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.

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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 expedient 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 molds (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 16/18S 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 anaerobic 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.

Table 2

| Sample source | + 16S PCR organism | Gram stain/culture |
|---------------|--------------------|--------------------|
| Brain abscess | S. intermedius      |                    |
| Brain abscess | S. pneumoniae      |                    |
| Brain abscess | P. aerogens        |                    |
| Pleural fluid | P. aerogens        |                    |
| Pleural fluid | S. pneumoniae      |                    |
| Pleural fluid | S. intermedius     |                    |
| Pleural fluid | P. aerogens        |                    |
| Spleen        | spleen             |                    |
| Ischemic bone | Ischemic bone      |                    |
| Femoral bone  | Femoral bone       |                    |
| Lung abscess  | Lung abscess       |                    |
| Right eye     | Right eye          |                    |
| Wrist abscess | Wrist abscess      |                    |
| Vertebral abscess | Vertebral abscess |                   |
| Right leg     | Right leg          |                    |

Table 3

| Sample source | + 16S PCR organism | Gram stain/culture |
|---------------|--------------------|--------------------|
| Leg           | Leg                |                    |
| Scalp abscess | Scalp abscess      |                    |
| Skin          | Skin               |                    |
| Scalp fluid   | Scalp fluid        |                    |
| CSF           | CSF                |                    |
| Pleural fluid | Pleural fluid      |                    |
| Spleen        | Spleen             |                    |
| Ischemic bone | Ischemic bone      |                    |
| Femoral bone  | Femoral bone       |                    |
| Lung abscess  | Lung abscess       |                    |
| Right eye     | Right eye          |                    |

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

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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 51) 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
that generated it must be compliant with CLIA. CLIA validation of WGS methods is critical to ensuring safety with regard to patient clinical care.

Methods. As a way to help facilitate WGS implementation, we sought to identify the challenges for the establishment and use of CLIA-compliant WGS workflows in SPHLs. An environmental scan was performed in which we assessed materials produced at CDC, by the Association of Public Health Laboratories (APHL), by a Next-generation Sequencing Tri-agency workgroup, as well as published papers and guidance. We also engaged stakeholders through conversations with SPHL partners, APHL, and several groups within CDC.

Results. Our analysis revealed relevant resources and key WGS validation materials were dispersed and difficult to locate. To address this, we developed a CDC Next-generation Sequencing Resource Roadmap, to house key materials. After we reviewed, selected, and collated the resources, our web developer created a visual roadmap webpage to guide the user through the resources. This roadmap was then reviewed and tested for initial use internally at CDC.

Conclusion. This communication tool has the potential to provide critical resources needed to develop functional WGS validation strategies.

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2299. Oral Microbiome of High-Risk Children: A Cohort Study
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Background. Vulnerable pediatric populations are at high risk of bloodstream infection (BSI) and sepsis, such as patients of the pediatric intensive care unit (PICU) and bone marrow transplant (BMT) wards. Previous research demonstrates that commensal gut anaerobes provide host resistance against colonization and infection with pathogens but data on other body sites is lacking. Characterization of overlap or differences in commensal microbes of the mouth can provide insight on factors that may put these populations at risk for invasive disease.

Methods. A cohort study of patients (0–18 years) at a large pediatric quaternary care center from 2017 to 2018 was conducted. Cohort group 1 children were admitted to the PICU, cohort group 2 patients were admitted to the BMT unit. Matching was by age range. Metagenomic sequencing of oral swabs and statistical analysis was performed. A retrospective review of causes of BSI in both groups from 2016 to 2018 was also conducted.

Results. Eighteen patients in the PICU group and 21 patients in the BMT group were identified. Common causes of BSI from 2016 to 2018 vary in each cohort (Figure 1). Unlike Enterobacteriaceae, which were more common in the BMT cohort, *Pseudomonas aeruginosa* was a more common cause of BSI among PICU patients. When evaluating the oral microbiomes, the number of reads for *Pseudomonas aeruginosa* was significantly higher in the PICU group compared with the BMT group (P = 0.0019, Figure 2).

Conclusion. Children in the PICU have a statistically significant difference in the frequency of oral colonization with *Pseudomonas aeruginosa* when compared with the BMT group. *Pseudomonas aeruginosa* was more common in the PICU. PICU children have a significantly increased oral colonization with *Pseudomonas aeruginosa*.

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2300. Molecular Epidemiology of Carbapenem-Resistant Klebsiella pneumoniae (CRKP) Causing Central Line Associated Blood Stream Infections (CLABSI) in Three ICU Units in Egypt
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Background. CLABSI caused by CRKP is associated with high mortality. Identification of the genetic basis for carbapenem resistance is crucial for selecting the proper antimicrobial therapy, and testing for bacterial clonality. We aimed to study the genetic basis of CRKP causing CLABSI in 3 ICUs, and use ERIC PCR to test for their clonality.

Methods. The study was conducted in a tertiary care hospital in Egypt from January 1, 2016 to December 31, 2017 after approval by the Institution Review Board. We enrolled all patients with CVGs in 3 ICUs. At least 2 sets of blood cultures were collected from each febrile patient by BACT/ALERT system (Bio Merieux, France), before starting an antibiotic. The pathogens and their antimicrobial susceptibility were detected by the VITEK 2 system (Bio Merieux, France). Phenotypic detection of carbapenemase activity was done by modified Hodge (MHT) test and Carba-NP test. Multiplex PCR was done to identify the carbapenemase genes. Molecular typing of carbapenem-resistant isolates was performed by ERIC-PCR.

Results. We enrolled 1,210 patients admitted for 17,785 ICU days. Central catheters were utilized in 53.3% of patients for a total of 11,014 central line days. Out of 130 Gram-negative CLABSI pathogens detected, we identified carbapenem resistance in 57 (43.8%); of which *K. pneumoniae* was the predominant pathogen (27 out of 57, 47.4%). By MHT and carba-NP, 63.79% of *K. pneumoniae* isolates were carbapenemase producers. Multiplex PCR revealed *bla*<sub>NDM</sub> in 48.14% and *bla*<sub>KPC</sub> in 33.33% of the *K. pneumoniae* isolates, whereas *bla*<sub>SHV</sub>, *bla*<sub>GES</sub>, *bla*<sub>TEM</sub>, *bla*<sub>CTX</sub>, *bla*<sub>OXA</sub>, *bla*<sub>GES</sub>, and *bla*<sub>CMY</sub> were not detected. ERIC-PCR analysis of 27 CRKP isolates showed genetic relatedness among only 5 KPC-positive and 2 producers, while most isolates were polyclonal.

Conclusion. We detected a high rate of carbapenem resistance among *K. pneumoniae* causing CLABSI showing *bla*<sub>NDM</sub> in 48.14% and *bla*<sub>KPC</sub> in 33.33%; and they were mostly polyclonal.

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