2023 APHL ID Lab Con
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Poster Abstracts

Monday, March 13, 2023

Diagnostic Microbiology

Poster 01M

High-Throughput, Automated Detection of *Candida auris* on the Cobas(R) 6800/8800 with the Cobas(R) omni Utility Channel

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Background: The US Centers for Disease Control and Prevention (CDC) has recognized *Candida auris* as an emerging fungal pathogen associated with multiple-drug resistance and healthcare-associated outbreaks. Despite the public health relevance, few diagnostic tests are available in the US; most laboratories utilize low throughput, laboratory-developed tests. This study utilizes the cobas® omni Utility Channel of the cobas® 6800/8800 Systems to assess the feasibility of conducting fully automated, higher throughput C. auris testing, using a primer/probe design currently employed by the Antimicrobial Resistance (AR Lab) Network.
Methods: Primers and probes from the C. auris AR Lab protocol were evaluated for system compatibility on the cobas® z480 analyzer using the cobas® omni Optimization Kit, then concentrations titrated to be most effective with the cobas® omni master mix. Once optimized, the assay was evaluated on a cobas® 6800 instrument with spiked specimens of a pure, titered, whole C. auris isolate.
Results: Un-modified, the AR Lab protocol had an LOD of approximately 50 CFU/mL (37.50 ± 0.19 Ct), or 35 CFU/mL (38.09 ± .74 Ct) with an adjusted RFI (Relative Fluorescence Index). This is equivalent to the published assay performance in Leach et al., 2019. Primer methylation has been demonstrated to enhance PCR specificity, and increase sensitivity, by reducing competition with non-productive reactions. Methylation of the AR Lab primers increased assay sensitivity to 10 CFU/mL for both a 500µL (36.51 ± 0.33 Ct) and 200µL (39.35 ± 0.08 Ct) extraction volume. The 200µL extraction volume was added to this protocol upon personal recommendation from S. Chaturvedi. Finally, pre-extraction incubation was evaluated for contribution to assay sensitivity; pre-extraction with Bacterial Lysis Buffer/Proteinase K increased sensitivity of a 500µL extraction volume (1 CFU/mL, 38.8 ± 0.37 Ct), but did not impact the 200µL extraction volume appreciably.
Discussion: This study demonstrated proof-of-concept application of the cobas® omni Utility Channel using a previously published AR Lab protocol for Candida auris. Primer/probe design of the AR Lab protocol was compatible with the cobas® omni Utility Channel; methylation of primers, however, increased assay sensitivity from 50 CFU/mL to between 1-10 CFU/mL depending on the input volume and use of pre-incubation steps. Studies evaluating the performance of this assay with previously characterized clinical specimens are ongoing.

References: Leach, L, Zhu, Y, & Chaturvedi, S. (2018). JCM, 56(2), e01223-17.
Schoenbrunner, NJ et al., (2017). Biol Meth Protoc, 1-10.

Presenter: Stephen McCune, Roche Diagnostics, Indianapolis, IN, stephen.mccune@roche.com

Poster 02M

Stability of the PilotTM COVID-19 At-Home Test After Exposure to Extreme Temperatures

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Background: Test to stay, test to treat, test to return to work. Testing has become a household practice within the COVID-19 pandemic response, especially with the availability of rapid antigen tests for non-prescription home use. These rapid antigen lateral flow tests ("rapid tests") are also of critical importance in public health initiatives to reach at risk populations, such as the homeless, homebound, schools, and congregate care facilities. But how reliable are these tests when temperature extremes are encountered? The purpose of this study was to examine the stability and performance of the PilotTM At-Home Test (SD Biosensor/Roche) after exposure to extreme temperatures.

Methods: The PilotTM rapid test kits were stored at five different temperatures: frozen (-4.0°F [-20.0°C]), refrigerated (42.8°F [6.0°C]), room temperature (68.0°F [20.0°C]), warm (98.0°F [36.7°C]), and excessive heat (118.0–126.0°F [47.8–52.2°C]) for either 24 hours, 48 hours, 1 week or 2 weeks. After the temperature excursion, kits were brought back [i.e. normalized] to room temperature for 60, 90, or 120 minutes before use. Performance was measured for five positive and five negative samples per each temperature, duration, and normalization variable examined. Line intensity was evaluated using a color intensity scale (0-100%).

Results: The tests performed as expected in all scenarios after storage, and all normalization conditions, at temperatures ranging from frozen (-4.0°F [-20.0°C]) to excessive heat (118.0–126.0°F [47.8–52.2°C]) over a period of time ranging from 24 hours to 2 weeks. Positives did not vary in color intensity across the temperature range or normalization time points examined.

Discussion: Both individual households and congregate living facilities rely on the data provided by COVID-19 rapid tests to make decisions around their health status. From a public health perspective, rapid tests provide critical insights on the need for patients to isolate, their ability to return to work/school, and on eligibility for antiviral therapies. Rapid test manufacturers provide recommendations for storage within specific temperature ranges, but exposure to extreme temperatures is a real-world variable that must be evaluated. This study demonstrated that despite exposure to a wide range of temperatures, the PilotTM test results are highly reliable simply by returning the test kit to room temperature for 60 minutes prior to testing. These results are consistent with those obtained following the current FDA EUA recommendation of allowing all rapid tests to sit at room temperature for two hours prior to use.

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Poster 03M

Public Health and M. tuberculosis-Complex Nucleic Acid Amplification (NAA) Testing: Finding A Balance

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Background: In June 2021 the State of Arizona Tuberculosis Program (AZ-TB) launched a 12-month NAA testing collaborative with the State Public Health Lab (AZ-Lab). AFB smear(-) clinical sputa, not normally triggering NAA reflux, would be assessed for possible NAA testing. A goal was to determine the right balance of testing based on resources and patient impact.

Intervention/Response: AZ-TB evaluated whether broader NAA testing, alongside the existing reflux after AFB sputum smear(+) result, would promote earlier treatment initiation. Inclusion criteria were: 1-public health submitters without in-house NAA capabilities; 2-clinical sputum sample; 3-AFB smear(-); and 4-no prior NAA or culture(+) within the last 365-days. AZ-TB reviewed samples daily and notified AZ-Lab which sample(s) to include.

Results: During the 12-month process, 214 samples met inclusion criteria, resulting in an increase of 12.5 NAAs per month. Four samples were AFB smear(-) and NAA(+), accounting for < 2% of all NAAs. Of those four, treatment started 1-day prior to NAA result for one patient and 3, 7, and 23-days after NAA result for the remaining three patients.

Conclusions: Highlights of the testing collaborative included exploring a deeper connection with laboratory data, automating morning emails for eligible samples to review, an expansion to selected correctional partners, and reenforcing the lab/program partnership. It's unclear if universal NAA testing on sputum smear(-) samples impacts treatment initiation and public health interventions among patients already followed by public health. The NAA testing collaborative continues and will be adapted based on ongoing analysis and building upon lessons learned.

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Poster 04M

Prevalence of *Mycoplasma genitalium* among High-risk Persons in Dallas County

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Background: *Mycoplasma genitalium* (MG), while not a nationally reportable or notifiable disease, is highly prevalent compared to other sexually transmitted infections (STI), e.g., Chlamydia, Gonorrhea, and Trichomonas. A previous national meta-analysis of MG by Lisa Manhart, Ph.D., and colleagues in 2020 reported a prevalence of sexually active women (19.80%) and men (16.50%) between the ages of 15 and 24. From these data, it is expected that prevalence in the population of individuals tested at the Dallas County Health and Human Services (DCHHS) Sexual Health Clinic (SHC) would represent high-risk for MG, and therefore warrant monitoring. Considering that persistent MG infection has been associated with adverse reproductive outcomes, determining prevalence is imperative to implement testing and direct treatment. It is the goal of this study to provide supportive evidence of MG prevalence in the DCHHS SHC population to justify a monitoring program.
Aims/purpose: To conduct a prevalence study, determining the burden of and risk factors for MG among the high-risk cohort of DCHHS SHC patients.
Methods: Collect ten (10) reserve urine specimens submitted for Gonorrhea, Chlamydia, and Trichomonas testing at the DCHHS SHC every day until 400 specimens are collected. To obtain clinical and demographic data, a retrospective review will be conducted using Greenway Laboratory Information System (LIS). All specimens will be de-identified and tested for MG utilizing the Aptima Mycoplasma genitalium Nucleic Acid Amplification Test (NAAT) Assay on the Hologic Panther System. Results will be analyzed for prevalence and adjusted ratios for sex, age, race, ethnicity, co-infection, and symptom status using logistic regression via STATA SE 16 (64-bit).
Results: At the time of abstract submission, more than half of the total specimen reserves have been collected. Of this preliminary cohort, comprised of female (51.43%) and male (48.57%) groups, disease prevalence for each is: Chlamydia (10.00%), Gonorrhea (8.57%), and Trichomonas (9.05%). Prevalence of co-infection is 3.81%, with the most common co-infection being Chlamydia and Gonorrhea. The prevalence of all STI's is the highest in those 25-34 years of age (45.00%), and second highest in those 15-24 years of age (23.50%).
Conclusions: In the reviewed literature, MG prevalence is similar to Chlamydia, often exceeding Gonorrhea (Getman, et al., 2016). If this trend follows in Dallas County, then MG is expected to be as prevalent as Chlamydia (10%) - constituting a public health concern. Risk factors for MG infection include young age and coinfection with other STIs (Manhart, et al., 2020). This trend is currently being observed in the data to date. Young age, specifically between ages of 25 and 34, have the highest prevalence of STIs. Though coinfection prevalence is currently observed to be low (3.81%), it is anticipated to rise with greater sample size. The results of this study will aid in providing a reference range for subsequent verification studies and onboarding of the Aptima MG Assay for patient testing.

