and the ESwab is submitted to the microbiology laboratory for bacterial culture, if indicated. Residual ESwab specimens were de-identified, cultured, and tested using the Alere and Roche molecular assays (at the time of de-identification the result of the GAS rapid antigen test that was performed on the same patient at the time of ESwab collection was noted). Following testing, ESwab specimens were frozen and tested on the Cepheid molecular assay within 6 months. In total 194 specimens were compared.

**Results.** Specimens positive by culture or in two of three molecular assays were considered true positives. The results can be seen in the Table below.

**Acknowledgements.** Molecular testing reagents and equipment were provided by Roche, Alere, and Cepheid.

**Disclosures.** All authors: No reported disclosures.

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### Table 1: Karius NGS Data

| Patient | Immunizations Up to Date | Organism ID | MPM (Molecules/μL) | Serotype |
|---------|--------------------------|-------------|---------------------|----------|
| 18 Months (CNMC) | Y | S. pneumoniae | 1,957,238 | 3 |
| 11 Months (Rady) | Y | S. pneumoniae | 9,122,698 | 3 |
| 28 Months (Rady) | Y | S. pneumoniae | 151,941,207 | 12A |
| 42 Months (Rady) | N | S. pneumoniae | 1,439,748 | 3 |

*Median MPM in non-HUS S. pneumoniae positive samples over the last 90 days was 1202 MPM.

**Disclosures.** S. Venkatakrishnamohan, Karius, Inc.: Employee, Salary. D. Hong, Karius, Inc.: Employee, Salary.

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### 2295. Streptococcus pneumoniae-Related Hemolytic Uremic Syndrome (pHUS) and the Identification of Matched Cross Country Serotypes by Plasma Next-Generation Sequencing (NGS)

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**Background.** Hemolytic uremic syndrome (HUS) describes a clinical presentation of kidney injury, microangiopathic hemolytic anemia and thrombocytopenia. Five to 15% of HUS cases are related to *Streptococcus pneumoniae* infection, most often meningitis or pneumonia. Despite the introduction of PCV13 and a decrease in invasive pneumococcal disease in children, the incidence of pneumococcal-related HUS (pHUS) cases is rising for unclear reasons. Efforts to determine whether certain serotypes increase the risk of pHUS are often hampered by negative cultures in patients with suspected pneumococcal disease. Direct microbiologic detection methods, such as next-generation sequencing (NGS), may be useful in identifying pHUS cases. We describe four children with pHUS from two institutions that were identified via NGS of cell-free plasma.

**Methods.** Four patients with HUS and negative initial cultures were identified. Blood was sent to Karius (Redwood City, CA) for pathogen detection via plasma NGS. Cell-free DNA was extracted and NGS performed. Human sequences were removed and remaining sequences were aligned to a curated pathogen database including over 1000 organisms. Organisms present above a predefined statistical threshold were reported. For serotyping by NGS, sequences were aligned to a collection of 90 serotype-associated cps alleles.

**Results.** In this case series, we report on four patients with pHUS identified via plasma NGS. These cases demonstrate the potential of NGS for pathogen detection and quantitation in plasma to assist in identification of culture-negative infections, as well as the potential to identify clusters of disease that would likely otherwise have gone undetected.

**Table 1:**

| Patient | Immunizations Up to Date | Organism ID | MPM (Molecules/μL) | Serotype |
|---------|--------------------------|-------------|---------------------|----------|
| 18 Months (CNMC) | Y | S. pneumoniae | 1,957,238 | 3 |
| 11 Months (Rady) | Y | S. pneumoniae | 9,122,698 | 3 |
| 28 Months (Rady) | Y | S. pneumoniae | 151,941,207 | 12A |
| 42 Months (Rady) | N | S. pneumoniae | 1,439,748 | 3 |

*Median MPM in non-HUS S. pneumoniae positive samples over the last 90 days was 1202 MPM.

**Disclosures.** S. Venkatakrishnamohan, Karius, Inc.: Employee, Salary. D. Hong, Karius, Inc.: Employee, Salary.

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### 2296. Development of a Sequencing-Based Assay for Detection of CMV Antiviral Resistance Mutations to Letermovir in UL56

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**Session:** 245. Molecular & Sequence Based Diagnostics Saturday, October 6, 2018: 12:30 PM

**Background.** Antiviral resistance to human cytomegalovirus (CMV) is a growing concern for immunocompromised patients on prolonged antiviral regimens, and CMV remains the most clinically significant infection following allogeneic hematopoietic-cell transplantation. Letermovir targets subunit 2 of the viral terminase complex (UL56) and is approved for CMV prophylaxis in adult stem cell transplant recipients. Resistance to letemovir is conferred by point mutations in the UL56 gene, and with the potential clinical need for antiviral resistance testing, we have developed a UL56 sequencing assay covering 23 identified resistance mutations. Here we summarize the performance characteristics of the UL56 antiviral resistance assay.

