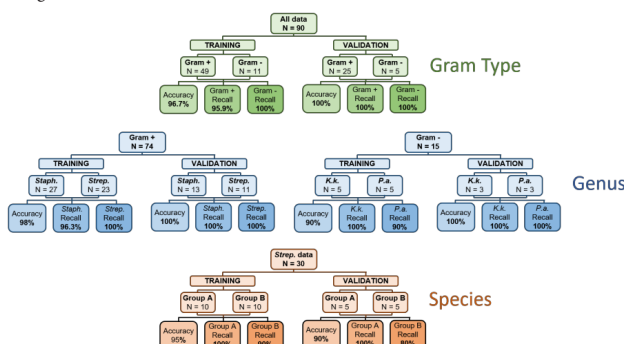


Figure 2: Statistical classification results



Conclusion: These results demonstrate the potential of the MasSpec Pen as a tool for clinical analysis of infected biospecimens.

Disclosures: Sydney C. Povilaitis, BA, MS Pen Technologies, Inc. (Other Financial or Material Support, Patent) Livia Eberlin, PhD, MS Pen Technologies, Inc. (Board Member, Shareholder)

663. Improved Detection of ESBL and AmpC Beta-Lactamase Producing Isolates of Enterobacteriaceae in Pediatric Patients with Bloodstream Infections Using Combined Genotypic and Phenotypic Antimicrobial Susceptibility Testing
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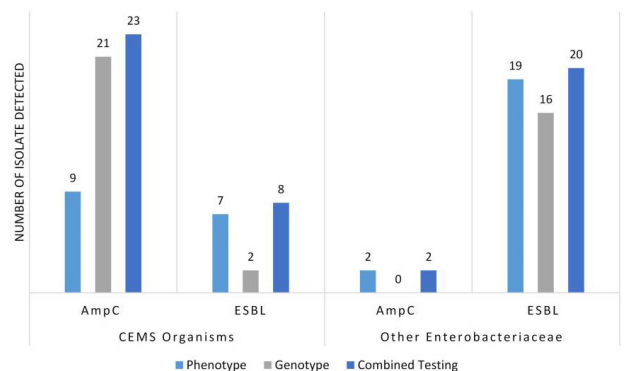
Session: P-25. Diagnostics: Bacteriology/mycobacteriology

Background: Infections secondary to pathogens resistant to third-generation cephalosporins (3GC), such as extended-spectrum (ESBL) and AmpC β-lactamase (AmpC) producing *Enterobacteriaceae*, are increasing. Currently, there are no recommendations regarding identification of AmpC in *Citrobacter*, *Enterobacter*, *Morganella* and *Serratia* spp. (CEMS organisms). This study's aim was to increase the detection of AmpC and ESBL producing *Enterobacteriaceae* in blood cultures from pediatric population by combining genotypic with phenotypic antimicrobial susceptibility testing (AST).

Methods: All first time *Enterobacteriaceae* isolates recovered from blood cultures of pediatric patients at CCHMC between January 2017 and December 2018 were evaluated. The Check-MDR CT103XL assay was used to determine the presence of AmpC and ESBL. AST was performed using the Vitek 2 platform. Phenotypic ESBL resistance was defined by resistant to either ceftaxime or ceftazidime using CLSI breakpoints. Combined ceftaxime resistance with ceftaxime or ceftazidime resistance was used to define phenotypic AmpC (EUCAST standards).

Results: There were 170 isolates, from 147 patients, with 21 (12.4%) AmpC and 18 (10.6%) ESBL genes detected. Using AST, 11 (6.5%) and 26 (15.3%) isolates met AmpC and ESBL phenotypic criteria respectively. However, 14 of the isolates with AmpC genes detected and 2 of isolates with ESBL genes detected failed to meet phenotypic criteria. In addition, 4 (19%) of 21 AmpC isolates were susceptible to ceftaxime and 3GC while both *E. coli* and *S. marcescens* genotypic ESBL isolates were susceptible to 3GC.

Number of AmpC- and ESBL- Producing Isolates Detected Using Phenotypic Method a,b, Genotypic Method and Combined Testing



Conclusion: We identified 16 (9.4%) isolates with resistance genes detected but that failed to meet phenotypic criteria.

Without molecular testing, patients with these isolates may have been treated with 3GC which could have resulted in treatment failure.

The addition of genotypic testing to AST improved the identification of AmpC and ESBL organisms and provided clinically relevant data to guide treatment of resistant organisms. Combined testing is also beneficial for infection control and epidemiological purposes.

Disclosures: All Authors: No reported disclosures

664. LiaX as a Surrogate Marker of Daptomycin Susceptibility in Multidrug-Resistant Enterococcus faecium Recovered from Cancer Patients

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Session: P-25. Diagnostics: Bacteriology/mycobacteriology

Background: Vancomycin-resistant *Enterococcus faecium* (VRE_fm) are leading causes of bloodstream infections (BSI) in patients (pts) with hematological malignancies (HM). Daptomycin (DAP) is commonly used to treat VRE BSI, but DAP non-susceptibility (DAP-NS) in pts with HM is increasing. Current methods to determine DAP minimum inhibitory concentrations (MICs) have poor reproducibility. DAP