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Poster 05M

GAMBIT (Genomic Approximation Method for Bacterial Identification and Tracking): A Methodology to Rapidly Leverage Whole Genome Sequencing of Bacterial Isolates for Clinical Identification

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Whole genome sequencing (WGS) of clinical bacterial isolates has the potential to transform the fields of diagnostics and public health. To realize this potential, bioinformatic software that reports identification results needs to be developed that meets the quality standards of a diagnostic test. We developed GAMBIT (Genomic Approximation Method for Bacterial Identification and Tracking) using k-mer based strategies for identification of bacteria based on WGS reads. GAMBIT incorporates this algorithm with a highly curated searchable database of 48,224 genomes. Herein, we describe validation of the scoring methodology, parameter robustness, establishment of confidence thresholds and the curation of the reference database. We assessed GAMBIT by way of validation studies when it was deployed as a laboratory-developed test in two public health laboratories. This method greatly reduces false identifications which are often detrimental in a clinical setting. Furthermore, the number of microbial whole genome sequences has rapidly increased since 2016 and will continue to exponentially increase. The highly curated and quality-controlled GAMBIT database, constituted of genomes from NCBI RefSeq, has not been updated since its launch in 2016. Currently the database contains ~50,000 genomes covering 1,445 species. The NCBI RefSeq database (source) now contains over 200,000 microbial genomes covering over 8000 species with at least two representative genomes (required for the GAMBIT database). We are in the process of updating the GAMBIT database with these additional genomes and adding eukaryotic microbial pathogens to the database.

Presenter: David Hess, Nevada State Public Health Laboratory, Reno, NV, dhess@unr.edu

Poster 06M

Clinical Identification of Dimorphic Molds Using the Bruker MALDI-TOF

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Objective: The Michigan Department of Health and Human Services (MDHHS) performs confirmation identification of the dimorphic molds (Blastomyces species, Histoplasma capsulatum and Coccidiodies species) from submitted culture isolates. Due to the discontinuation of the Hologic Accuprobe assay, MDHHS decided to validate MALDI-TOF as our primary confirmation identification method of dimorphic molds. This required assessing inactivation and extraction methods in addition to alternative spectral libraries as Bruker's libraries currently do not contain representative spectra for these molds.

Study Design: The validation included inactivation, culture age, phase (yeast or mold), media, reproducibility, sensitivity, and specificity studies. Cultures included within the study comprised of isolates previously identified using the Hologic Accuprobe assay. The inactivation study included a heat (90 ± 5° for 30 minutes) inactivation method and alcohol deactivation using 70% and 100% alcohol with a 3-minute exposure). Multiple extraction methods were tested including the CDC Identification of Filamentous Fungi for Bruker Biotyper, Bruker Cultivation and Sample Preparation for Filamentous Fungi, Vitek Mold Sample Preparation, and the NIH MALDI TOF MS Extraction Procedure for Molds grown on Solid Media. Each of these procedures were analyzed with each of the dimorphic molds and an in-house procedure was developed from them. This method most closely resembled the NIH technique and was used for the entirety of the validation study. Isolates were tested on Sabourand Dextrose (SAB), Kellys agar, and Cysteine agar using both phases and culture ages ranged from week 1 to 4. Inter and intra-assay reproducibility was assessed along with sensitivity and specificity. MALDI scores > 1.7 obtained from the Bruker Biotyper Sirius One using the NIH fungal library were considered acceptable for full reporting (including to species level for Histoplasma capsulatum).

Results: Heat inactivation impacted spectral quality while chemical inactivation with 70% alcohol for 3 minutes was found to kill all isolates without impacting MALDI-TOF score quality. MDHHS found the NIH extraction method to work best in terms of spectral quality and workflows. Reportable results were obtained for Blastomyces sp. and Coccidiodies sp. regardless of phase or isolate age. Histoplasma capsulatum failed to produce reportable scores at 4 weeks for the yeast phase and 1 week for mold the phase. A 93% (13/14) intra-assay and 100% (5/5) inter-assay reproducibility were observed. One isolate failed to produce a score during retesting for the intra-assay study. No misidentifications were observed. Overall, a 98.6% (74/75) sensitivity and 100% specificity were observed.

Conclusions: MDHHS found this assay to be an acceptable alternative for clinical identification of dimorphic molds. The test is cost effective while providing a sufficient turnaround time with high specificity and sensitivity. As with any testing, MALDI results will be used in conjunction with microscopic identification. MDHHS will continue to investigate new libraries as they become available."

Presenter: Tonya Heyer, Michigan Department of Health and Human Services, Lansing, MI, heyert@michigan.gov

Poster 07M

Rapid Testing Methods to Combat Penicillin Resistant *Neisseria meningitidis*

A. Peifer, A. Kidney, S. Jose, G. Nattanmai, L. Randall, T. Halse, M.-C. Rowlinson and K. Mitchell, Wadsworth Center, Albany, NY

Neisseria meningitidis is a gram-negative diplococci and is the causative agent of invasive meningococcal disease (IMD). N. meningitidis has 12 serogroups differentiated by the content of their capsular polysaccharides. Of these 12 serogroups, IMD is predominantly caused by B, C, and Y. Meningococcal disease is fatal in 10-15% of patients, with another 20% of patients suffering from other long term neurological sequelae. Prompt antibiotic treatment is critical due to the severity of IMD. Antibiotic resistance in N. meningitidis in the past has been uncommon, and cases of meningococcal disease are typically treated with penicillin, ampicillin, cefotaxime, or ceftriaxone. Recently, there has been an increase of ciprofloxacin and penicillin-resistant, β-lactamase-producing N. meningitidis serogroup Y cases in the United States, thus healthcare providers should ascertain susceptibility of meningococcal isolates to penicillin for starting treatment. A CDC report indicated that 33 cases of N. meningitidis serogroup Y were reported between 2013-2020 and all isolates contained a blaROB-1 β-lactamase enzyme gene conferring resistance to penicillins. To support routine antibiotic susceptibility testing (AST) on N. meningitidis isolates Wadsworth Center developed a real-time PCR assay to detect the presence of blaROB1 in N. meningitidis isolates to help predict penicillin susceptibility faster than AST alone, contributing to the timely application of the correct antibiotic in cases of IMD. The limit of detection was determined to be 2.7 colony forming units per PCR reaction with a 99% efficiency. The specificity of our assay was tested using a panel of 80 isolates, including 50 different microbial species and 89 different antibiotic resistance genes, with a total of 30 β-lactamases included. The assay was determined to be reproducible with a coefficient of variation of ≤1.53%. A screen of 51 clinical isolates was performed retrospectively and 2 isolates were determined to be blaROB1 positive and resistant to penicillins. This assay represents a rapid and cost-effective method for screening N. meningitidis to predict penicillin resistance. Additional studies are underway to validate this assay for use on blood and cerebral spinal fluid samples positive for N. meningitidis.

Presenter: Kara Mitchell, Wadsworth Center, Albany, NY, kara.mitchell@health.ny.gov

Poster 08M

Comparison of a Commercial Kit and Traditional Culture Method for Detection of Salmonella spp. on Environmental Samples

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Nosocomial salmonellosis in hospitalized animals is a recognized hazard, especially in large animal clinics. Mitigation of outbreaks, as well as active surveillance efforts, require an effective and time-sensitive diagnosis. A standardized culture method for detecting Salmonella spp. in environmental samples using a 48-hour enrichment step results in a 5-day turnaround time for negative results. RapidChek® SELECT™ Salmonella (RCSS) test system offers detection of organisms in 22-44 hours through double enrichment and detection by a lateral-flow immunoassay (LFIA). At the Indiana Animal Disease Diagnostic Laboratory (IADDL), we compared the performance of RCSS to a standard tetrathionate-enriched culture-based protocol for detection of Salmonella spp. in environmental samples submitted by the large animal Purdue Veterinary Hospital (LA-PVH). The study included 20 environmental samples collected at the LA-PVH using electrostatic wipes and 20 wipes artificially spiked with either 10^0 or 10^1 CFU of ATTC25923 S. Typhimurium. RCSS consistently detected S. Typhimurium at a spiking level of 10^1 CFU, and 40% of samples spiked with 10^0 CFU; whereas standard Salmonella culture failed to recover organisms from all samples inoculated with 10^0 CFU and recovered only 40% of replicates with a 10^1 CFU inoculum. A clear time advantage coupled with higher sensitivity led to the adoption of RCSS as the IADDL's primary method for testing environmental samples submitted by the LA-PVH. Eight-hundred and seventy-two environmental samples were tested over 12 months. One hundred and twenty-four samples tested positive for Salmonella spp. per RCSS LFIA. Culturing positive samples on Xylose-Lysine-Tergitol-4 agar with subsequent identification of isolates through matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) analysis was chosen as the confirmation method. Salmonella spp. was confirmed in 57 out of the 124 LFIA-positive samples. Fifty-two of the 124 positive samples grew bacteria that showed morphologic characteristics consistent with Salmonella spp. but identified as a different bacterium per MALDI-TOF. Isolates identified as E. cloacae, E. xiangfangensis, E. hormachei, E. kobei, E. asburiae, C. freundi, C. braakii, C. sedlakii, K. pneumoniae, P. aeruginosa, and P. stutzeri. Thirty-six samples with positive LFIA results but negative Salmonella growth were tested for the presence of Salmonella spp. nucleic acid by PCR, and all tested negative. When the RCSS was used to test samples spiked with 41 pure isolates of various bacteria, the LFIA reacted to 4/41 isolates corresponding to E. cloacae (2), K. pneumoniae, and C. freundii. Our data suggest that an estimated 54% of positive RCSS LFIA results obtained in our lab during the analyzed period could be false positives; nevertheless, the benefits of this method still prevail over traditional culture. Here, we report the performance data of RCSS and guide how to report results obtained using this system.