**Methods.** This assay uses automated nucleic acid extraction followed by CMV UL56-specific polymerase chain reaction (PCR). PCB products are subjected to cycle sequencing and capillary electrophoresis, and the resulting sequences are analyzed for the presence of known resistance mutations between codons 229 and 369 of the UL56 gene. The assay’s limit of detection (LOD), precision and accuracy were validated in accordance with accepted regulatory standards using multiple laboratory and clinical CMV strains.
Results. The LOD was determined to be 99 IU/mL. Precision was demonstrated with multiple sample replicates over three days of testing, with 100% amino acid concordance within the region of interest (ROI). The assay also accurately identified 100% of amino acids within the ROI of 30 unique CMV-positive de-identified clinical samples. While some polymorphisms were detected, no mutations conferring resistance were identified in the clinical samples tested, which is in agreement with the literature indicating that naturally occurring polymorphisms in the UL56 gene have not been shown to confer resistance to letermovir.

Conclusion. The CMV UL56 antiviral resistance assay was shown to be a rapid and sensitive means of detecting mutations conferring letermovir resistance. This expands current CMV antiviral resistance testing, which includes UL54 and UL97 sequencing, and provides physicians with the ability to monitor for the emergence of antiviral resistance mutations to all current FDA-approved anti-CMV drugs.

Disclosures. J. Grantham, Viracor Eurofins Clinical Diagnostics: Employee, Salary. J. Nutt, Viracor Eurofins Clinical Diagnostics: Employee, Salary. A. Tyler, Viracor Eurofins Clinical Diagnostics: Employee, Salary. E. Bixler, Viracor Eurofins Clinical Diagnostics: Employee, Salary. M. Altrich, Viracor Eurofins Clinical Diagnostics: Employee, Salary. S. Kleibooker, Viracor Eurofins Clinical Diagnostics: Employee, Salary.

2297. The Diagnostic Yield of 16/18S rRNA PCR of Sterile Site Samples in Pediatric Patients

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Background. 16S ribosomal RNA (rRNA) and 18S rRNA gene polymerase chain reaction (16/18S PCR) with sequencing can provide expeditious bacterial or fungal pathogen identification from sterile site samples (cost $474/PCR). Our objective was to assess the utilization and diagnostic yield of 16/18S PCR of sterile site samples in pediatric patients.

Methods. Patients’ sterile site fluid or direct tissue specimens were collected and cultured at Lurie Children’s Hospital of Chicago and sent to Northwestern Memorial Hospital for 16/18S PCR as clinically indicated. Clinical data were reviewed including PCRs, cultures, and medical conditions.

Results. 16/18S PCR testing increased over the study period. In total, 177 samples were sent for 16S and/or 18S PCR from 146 patients (January 2016–April 2018). Osteoarticular, CSF, pleural fluid and organ tissue (n = 28; lung=19, chest mass=2, liver=2, spleen=2, etc.) sites were most frequent. The yield of 16/18S PCR by source is listed in Table 1. Twenty-eight of 156 samples for 16S PCR were positive (17.9%); 21 with a single organism ID, one with two organisms, and 6 indeterminate. (Table 2). Of negative 16S PCR samples, one grew Mycobacterium avium complex in culture. 18S PCR was performed on 108 unique samples; 7 were positive (6.5%, Table 3). For 4 positive 18S PCRs, a fungus also grew in culture with 3 concordant results and one discordant. Two negative 18S PCR samples grew Phellinus spp., Blastomyces dermatitidis. All patients (100%) with positive 18S PCR were immunocompromised compared with 21% (6/28) with positive 16S PCR. Both 16S and 18S PCRs were sent on 87 samples of which 16S PCR was positive in 5, 18S PCR was positive in 3, and none had both 16S and 18S PCRs positive.

Conclusion. 16/18S PCR can provide important infectious pathogen diagnostics. 16S PCR should be sent only if bacterial culture is negative with higher yield sites being brain, abscess, pleural effusion, bone/joint and CSF. 16S PCR appears useful if an anacrobic pathogen is likely but conditions are not optimal for recovery. 18S PCR is highest yield in patients at risk of fungal disease. 16 and 18S PCRs were often sent together, likely reflexively. Selective or sequential testing may be advisable for most cases, guided by the clinical index of suspicion. Best practices to optimize resource utilization and clinical impact are evolving.

Disclosures. All authors: No reported disclosures.

2298. Identifying and Addressing Implementation Barriers to Whole-Genome Sequencing (WGS) in State Public Health Laboratories

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Session: 245. Molecular & Sequence Based Diagnostics
Saturday, October 6, 2018: 12:30 PM

Background. The past decade has witnessed revolutionary advances in DNA sequencing, bioinformatics, and related technologies. The Advanced Molecular Detection (AMD) program at the Centers for Disease Control and Prevention (CDC) is a catalyst for bringing advanced DNA sequencing and related technologies to the forefront for combating a wide range of infectious disease threats by the US public health system, resulting in quicker detection of outbreaks and more effective public health responses. Bacterial whole-genome sequencing (WGS) has many applications in public health and is now being implemented in several areas both at the CDC and in state public health laboratories (SPHLs). While SPHLs have overcome a variety barriers to the implementation of WGS technology, only a small percentage of SPHLs using bacterial WGS (3 out of 26) have validated workflows that comply with regulations set forth by the Clinical Laboratory Improvement Amendments of 1988 (CLIA). If a piece of data has the potential to make it back to a patient’s record, then the laboratory