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Poster 09M

Moving from Drive-through to Home Collection for COVID-19

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Test Iowa is a program that offered readily available COVID testing services to Iowa residents using drive-through sites. From July 2020 through June 2021, 564,622 nasopharyngeal COVID tests were performed. First year successes included instrumentation improvements such as incorporating liquid handlers into the workflow to reducing rework and aligning testing strategies with other areas of the laboratory to consolidate purchasing streams. A 24-hour turnaround time was maintained for reporting results, except during times of extremely high demand where over 6,600 samples per day arrived at the laboratory. Other successes of the program included the nimbleness to respond to surges due to a simplified sample receiving process, a training program that broke the testing process into manageable steps, and redundancy in instrumentation and supply chain management. In April 2021, the Iowa Department of Public Health determined that the COVID-19 drive-through testing sites were no longer sustainable but still needed testing available for those who did not have ready access to a testing site. By then, testing had become widely available in physician offices and pharmacies throughout Iowa but there were still underserved population areas. By July 15, 2021, SHL had established a program to prepare kits, receive orders for kits, distribute kits, receive saliva samples, process tests and report results. The goal was to have a two-day average turnaround time from collection to result. What was thought to be a low demand testing scenario, turned out to be a significant resource. Within three months, SHL had built 278,311 kits, received 170,287 orders for kits, processed 43,759 tests, detected 5,979 positive samples, and met the two-day average turnaround time. During the surge in testing caused by the delta variant (October 1 to December 31, 2021), 100,683 kits were ordered, 88,139 tests performed, and 15,402 positives detected. During the omicron variant surge (January 1 to March 1, 2022) there were 181,356 tests kits ordered, 100,159 tests performed, and 32,144 positives detected. Home collection of samples proved to be a significant resource in the battle against COVID in Iowa. It serves as an excellent example of the value of home collection of samples and submitting to the public health laboratory.

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Poster 10M

Chagas Disease Molecular Detection at The Center for Disease Control and Prevention (CDC)

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Chagas disease is caused by the protozoan parasite *Trypanosoma cruzi*, which can cause cardiomyopathy in chronically infected individuals. There are an estimated 8 million persons living with Chagas disease in Mexico, Central and South America, and, through immigration, Chagas disease poses a public health challenge to the U.S. Selecting the most appropriate diagnostic test for Chagas disease requires knowledge of the parasite lifecycle in the infected host and the phase of Chagas disease. PCR testing is only informative in certain circumstances. As a national reference lab, in support of state public health programs, the Parasitic Diseases Branch in the Centers for Disease Control and Prevention (CDC) has developed an algorithm for Chagas disease diagnostic testing. At CDC, we use a multi-target TaqMan qPCR approach to detect acute infection and monitor for reactivation in chronically infected individuals who are immunosuppressed, most commonly post heart transplantation. Risks for acute infection that are indications for PCR testing include: recent vector exposure (e.g., a triatomine bug bite), a neonate born to a mother with confirmed infection, and receipt of a transplanted organ from an infected donor. PCR testing is indicated to monitor for Chagas disease reactivation when a chronically infected patient is immunosuppressed such as after solid organ (especially heart) or during conditioning for human stem cell transplantation. The utility of PCR testing is very low and is not indicated for diagnosis of chronic phase infections. In those cases, serological testing is the appropriate method. Over the last eight years, we have detected 11 cases of acute infection acquired via various modes of transmission and monitored 66 cases of Chagas disease reactivation in chronically infected patients. Testing at CDC using this algorithm is an important resource as there are only a few other institutions nationwide offering Chagas disease molecular testing.

Presenter: Yvonne Qvarnstrom, Centers for Disease Control and Prevention, Division of Parasitic Diseases and Malaria, Parasitic Diseases Branch, Atlanta, GA, bvp2@cdc.gov

Poster 11M

Multiplex Molecular Assays to Improve Workflow and Performance for Detecting and Subtyping *Haemophilus influenzae* and *Neisseria meningitidis*

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Haemophilus influenzae and *Neisseria meningitidis* are two prevalent causes of bacterial meningitis in the United States (CDC). There are six serotypes of H. influenzae and N. meningitidis (a-f, and A, B, C, W135, X, and Y, respectively), known to cause the highest proportion of infections in people. Molecular detection and characterization of the described H. influenzae and N. meningitidis subtypes by real-time PCR (RT-PCR) is an established method at the Minnesota Department of Health Infectious Disease Lab (MDH-IDL). However, the single plex design of the assays require a cumbersome and tedious setup process. The time-intensive setup requirement using the current methodology is an opportunity for a novel multiplex RT-PCR design.

MDH-IDL is developing a multiplex RT-PCR assay as an APHL Infectious Disease Fellowship project to offset the burden of molecular testing for H. influenzae and N. meningitidis. The improved assay includes all relevant targets with a drastic reduction in the number of reaction mixes required. A cross-platform analysis using the Qiagen QIAcuity digital PCR (dPCR) instrument is also evaluated. Performance characteristics of the existing and multiplex assays are compared here, demonstrating assay performance and workflow improvement.

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Poster 12M

Multicenter Evaluation and Validation of a Customized MIC Panel for Antimicrobial Susceptibility Testing of *Mycobacterium tuberculosis*

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Objective: A multi-center study to validate a customized minimum inhibitory concentration (MIC) method for phenotypic antimicrobial susceptibility testing (AST) in *Mycobacterium tuberculosis* complex (MTBC).
Study Design: Validation of a customized MIC panel was conducted at two sites, the State of Florida Bureau of Public Health Laboratories (BPHL) and the New York State Wadsworth Center (WC). The customized MIC plate was developed in collaboration with the Florida TB Physician's Network to include drugs that have recently become important in the treatment of tuberculosis (TB), including bedaquiline, linezolid and clofazimine. A total of 12 drugs were included on the 96-well plate, manufactured by TREK Sensititre (ThermoFisher). A standardized protocol was developed, including specifications about plate inoculation, incubation/plate-reading time (read at 10, 14 and 21 days), and method for interpreting results (e.g., sharing pictures of plates to minimize subjective interpretation). A total of 50 plates per site were allocated to test a panel of pre-characterized isolates. All of these strains were from BPHL and WC cases, with the exception of two linezolid resistant strains that had to be purchased.
Results: 50 plates per site were tested with the panel of pre-characterized isolates according to the same standardized protocol. Isolates tested included 22 pan-susceptible strains of varying lineages, 10 multidrug resistant (MDR), 5 extensively drug-resistant (XDR) or pre-XDR strains, and 14 strains with a variety of resistance patterns including resistance to bedaquiline and clofazimine, heteroresistance to fluoroquinolones and low-level rifampin resistance. Since there was no comparator method for many of the drugs on the panel and clinical concentration or breakpoints may not be established in the U.S., World Health Organization guidelines were followed where applicable. Results from customized MIC panel were compared to prior phenotypic results (either CRYPTIC MIC panel or current BPHL or WC AST methods) and genotypic results (whole genome sequencing). There was general concordance although some strains had elevated MICs for rifampin or isoniazid at 21 days. One MDR-TB isolate from 2015 was resistant to bedaquiline and clofazimine by MIC which was unknown because it was not detected by the WGS pipeline being used in 2015. It was rerun through the updated pipeline which confirmed mutations associated with resistance to these drugs. Another important note with the MIC plates was that the meropenem activity degraded with time, and before plate reading was completed. Therefore, no reliable data could be collected for meropenem and due to its reduced stability is not a candidate to be included on future customized MIC plates.
Conclusions: MIC testing is a valuable method for AST of MTBC, however there are challenges in validation and implementation especially in a low-incidence drug resistant population. Nevertheless, in light of new drugs and new drug regimens for drug-resistant TB, e.g., BPaL regimen (bedaquiline, pretomanid and linezolid), and a move to oral regimens for treatment, this testing will become more crucial to ensuring that TB patients receive the appropriate therapy.

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Poster 13M

Comparison of Enzyme-linked Immunosorbent Assays for the Diagnosis of Dengue Virus Infections

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Introduction: Dengue virus (DENV) is a mosquito-borne flavivirus that causes a febrile illness in humans, occasionally leading to severe hemorrhagic fever and shock syndrome. After viraemia, the laboratory diagnosis is based on antibody detection which can be complicated by cross-reactivity of the antibodies with other flaviviruses, mainly due to antigenic substrates containing the highly conserved viral glycoprotein E. Flavivirus serological assays based on the more species-specific non-structural protein 1 (NS1) have demonstrated a higher specificity. Here, we compared the performance of two ELISAs based on different DENV antigens.

Methods: The study included 124 follow-up samples (day 0-196) from DENV-infected travellers. Additionally, we examined sera from 688 healthy individuals and 95 patients with previous flavivirus contact, i.e. vaccination against or infection with tick-borne encephalitis virus (TBEV), yellow fever virus (YFV), hepatitis C virus (HCV), West Nile virus (WNV) or Zika virus (ZIKV).

Samples were analyzed for anti-DENV IgM and IgG using the Anti-Dengue Virus Type 1-4 ELISA (Euroimmun AG, Lübeck, Germany) based on purified DENV particles and recombinant glycoproteins E of DENV1-4. In addition, IgG reactivity against DENV NS1 was examined by an ELISA (Euroimmun) coated with recombinant NS1 of DENV1-4.

Results: Of the samples taken on day 0, anti-DENV1-4 antibodies were positive in 36% (IgM) and 40% (IgG), but only 13% reacted positively in the NS1-based IgG ELISA. Among sera obtained between day 6 and 14, 100% were anti-DENV1-4 positive for both IgM and IgG, while anti-DENV NS1 IgG positivity was detected in 70% of cases. In samples taken later, the prevalence of anti-DENV1-4 IgM steadily decreased, whereas both IgG ELISAs reached positivity in 100%.

Specificities of the different ELISAs were 94-99% with respect to healthy individuals. However, in the cross-reactivity panel, the NS1-based IgG ELISA was more specific than the anti-DENV1-4 IgG ELISA (83% vs. 49%), with deviations observed in TBEV-vaccinated (100% vs. 68%) and WNV-infected (93% vs. 33%) patients. Highest cross-reactivity (100%) occurred with anti-ZIKV IgG antibodies.

Conclusion: The Anti-Dengue Virus Type 1-4 ELISA IgM and IgG is capable of detecting all DENV infections in the time window predestined for serodiagnostics, thus presenting a valuable screening tool for acute infections and epidemiological studies. By comparison, the NS1-based IgG ELISA has the advantage of reduced IgG cross-reactivity, but limited sensitivity in early stages of infection.

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Poster 14M

Development of a Novel NS1-based ELISA for the Detection of Specific IgG Antibodies against Japanese Encephalitis Virus

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Introduction: The performance of a newly developed anti-JEV IgG ELISA based on the species-specific non-structural protein 1 (NS1) to support diagnosis of infection with Japanese encephalitis virus (JEV) was evaluated.
Methods: Panel A: 25 sera from acutely infected Vietnamese patients in which anti-JEV IgM was detected by JEV IgM Capture ELISA (DRG International) based on JEV-derived recombinant antigen (JERA), and confirmed by Flavivirus IFA Mosaic (EUROIMMUN). Results were compared to each other and to the Jera-based IgG and IgM ELISAs. Panel B: 102 sera with seropositivity for glycoprotein E (gG) against tick-borne encephalitis (TBEV), yellow fever (YFV), hepatitis C (HCV), West-Nile (WNV), Zika (ZIKV) or dengue virus(DENV). Panel C: 50 sera from healthy blood donors. All samples were tested for anti-JEV IgM and IgG antibodies using ELISAs (EUROIMMUN) based on gE and the novel Anti-JEV-NS1 IgG ELISA (EUROIMMUN). Borderline results were considered as positive.
Results: Panel A: total agreement between the ELISAs was 100% (IgM: gE vs. JERA), 92.0% (IgG: gE vs. JERA) and 76.0% (IgG: NS1 vs. gE). Panel B: IgG reactivity rates between the gE- and NS1-based ELISAs were similar for anti-HCV (0%) and anti-ZIKV (100% vs. 91.7%) positive samples. NS1-based testing was considerably less cross-reactive in individuals seropositive for TBEV, YFV, WNV or DENV. Panel C: 8.0% and 2.0% were IgG positive in the gE- and NS1-based ELISAs, respectively (specificity 92.0% vs. 98.0%).
Conclusion: The use of NS1, compared to gE, increases the specificity for detecting anti-JEV IgG antibodies. Serological cross-reactivity and multiple flavivirus infections, however, cannot be excluded due to overlapping distribution areas.

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Poster 15M

Comparison of Diagnostic Performance of Two Aspergillus Antigen ELISAs

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Introduction: Aspergillus spp. are ubiquitous and opportunistic pathogenic fungi. Inhalation of their spores can cause life-threatening invasive aspergillosis (IA) in immunocompromised patients. Current diagnostic guidelines recommend serial screening for Aspergillus antigen in serum or bronchoalveolar lavage fluid samples from patients at risk of IA. Only few enzyme-linked immunosorbent assays (ELISAs) based on monoclonal antibodies with high specificity for Aspergillus antigens are commercially available. We assessed the serodiagnostic performance of the novel Aspergillus-specific galactomannoprotein (GP) ELISA (EUROIMMUN) by comparing it to the Platelia Aspergillus galactomannan (GM) ELISA (Bio-Rad).
Methods: Diagnostic performance of the two ELISAs was assessed by analysing serum samples from 43 patients with IA and 77 without IA. The agreement between manual versus automated processing of the GP ELISA using the EUROIMMUN Analyzer I was evaluated in sera from 16 patients with IA and eight fungus-spiked pooled sera.
Results: The GM ELISA identified 24 true positive and 77 true negative samples, whereas the GP ELISA identified 27 true positive and 74 true negative samples. The two assays had a positive and negative agreement of 90 % and 88 %, respectively. The overall agreement between the ELISAs was substantial (Cohen's $\kappa = 0.65$). Nine IA patients were correctly identified with the GP ELISA but missed by the GM ELISA. The GP ELISA classified two IA patients as negative for Aspergillus antigen, which were identified by the GM ELISA. Manual and automated processing of GP ELISAsagreed almost perfectly (Cohen's $\kappa = 0.91$, one discrepant sample was close to cut-off).
Conclusion: The GM ELISA correctly identified more patients without IA, whereas the GP ELISA correctly identified more patients with IA. Although each test identified cases that were not detected by the competitor, their performance in the serodiagnosis of IA was comparable. Overall, the assays are similarly suitable for screening of patients at risk for IA. However, serial testing and use of further diagnostic tools are recommended by standard guidelines. Furthermore, our results demonstrated that the GP ELISA can be reliably processed on fully automated systems, thus providing high-throughput analysis for monitoring patients at risk of IA.

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Poster 16M

Sensitive Detection of Antibodies in Patients with Acute *Coxiella burnetii* Infection

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Introduction: *Coxiella burnetii* bacteria cause a zoonotic disease called Q fever. Differentiation between acute and chronic Coxiella infection is possible via serological detection of specific antibodies against Coxiella surface lipopolysaccharides, which exhibit antigenic phase variation over the disease course (acute: IgM/IgG against phase 2 antigens; chronic: IgG against phase 1 antigens). Here, we evaluated the diagnostic accuracy of three phase-specific Anti-Coxiella burnetii ELISAs from EUROIMMUN.
Methods: The study included sera from 23 patients with acute Coxiella infection (23 primary, 17 follow-up samples) and 12 patients with chronic Coxiella infection (12 primary, 6 follow-up samples). The infection status had been classified based on composite results obtained by the SERION ELISA classic *Coxiella burnetii* Phase 2 IgM / IgG (Virion\Serion) and in-house complement fixation tests (CFT) based on Virion\Serion Phase 1 and 2 antigens. 47 samples were classified as seronegative based on SERION ELISA classic Coxiella burnetii Phase 2 IgM/IgG (Virion\Serion). Samples were analysed using Anti-*Coxiella burnetii* Phase 2 ELISA (IgM/IgG) and Anti-Coxiella burnetii Phase 1 ELISA (IgG) (EUROIMMUN).
Results: The Anti-Coxiella burnetii ELISAs correctly identified acute-phase and chronic-phase marker for an infection (detection rates = 100%). 5 of the primary acute-phase samples were IgM negative or borderline in the SERION ELISA classic *Coxiella burnetii* Phase 2 IgM/IgG but positive in the Anti-Coxiella burnetii Phase 2 ELISA (IgM). The respective follow-up samples were positive for acute infection and indicated seroconversion. In the negative panel, assay specificities amounted to 97.9% (Phase 2 IgG and Phase 1 IgG) and 78.7% (Phase 2 IgM).
Conclusion: The EUROIMMUN Phase 2 IgM ELISA provides higher sensitivity for detection of antibodies in the acute phase, enabling early diagnosis of Coxiella infections. Anti-Coxiella reactive samples in the preselected negative panel could include false positives due to cross-reactivity (with antibodies against other gram-negative bacteria or interferences with e.g. rheumatoid factors or autoantibodies) or a low assay specificity. Alternatively, they may represent true positive results due to lower sensitivity of the comparison test or previous infection with Coxiella. Confirmation would require follow-up testing. Overall, the EUROIMMUN Anti-Coxiella burnetii ELISAs are sensitive assays to support the laboratory diagnosis of a Coxiella infection.

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Poster 17M

Novel ELISA Based on Purified and Recombinant Antigens from *Toxocara canis* Exhibits a High Diagnostic Sensitivity

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Introduction: Toxocariasis is a zoonosis that is mainly caused by the dog roundworm *Toxocara canis* and the cat roundworm T. cati. The prevalence of T. canis-specific antibodies in humans is 2-5% in Germany. Many infections with Toxocara spp. have a subclinical course, but can develop into a severe disease, with visceral, ocular, or neurotoxic manifestations. While first serological antibody detection methods were based on the whole lysate from T. canis larvae, meanwhile Toxocara excretory/secretory (TES) antigens from larvae cultures are used. Here, we evaluated the diagnostic performance of a new ELISA based on a highly purified larval antigen from T. canis and recombinant TES antigen.

Methods: [1] Comparison with an external quality assessment scheme (NEQAS, UK) encompassing 9 positive and 8 negative sera.

[2] Agreement study with a commercial Toxocara canis IgG ELISA (based on T. canis antigens; IBL International, Germany) including 117 sera pre-characterised by IBL ELISA.

[3] Specificity was evaluated with respect to a control panel containing 586 sera from healthy blood donors, 98 from pregnant women and 197 from children as well as a cross-reactivity panel (135 sera from patients infected with Strongyloides, Schistosoma, Ascaris, Trichinella and other parasitic/bacterial infections).

Results: Results obtained with the Anti-Toxocara ELISA were 100% in agreement with the NEQAS target values (sensitivity: 100%, specificity: 100%).

The agreement between the two methods was substantial (79%, κ=0.57). The EUROIMMUN ELISA found 86 and the IBL ELISA 71 positive samples (negative predictive value=1). The EUROIMMUN ELISA found 23 and the IBL ELISA 41 negative samples. Thirteen discrepant samples were positive in the EUROIMMUN ELISA but negative in the IBL ELISA.

The Anti-Toxocara ELISA was reactive in 25.9% of samples in the cross-reactivity panel and in 4.5% of the samples from healthy individuals, yielding a combined specificity of 92.6%.

Conclusion: The novel Anti-Toxocara ELISA is a valuable screening test for sensitive serological detection of T. canis infections. Possible past infections or double infections with other parasites should be considered when evaluating the test result. A positive ELISA test result could be confirmed by a corresponding Western blot, which is available from EUROIMMUN.

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Poster 18M

Description of a Custom Linux Workflow for Targeted Amplicon Deep Sequencing

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We recently developed a pan-parasitic targeted amplicon deep sequencing (TADS)-based assay with significant potential for diagnostic utility. This assay involves PCR amplification of part of the eukaryotic 18S rDNA prior to Illumina sequencing and was designed for application to DNA extracted from human blood and tissues for detection of a range of parasitic pathogens in clinical diagnostic settings. However, continued use of proprietary software (i.e., Geneious prime) for data analysis combined with reliance on manual data processing steps limits our ability to customize workflows and increases analysis turnaround times. Here we describe an improved workflow based on bash, python, and freely available sequence analysis software that increases computation speeds and automates numerous steps.

The workflow uses FastQC software and BBTools to perform initial trimming, merging, and quality control (QC) checks on fastq sequence files. Samples with less than 17,000 reads and/or a mean phred quality score < 20 are removed. To speed up BLASTing steps, the workflow takes advantage of the CD-Hit program which bins identical reads into clusters based on one representative sequence. Each sample is now defined by multiple clusters that each represent the unique haplotypes detected for that sample. Thus, rather than BLASTing individual reads, we BLAST a representative of each haplotype, reducing computation times. Clusters represented by less than 20 merged reads are excluded. Remaining representative sequences undergo a BLASTN search against a database of human 18S rDNA sequences. Hits towards this “Exclusion Database” are discarded. Finally, BLASTN is used again to search remaining sequences against a “Reference Database” containing a curated set of 18S sequences from 234 parasite taxa, including various apicomplexan and helminth parasites often infecting humans.
This being a qualitative assay, it is necessary that the workflow establishes a minimum coverage cutoff multiplier, that considers index crosstalk (i.e., sample bleeding), with the following formula:
u_contam_all +(4*S.D.) where u_contam_all is the mean proportion of contaminating reads present in negative controls due to index cross talk (low-level parasite hits in our negatives) and S.D. equals the standard deviation. At least 4 blood or tissue negative controls are necessary in every sequencing run to ensure an accurate mean and standard deviation is computed. This value is then multiplied by the total reads for each sample to establish the minimum number of reads in a sample needed to match a parasite sequence in order to be considered ‘positive’. The workflow ends by printing resulting matches within in a summary table.

When running 10 identical sets of paired Illumina reads from human clinical samples known to contain parasitic pathogens; our Geneious workflow processed each set of paired fastq files (a set for each sample) at an average of 8.26 minutes, while this workflow took 0.521 seconds per sample. The quality metrics provided as part of standard QC and quality assurance (QA) from the fastqc report are also recorded along with any samples that failed and why. This Linux workflow allows for further customization to make the bioinformatic analysis of our TADS assay more streamlined and easier to use, even for lab personnel with little to no bioinformatic experience. The increased speed and generation of QA and QC information makes this novel TADS assay more amenable to clinical diagnostic settings."

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Poster 19M

Optimization and Verification of an Automated Analyte-specific Reagent Assay for *Mycoplasmoides genitalium* Macrolide Resistance Detection in Primary Clinical Specimens

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Background: Centers for Disease Control and Prevention sexually-transmitted infection treatment guidelines for *Mycoplasmoides genitalium* are predicated on macrolide resistance detection assays following a positive molecular screen. However, M. genitalium macrolide resistance detection assays are not widely available in the United States. Here, we optimized parameters for an analyte-specific reagent (ASR) macrolide resistance detection assay on an open access analyzer and evaluated macrolide resistance detection in a clinical specimen set.

Methods: Real-time RT-PCR was performed on the Panther Fusion system (Hologic). Extraction reagents, internal control RNA additive, and lyophilized cartridges containing enzymes and nucleotides were acquired from the manufacturer. M. genitalium macrolide resistance (A2058C, A2058G, A2058T, A2059C, A2059G) and internal control primer/fluorescent probe sets, KCl, and MgCl2 (PPR solution) were titrated by the user. PPR solution optimization was challenged with M. genitalium target mutation and M. genitalium wild-type (negative control) RNA constructs. Clinical verification utilized first-void urine and urogenital swab specimens in Hologic transport medium.

Results: Internal control RNA was amplified (0.6 micromolar primer concentration) and detected (0.4 micromolar probe concentration) in all testing. 1.2 micromolar M. genitalium primer and 0.8 micromolar M. genitalium probe concentrations yielded an 80% rate of mutation detection when challenged with 10,000 copies of wild-type RNA. False detections were reduced when M. genitalium primer and probe concentrations were lowered to 0.6 micromolar and 0.4 micromolar, respectively. A 44.2% rate of false detection was observed when 3 mM MgCl2 was utilized. This rate was reduced to 7.5% with 2 mM MgCl2. These differences were independent of KCl concentration. However, when PPR solution optimization was challenged by an A2058G mutation construct, 60 mM KCl yielded lower cycle threshold values and increased fluorescence generation when compared to 40 mM KCl ($P \leq 0.03$). Lower limit of A2058G mutation detection was 5000 copies (20/20 detections). Utilization of a baseline correction slope limit of 250 units further mitigated false detection from wild-type RNA at challenges up to 3.3 billion copies. Macrolide resistance was detected in 367/543 (67.6%) clinical specimens initially screening positive for M. genitalium by commercial transcription-mediated amplification (Hologic). These data included 231/328 (70.4%) rectal swab specimens and 99/164 (60.4%) male urine specimens derived largely from men who have sex with men. The increased rate of macrolide resistance detection from rectal swab specimens versus that from male urine specimens was significant ($P = 0.03$). Overall resistance detection rates did not vary by gender ($P = 0.31$). Specificity of the M. genitalium macrolide resistance detection assay was 100% (54 determinations, including 18 specimens that were positive for Chlamydia trachomatis, Neisseria gonorrhoeae, or Trichomonas vaginalis).

Conclusion: M. genitalium primer/probe, MgCl2, and KCl concentrations of 0.6 micromolar/0.4 micromolar, 2 mM, and 60 mM, respectively, allow for accurate detection of M. genitalium macrolide resistance with Panther Fusion ASR, particularly when baseline slope correction is enabled.

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Poster 20M

Evaluation of Updated Aptima Combo 2 Assay in a High-prevalence United States Sexually-transmitted Infection Community

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Background: In 2019, a variant of *Chlamydia trachomatis* that escaped detection by the 23S rRNA-based Aptima Combo 2 Assay (Hologic) was elucidated from more than 200 specimens in Finland that subsequently tested positive by the 16S rRNA-based Aptima Chlamydia trachomatis Assay (Hologic). The majority of sequenced discrepant specimens revealed the single nucleotide polymorphism C1515T in 23S rRNA. In the same year, reports of C. trachomatis diagnostic escape variants surfaced in other Scandinavian countries, while adding C1514T and G1523A as contributing single nucleotide polymorphisms. The C. trachomatis variant phenomenon was observed to a limited extent in England, though a potential influence of concomitant *Neisseria gonorrhoeae* positivity was hypothesized toward the decreased detection of C. trachomatis by the Aptima Combo 2 Assay. One United States study of 401 specimens failed to detect the C1515T C. trachomatis variant but did report two variants possessing A1518G and G1526A polymorphisms. The purpose of this investigation was to evaluate the updated Aptima Combo 2 Assay for C. trachomatis and N. gonorrhoeae detection in a high-prevalence STI community in the United States.

Methods: A convenience sampling of 784 archival first-void urine specimens, 254 vaginal swabs, 2 endocervical swabs, and 4 urethral swabs was gathered from a Milwaukee, WI laboratory. All urogenital specimens were stored in appropriate Hologic transport medium, assayed within 30 days by the Aptima Combo 2 Assay (predicate device), and archived at -70°C for up to one year. Extragenital specimens (41 pharyngeal swabs, 29 rectal swabs) collected in Hologic transport medium were assayed by the predicate device and archived at -70°C for up to 60 days. All specimens were originally collected from April 2021 through October 2022. Retrieved specimens were assayed by an updated Aptima Combo 2 Assay (investigational device) designed to cover C. trachomatis variants described in 2019.

Results: Positive agreement for C. trachomatis detection from urogenital and all specimens was 608/626 and 633/652 (both 97.1%), respectively. Positive agreement rates for N. gonorrhoeae detection were 94.4% for urogenital specimens and 94.7% for all specimens ($P \leq 0.03$ versus C. trachomatis positive agreement). Most discordant results reflected positive (predicate device) to negative (investigational device) shifts for both targets, suggesting that archival storage may have been deleterious to target stability. Negative agreement for C. trachomatis detection was 412/418 (98.6%). Six discrepant specimens positive with the updated Aptima Combo 2 Assay were derived from urine (4) and vaginal swab (2) specimens. The six specimens yielded a positive N. gonorrhoeae result by both predicate and investigational devices.

Conclusion: The updated Aptima Combo 2 Assay demonstrated acceptable performance characteristics within an archival clinical study set. A small number (n = 6) of Aptima Combo 2 Assay C. trachomatis-negative/updated Aptima Combo 2 Assay C. trachomatis-positive discordant specimens were observed in this high-prevalence population and may be associated with concomitant detection of N. gonorrhoeae RNA. Additional studies are necessary at the molecular level to determine the source of these assay discrepancies, including sequencing to determine if they are due to the presence of a C. trachomatis variant.

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Poster 21M

Detection of Acute HIV-1 Infection in a 16-Sample Pool Using the Aptima HIV-1 Quant Dx Assay

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Introduction: The detection of acute HIV-1 infection is crucial in the fight to end the HIV Epidemic. Nucleic acid amplification testing (NAAT) is superior to serological testing for early detection within days after transmission because the virus is replicating at high levels and serological markers are undetectable (i.e., window period). The New York City Department of Health and Mental Hygiene Public Health Laboratory (NYC PHL) employs an alternative testing algorithm that screens pooled patient samples using the APTIMA HIV-1 Qualitative Assay to detect acute HIV-1 infection from patients who tested negative by a rapid antibody test. Since 2009, this approach has identified 357 acute HIV-1 infections among 130,000 specimens, but it is labor intensive and has longer turnaround times. The purpose of this study is to validate and implement an automated HIV-1 NAAT method that would decrease hands-on time, staffing, and turnaround time for diagnosis of acute HIV-1 infection.

Method: A 16-sample pooling protocol was validated using the APTIMA HIV-1 Quant Dx Assay performed on the Hologic Panther instrument. The limit of detection (LoD) was determined using a serial dilution series of pooled plasma samples created from a known positive sample (300,000 copies/mL). Accuracy was assessed using 30 HIV-1 positive specimens previously tested by APTIMA HIV-1 RNA Qualitative Assay. These specimens were used to contrive 16-sample pools by diluting 1:10 with negative plasma. A total of 40 pools (30 positive and 10 negative) were tested. Reproducibility was determined by testing samples at high, medium, and low viral concentrations (10-1, 10-2, and 10-3) on three different days by two technologists. Positive pools were deconstructed and individually tested to determine which sample(s) within the pool is positive. Assay hands-on time, staffing, and test result turnaround time were compared to the previous manual pooling method.

Results: The Aptima HIV-1 Quant Dx assay detected < 30 copies/ml at the 10-4 dilution of the original pool, which corresponds to the LoD determined by the manufacturer. The accuracy study showed that the results obtained from an individual specimen and its pool were in 100% agreement, and comparable to previous test results from the HIV-1 qualitative assay. The results from all dilution levels demonstrated 100% reproducibility (day to day and tech to tech). After implementation, 11 HIV-1 acute infection cases were identified among 7,000 specimens between February 2022 and August 2022. Compared to the previous manual pooling method, the new assay automated many steps of the procedure, which reduced hands-on time by ~3.5 hours and decreasing turnaround by at least 24 hrs in most cases.

Conclusion: Our verification and clinical data showed that the Aptima HIV-1 Quant Dx assay can be efficiently used for testing of a combined pool of 16 clinical specimens. The assay is highly reproducible and accurate, and routine use of this assay has minimized labor and shortened turnaround time while maintaining the ability to detect acute HIV-1 infection.

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Poster 22M

Evaluation of Analyte Specific Reagents for Molecular Detection of Macrolide Resistance in *Mycoplasma genitalium*

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Background: *Mycoplasma genitalium* (Mg) is a common cause of urethritis and is frequently resistant to azithromycin (AZM-R). CDC STI Treatment Guidelines recommend resistance-guided therapy with use of AZM when Mg is susceptible and moxifloxacin when it is resistant. However, there is no FDA-approved commercially available assay for Mg macrolide resistance-mediating mutation (MRM) detection. We evaluated the performance of Hologic analyte specific reagents (MG-mac-Res-ASR) for detection of Mg MRM using remnant nucleic acid amplification test (NAAT) specimens and well-characterized clinical Mg strains.
Methods: We used the MG-mac-Res-ASR to test 41 Mg-negative urine specimens spiked with different Mg clinical strains that had previously been characterized for MRM by PCR and bi-directional Sanger sequencing of Mg 23S rRNA to identify mutations at nucleotide positions 2058 and 2059 (8 A2058G, 2 A2058C, 19 A2059G, 12 wild type). We then tested 463 remnant Aptima Mg positive specimens (urine, vaginal, rectal, and pharyngeal) with no previous MRM characterization.
Results: Mg-mac-Res-ASR and sequencing results showed 100% agreement among the 41 previously characterized Mg strains (29 macrolide-resistant, 12 macrolide-susceptible). 255 of 463 (55.1%) remnant Aptima Mycoplasma genitalium assay (Hologic) positive specimens were positive for MRM (CT values 25.1–40), 207 (44.7%) were wild type, and 1 (0.2%) gave an invalid result. The assay showed no cross reactivity using a specificity panel of other microbial species.
Conclusions: MG-mac-Res-ASR accurately detected Mg macrolide resistance. When validated as a laboratory developed test (LDT), this assay can be used to detect AZM-R Mg and guide treatment of Mg infections, as recommended in the 2021 CDC STI Treatment Guidelines.

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Poster 23M

Interlaboratory Comparison Exercise (ILC) of SARS-CoV-2 Molecular Detection Method Used by Veterinary Diagnostic Laboratories

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Ensuring reliable detection methods for SARS-CoV-2 is critical as veterinary diagnostic labs are testing both human and animal samples. The US Food and Drug Administration (FDA) has previously organized two collaborative inter-laboratory comparisons (ILC) exercises to veterinary diagnostic laboratories for evaluating their RT-qPCR methods routinely used for clinical testing. The results showed that the participants' methods were sensitive and specific for detecting SARS-CoV-2 RNA spiked in viral transport medium. To further assess the RT-qPCR methods routinely used to detect emerging SARS-CoV-2 variants, the current ILC3 was conducted to detect the Delta and Omicron variants spiked in canine nasal matrix or viral transport medium. Samples included inactivated Delta variant at levels ranging 25 to 1,000 copies per 50 mL of nasal matrix and Omicron variant at 1,000 copies per 50 mL of transport medium. Feline infectious peritonitis virus (FIPV) RNA was used as a confounder for specificity assessment. Participants used their routine diagnostic procedures for RNA extraction and RT-qPCR. The overall results showed 93% detection for Delta and 97% for Omicron at 1,000 copies per 50 uL (22-200 copies per reaction). The overall specificity was 97% for blank samples and 100% for blank with FIPV. There were no significant differences in Ct values for samples at the same virus levels between N1 and N2 markers, nor between the two variants. The results indicated that all ILC3 participating laboratories were able to detect both Delta and Omicron variants. The canine nasal matrix did not significantly impact SARS-CoV-2 detection. This knowledge will lead to higher confidence in laboratory detection of current and new SARS-CoV-2 variants and aid in establishing reasonable cutoff parameters for these diagnostic methods. This work plays a critical role in public health as part of the continued pandemic response of these laboratories.

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Poster 24M

Withdrawn

Poster 25M

An Automated High-Throughput Extraction and Real-Time PCR Solution for Rapid Monkeypox Detection

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Objective: Real-time PCR is a rapid method for tracking the spread of monkeypox virus (MPXV) which is critical for public health decision making. Here, we present a series of studies on an assay, from sample extraction to detection within 2.5 hours, using the PerkinElmer's chemagic™ 360 system (96-samples/extraction) and the PKamp™ Monkeypox Virus Real-Time PCR RUO Kit (15 µL/rxn both 96- and 384- PCR instruments).
Study designs: (1) Limit of detection (LOD) was confirmed at 1X and 1/3X LOD (n=20) using genomic DNA and synthetic DNA (covers 80%-85% genome region) of MPXV in a simulated clinical matrix (VTM plus human cells). (2) MPXV inclusivity (BLASTN of GISAID's EpiPox™ database) and (3) cross-reactivity of 34 FDA-recommended microorganisms (NCBI database) were evaluated by in silico analysis. (4) 20 FDA-recommended substance interference was tested at clinically relevant concentration with 3X LOD synthetic MPXV DNA in a simulated clinical matrix (n=3). (5) Matrix equivalence was performed by comparing simulated clinical matrix and pooled monkeypox virus negative clinical matrix (lesion swab in VTM), with 0 (negative), 1X, and 3X LOD synthetic MPXV DNA (n=20+). (6) 20 MPXV positive and 20 MPXV negative specimens collected in VTM were tested for comparing results provided by the NYPHL using CDC LRN FDA-cleared Non-Variola Orthopoxvirus Real-Time PCR reference method. Chemagic™ 360 system with chemagic Viral DNA/RNA Kit special H96 (CMG-1033-S) for extraction and Applied Biosystems™ 7500 Fast Dx, QuantStudio™ Dx 96, QuantStudio™ 5 384, or Bio-Rad® CFX384™ Real-Time PCR instruments were included in the studies.
Results: (1) 1X LoD with MPXV gDNA is at 6E3 copies/mL for all four PCR instruments, and with synthetic MPXV DNA is at 222 cp/mL for 7500 Fast Dx and within 3-fold for the other three instruments which is at 666 cp/mL. (2) In silico analysis from September 30th, 2022, shows 100% alignment within Clade I (33 full-length sequences) and Clade II (1368 full-length, high coverage sequences) except for one sequence which had one mismatch and is not predicted to impact detection due to its location. (3) 9/34 microorganisms show >80% homolog on one or two of the primer/probe and chance of them to have cross-reactivity to MPXV is predicted to be low. (4) 20 evaluated substances at its clinically relevant concentration were confirmed not to interfere with MPXV detection at 3X LOD. (5) Simulated clinical matrix and pooled negative clinical matrix shows equivalency within 1-3 times LOD. (6) Specimen evaluation shows 100% agreement with the CDC reference method.
Conclusions: The PKamp™ Monkeypox virus real-time PCR RUO assay paired with the automated chemagic™ 360 extraction system provides a sensitive and scalable solution for monkeypox virus detection.

For research use only. Not for use in diagnostic procedures.

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Global Health/One Health

Poster 26M

Approaches for Improving the Turnaround Time for Viral Load Testing Services in the Eastern Province of Zambia

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Background: The Eastern Provincial Health Office experienced viral load (VL) backlogs with a high turnaround time (TAT) of average 119 days beyond the national established guidelines of 14 days. This caused a delay in test results and a potential reduction in the VL testing numbers, with prospects of affecting provision of timely healthcare.
Methods: Strategies and potential outcomes of mitigating the high TAT were evaluated and implemented from January 2018 to December 2019, focusing on the available laboratory testing space, capacity of the testing platform, the number of VL hubs and location from the testing laboratory, adequacy of sample preparation equipment, and the existing courier system.
Results: A new molecular testing laboratory was refurbished at Chipata Central Hospital with the installation of a higher throughput VL testing machine, the Cobas 4800, and specimen storage refrigerators. Zonal mapping of 10 VL hubs was completed, and where capacity was built with equipment such as refrigerators and centrifuges. Eight Laboratory hubs and 69 health facilities had Disalink and e-labs, the remote Laboratory Information Systems (LIS) for sample registration and result return installed and interfaced with the LIS, Disalab, at the testing laboratory. The Laboratory hubs were also provided with data capturing registers, thermometers, and onsite mentorship. In addition, ten motorbikes and one vehicle were procured to support the existing intra- and inter-district courier system. Subsequently, the molecular laboratory cleared the 15,324 VL backlog with the provincial pre and post-data analysis illustrating TAT improvement from 119 days to within 14 days. Viral load tests equally increased by 63.5%, attributed in part to the improved laboratory diagnostic services implemented.
Conclusion: Capacity building of laboratory systems with critical infrastructure and equipment is key to the improvement of diagnostic services. It is thus, cardinal that programs are evaluated and supported to attain the intended outcome.

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Poster 27M

Withdrawn

Poster 28M

Centralizing and Reporting AMR Data in Ethiopia

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Problem Studied: In Ethiopia, many international partners are working to strengthen the public health system and minimize the impact of infectious diseases. However, Anti-Microbial Resistance (AMR) has not typically received the same attention in Ethiopia as communicable diseases. AMR has become a public health risk factor and a serious issue within society and for the country over the past few years. In this modern world, antibiotic resistance not only negatively impacts humans/animals/environment, but also has unmeasurable impact on the local economies. The Ethiopian Public Health Institute (EPHI) is responsible for monitoring AMR through collecting data in diverse ways from health facilities throughout Ethiopia. Many of the laboratories in these health facilities use WHONET, a system developed by the World Health Organization (WHO), for AMR data capturing and reporting. However, the laboratories are challenged by the manual data entry needed into WHONET in addition to the reporting required to EPHI.

Methodology: With AMR data becoming crucial for Ethiopia's public health systems, the Association of Public Health Laboratories (APHL), in collaboration with the US Centers for Disease Control and Prevention (CDC), is strengthening data management and reporting of AMR data in Ethiopia through a centralized system. A multi-pronged approach is being utilized to 1) empower the laboratory workforce through training with a focus on systems interoperability to enable sharing of data 2) implement technology solutions in AMR data capturing to reduce manual data entry and 3) centralize AMR laboratory data at EPHI to enable a single destination for better decision making by the Ministry of Health.

Results: Over the last year, EPHI and APHL have collaborated to develop detailed plans to combat AMR data management issues with technological solutions that will be feasible in Ethiopia. EPHI and APHL recognize a stepwise approach is essential to impact AMR; use of appropriate technology will improve the data capturing process and EPHI's use of data to facilitate strengthening data analysis for evidence-based decision making. The initial step required gathering baseline data on the AMR testing laboratories in which equipment, testing capacity, and SOPs were documented. The baseline assessments informed the team that while automated analyzers are available, these were not being used due to limited availability of reagents. That made the laboratories unable to automate the data flow. The team pivoted to identify tools to enable AMR laboratories to share results from any testing method used and focus on centralizing the AMR data in a national laboratory repository.

Conclusion: APHL and EPHI's collaboration is focused on the long-term goal to have "Quality data for quality decision making". Putting the onus of sharing data on laboratories conducting AMR testing and locating the central data repository at EPHI leads to a better understanding by health facilities of the need to routinely report AMR data. An immediate next step is to develop dashboards based on the repository to enable testing laboratories and EPHI to view the data submitted.

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Poster 29M

Genetic Characterization of *Strongyloides fuelleborni* Infecting Free-ranging African Vervets (*Chlorocebus aethiops sabaeus*) on the Caribbean island of St. Kitts

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Human strongyloidiasis is an important neglected tropical disease primarily caused by the nematode *Strongyloides stercoralis*, and to a lesser extent *Strongyloides fuelleborni* which primarily infects non-human primates. Zoonotic sources of infection have important implications for control and prevention of morbidity and mortality caused by strongyloidiasis. Recent molecular evidence suggests that for S. fuelleborni, primate host specificity is variable among types across the Old World, and consequently that these types likely vary in their capacity for human spillover infections. Populations of free-ranging vervet monkeys (*Chlorocebus aethiops sabaeus*), introduced to the Caribbean Island of St. Kitts from Africa, are increasingly involved in conflicts with humans and concern has arisen as far as their role as a source of zoonotic infections. In this study, we sought to determine the genotypes of S. fuelleborni infecting St. Kitts' vervets to explore whether they are potential reservoirs for human-infecting S. fuelleborni types. Fecal specimens were collected from St Kitts' vervets and S. fuelleborni infections were confirmed microscopically and by PCR. S. fuelleborni genotypes were constructed from positive fecal specimens using an Illumina amplicon sequencing-based genotyping approach targeting the mitochondrial cox1 locus and 18S rDNA hypervariable regions I and IV of Strongyloides species. Phylogenetic analysis of resultant genotypes supported that S. fuelleborni from St Kitts' vervets is of an exclusively African variety, falling within the same monophyletic group as an isolate detected previously in a naturally infected human from Guinea-Bissau. This observation suggests that St Kitts' vervets could function as potential reservoirs for zoonotic S. fuelleborni infection, which warrants further exploration.

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Poster 30M

The Prevalence of Areca Nut Consumption and Self-Reported Oral Cancers Among Rural Communities In Karachi, Pakistan: A Community-Based Cross-Sectional Survey

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Background: The seed of the areca palm is chewed recreationally by approximately 600 million people globally. Chewing areca is strongly associated with oral cancers. In Karachi, Pakistan, the practice is common, with one study reporting 34.3% to 50% (areca nut products and areca nut mixed with tobacco products) in a squatter settlement. In Pakistan, oral cancers are second only to breast cancer in prevalence. Oral cancers are highly preventable; the high prevalence represents an unmet public health need. Further, local prevalence statistics are needed to inform successful prevention interventions to targeting the most vulnerable populations.
Methods: We enrolled 760 participants among the rural population of Karachi, Pakistan, between December 2021 and February 2022. This cross-sectional study assessed the prevalence of self-reported areca nut use and self-reported oral cancers stratified by age, gender, and ethnicity.
Results: 74% of the study population were areca nut users. There were significant associations with gender, age, and ethnicity. Among areca nut users, 10.3% had self-reported oral cancers.
The areca nut consumption among males was 87% compared to females with 53% and children with 60%. Adult daily use of areca nut is 76.5% compared to children with 60%. Among ethnic groups, the Muhajir population had a higher areca nut consumption (74%) than lower areca consumption among Balochis (55%). The 18-34 age group consumed more areca nut (87%) than individuals of ≥65 years at about 62%. The cancer prevalence was higher among females (17.5%). The overall awareness rate among regarding adverse health impacts among the the study population was 27%.
Discussion & Conclusion: Areca nut use appears to be common in this rural population, with an overall prevalence higher than the national reported rate. There is room to improve population awareness of adverse health impacts. Gender, age, and ethnic distinctions suggest where to focus public health efforts.

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Poster 31M

Improving HIV Viral Load Coverage Through System Strengthening, Data Audit and Triangulation in Lusaka Province Zambia

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Background: Lusaka Provincial Health Office (LPHO) experienced low VL coverage in quarter 3 of 2022 despite PCR laboratories clearing the backlog. The discrepancy between the number of VL specimens collected, tested and results transmitted to referral sites from testing sites, a potential reduction in VL coverage (VLC). We conducted mentorship and VL data audit to determine factors associated with low VLC and provide solutions to improve VLC.
Methods: Comparative analysis of VL testing coverage (VLTC) baseline audit in July 2022 and endline audit in September 2022 in 9 selected health facilities (HF) was conducted. Tx-curr, Tx-curr PVLS Denominator and Numerator were evaluated, and VLC calculated. Electronic data tools such as Laboratory Information System – LIS (Disalab, DisaPOC and Disalink) and SMARTCARE were used to enter data from Disalab extracted line list spreadsheet and VL results printed at the HF from LIS. The process of VL specimen/result management was mapped. Healthcare personnel were mentored in VL management cascade.
Results: Endline data audit was conducted across 9 HF showed improvement in VLTC compared to the baseline audit. Ngwerere Rural Health Centre (RHC) (70% to 90%), Chongwe RHC (79% to 94%), Chongwe District Hospital (78% to 89%), Luangwa RHC (77 to 97%), Chipata General Hospital (79% to 91%), Matero General Hospital (72% to 90%), Chilanga Health Centre (79% to 88%), Kafue Estates Health Centre (70% to 94%) and Lusitu RHC (83% to 92%). 7/9 (78%) attained set target of 90% VLC, 2/9 (22%) did not attain the 90% VLC. Average VLC for 9 HF was 92%, 76% before intervention, showing percentage increase of 16% post intervention. On-site mentorship in LIS usage, SMARTCARE and documentation in HF registers was done. SMARTCARE upgrade, poor appointment system, drug dispensantion, lag in updating SMARTCARE and lack of coordination among healthcare staff contributed to low VLC.
Conclusion: Capacity building, effective usage of LIS, SMARTCARE to automate digital VL results return a good contributor to improved and sustained VLC. Timely documentation of results in registers and weekly HF coordination meetings will strengthen the system and increase VL coverage.

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Tuesday, March 14, 2023

Biosafety and Biosecurity

Poster 01T

Zambia Waste Management Strengthening Project Implementation for Public Health Facilities Supporting Conventional Viral Load and Early Infant Diagnostic Testing Services

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Background: Concerns of the use of guanidinium thiocyanate routinely used for HIV viral load (VL) and HIV early infant diagnosis (EID) prompted a review of waste management (WM) procedures at public health laboratories. Zambia Ministry of Health (MOH) and US Centers for Disease Control and Prevention (CDC) initiated a review of WM that was carried out by the Association of Public Health Laboratories (APHL) and the Centre for Infectious Disease Research in Zambia (CIDRZ).
Methods: A stakeholder meeting was convened to consider strategies, activities, and expected outcomes. A detailed survey document was used by an MOH, APHL, and Zambia Environmental Management Agency team to assess 21 facilities that support VL and EID testing. The assessments evaluated waste handling, personnel training, personal protective equipment (PPE), and waste treatment technologies.
Results: The assessments identified several weaknesses in waste management practices including a) 81% of facilities not properly segregating waste, b) 90% of facilities had inadequate personal protective equipment, c) 81% of facilities lacked autoclaves, and 100% of facilities lacked properly operational incinerators. Based on evaluation of facility waste streams, incineration was identified as the best option for waste disposal and an improvement plan developed and implemented. 11/21 (52%) of facilities required incinerator replacement, and 10/21 required service and repair of the existing incinerators. Eight (08) 70Kg/hr MacroBurns and three (03) 50Kg/hr Inciner8 incinerators were installed in 11 facilities; 10 incinerators of varying types were repaired at 10 sites and 48 operators from the 21 sites were trained.
Conclusion: Engagement and empowerment of the laboratory and environmental health staff at the facilities is cardinal for the development of correct WM activities and sustainability. Routine reporting of WM operations is essential to maintain safe and effective waste disposal and spread repair and replacement cost over many budget years to sustain sufficient operations.

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Poster 02T

Effective Communication with Laboratory Professionals during an Emergency Response

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Laboratory professionals are on the front lines of any public health emergency response. Effective communication empowers laboratory professionals to ensure quality testing and timely result reporting, which in turn protects patients and saves lives. In the ever-evolving emergency response landscape, a clear and coordinated communication effort is of paramount importance.

On January 21, 2020, CDC activated its agency-wide response to the COVID-19 pandemic, and laboratories and testing sites across the country prepared for a surge in SARS-CoV-2 testing. At the onset of the pandemic, information for laboratories and testing sites was limited – and as the situation evolved, the need for prompt, credible information increased. To help bridge this information gap, CDC's Division of Laboratory Systems (DLS) implemented a three-pronged approach to optimize communication with the laboratory and testing community: hosting regular calls, supporting the development of a robust portfolio of content for CDC's COVID-19 Information for Laboratories website, and using the Laboratory Outreach Communication System (LOCS) to disseminate relevant email updates.

On March 23, 2020, DLS convened the first Clinical Laboratory COVID-19 Response Call. This was the agency's first large-scale, COVID-19 response-related partner call specifically for the laboratory and testing community. Agenda topics have included laboratory testing, anatomic pathology, biosafety, and laboratory data reporting. In 2021, DLS convened 23 of these calls with an average of 1,000 participants at each. In 2022, DLS renamed these to LOCS Calls to expand the scope of these calls to cover like the current monkeypox outbreak.

CDC has created a robust Information for Laboratories about Coronavirus (COVID-19) website, with DLS serving as a content expert for biosafety and laboratory testing. Hot topics include testing strategies, specimen collection, antigen testing, pooling, nucleic acid amplification tests (NAATs), and point-of-care and rapid testing. CDC has also published a Frequently Asked Questions (FAQs) about COVID-19 for Laboratories page. Most recently, when CDC activated its response to the monkeypox outbreak, DLS subject-matter experts and communicators sought to develop and optimize biosafety and testing guidance for Monkeypox Virus to support the laboratory and testing community as they faced a nationwide surge in testing.

CDC uses LOCS to disseminate email updates to the laboratory and testing community about new resources, regulatory guidance, the Clinical Laboratory COVID-19 Response Calls, and other topics related to emergency preparedness. Laboratories and testing sites can also contact CDC directly to request technical assistance or ask questions to support their work. LOCS has a distribution list of approximately 100,000 laboratory and testing professionals and DLS continues to receive daily opt-in requests.

DLS communication efforts use a scalable communication strategy based on existing and new communication channels, to reach the target audience and empower them to take action. In an emergency response, it can feel like change is the only constant, but we can keep timely effective communication front and center as we work together to protect people and save lives."

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Emerging Technologies

Poster 03T

Comparison of Four Commercial Kits to Extract Bacterial Chromosome and Plasmid DNA for Long-read Sequencing

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Third-generation sequencing technologies have undergone significant improvements in sequencing costs, accuracy, and sequencing read lengths. The critical factor for successful long-read sequencing is the extraction of high molecular weight DNA of sufficient purity and quantity.

We compared four commercial kits for the extraction of genomic DNA from the eight bacterial species. Three kits (Circulomics Nanobind CBB Big DNA kit®, ZymoBIOMICS DNA Miniprep Kit®, and Fire Monkey High Molecular Weight DNA extraction®) are based on manual extraction methods and one automated extraction method (Roche MagNaPure 96®), which relies on different principles (either mechanical lysis or chemical lysis) for DNA purification. We selected four Gram-negative and four Gram-positive bacterial species, including Acinetobacter baumannii, Klebsiella pneumoniae, Pseudomonas aeruginosa, group D Salmonella, Enterobacter faecium, Streptococcus agalactiae, Streptococcus suis, and Staphylococcus aureus, for subsequent nanopore sequencing. We assessed and compared the quality of genomic DNA (i.e., purity and concentration) obtained from the four kits. The molecular weight of the extracted DNA was determined visually using TapeStation.

The concentration of DNA and DNA quality of the extraction kits used in the study varied significantly. For gram-positive bacterial DNA extraction, we found that the ZymoBIOMICS kit® provided the highest DNA purity (260/280: 1.77) while the Circulomics kit® performed best in terms of molecular weight size of DNA for both Gram-positive and Gram-negative bacteria (> 60 kilobases). While both Circulomics® and Fire Monkey® kits required more hands-on time (2 hour and 1.5 hours, respectively), longer DNA molecular weight sizes were obtained which facilitate longer sequencing read lengths. The Roche system has lower hands-on time (10 min) to other DNA extraction kits (45 min to 3 hours). However, the cost of the instruments is high (~70,000 USD), and the consistency of DNA concentration is low between the same species.

Disclaimer - Use of trade names is for identification only and does not imply endorsement by [the Centers for Disease Control and Prevention/the Agency for Toxic Substances and Disease Registry], the Public Health Service, or the U.S. Department of Health and Human Services.

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Poster 04T

Strengthening Georgia Public Health Laboratory Surge Capacity for Flu Surveillance using Standard BioTools BiomarkTM HD Array Platform

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Background: Pandemics, surveillance and plethora of infectious disease outbreak investigations has necessitated the importance of establishing the high throughput testing capacity to support emergency preparedness and surge activities. Standard Bio Tools (BiomarkTM HD) is a nanofluidic automated real-time PCR system that relies on microfluidic technology with arrays of integrated fluidic circuits (IFCs). The instrument uses an array of non-fluidic chips called dynamic arrays for qPCR, which allows for 9,216 PCR reactions (96.96 chip format; 96 samples × 96 assays) in a single qPCR run (www.fluidigm.co.za). We aimed to validate and deploy BiomarkTM HD platform to expand Georgia Public Health Laboratory flu surge capacity for assisting both outbreak investigations and surveillance activities.
Objective: To validate the performance of Standard Bio Tools (BiomarkTM HD) array platform to detect influenza A subtypes and influenza B lineages using CDC Flu subtyping reagents to expand GPHL surge capacity.
Method: A total of 230 specimens (170 positive for Flu A and 60 positive for Flu A) was included in this validation. The assay setup (192.24 IFC) includes RNA extraction, IFC setup and RT-PCR with Janus and BioMark HD. The performance characteristics of this platform was compared with conventional RT PCR for flu subtyping.
Result: Overall, the assay performance showed 100% agreement between reference and Fluidigm pathogen detection RT-PCR kit using CDC Flu subtyping reagents. The introduction of preamplification demonstrated higher sensitivity and no cross reactivity was observed. The precision data showed 98.33% agreement. The limit of detection was determined as 10 copies/µl (equivalent to CT 23, > 95% of the low dilution replicates were detected). Based on the validation study, the reportable CT ranges including > 23 as “Not detected”, specimens with CT equal to 5 and < 23 as “Detected” and CT < 5 as “Inconclusive”. The specimens with CT < 5 will be reflexed to CDC subtyping assay.
Conclusion: Microfluidics technology using integrated fluidic circuit (IFC) has not been widely evaluated for influenza testing. The implementation of high throughput IFC array at GPHL has improved the flu surge testing capacity and can test approximately 162 specimens per run to support surveillance and outbreak investigations. Most importantly, this assay has improved the turnaround time with limited staffing capacity.

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Poster 05T

Detection of SARS-CoV-2 in Municipal Wastewater Using Stilla Naica® Digital PCR Platform as Part of Promega Enviro® SARS-CoV-2 WBE Workflow

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Early in the COVID-19 pandemic, studies showed that SARS-CoV-2 viral RNA could be detected in the feces of infected individuals. Detection of SARS-CoV-2 in wastewater can indicate the presence and/or prevalence of COVID-19 within a community and provide crucial information about upcoming disease outbreaks, an approach known as wastewater-based epidemiology (WBE). WBE studies require both effective methods for concentration and purification of viral RNA in addition to robust and quantitative RT-PCR methods for accurate acquisition and interpretation of WBE results. Here we describe a coupled workflow for both purifying and detecting SARS-CoV-2 RNA from municipal wastewater (WW). Total nucleic acid (TNA) was isolated from WW using the Promega Maxwell® RSC Enviro TNA kit, which has been demonstrated to provide high yields of viral TNA with minimal carryover of PCR inhibitors. Following TNA purification, the Naica® digital PCR platform from Stilla Technologies was utilized in conjunction with Promega GoTaq® Enviro Wastewater SARS-CoV-2 system to specifically detect and quantify viral RNA in TNA samples. This detection method allows for sensitive, multiplexed detection of multiple SARS-CoV-2 genomic targets in a single reaction. PMMoV, a ubiquitous virus in municipal WW that is often used as a process control and for normalization of SARS-CoV-2 signal, is also detected in a discrete fluorescent channel. To assess the effect of PCR inhibitors present in TNA samples, an endogenous internal amplification control (IAC) template is amplified concurrently along all WW TNA samples. Raw data was analyzed and viral titers were quantified using the Stilla Crystal Miner Software. Our results demonstrate that this method is a sensitive, robust, and repeatable solution for SARS-CoV-2 wastewater-based epidemiology (WBE).

Presenter: Nathan Feirer, Promega Corporation, Madison, WI, nathan.feirer@promega.com

Poster 06T

Fully Automated Isolate Whole Genome Sequencing Platform to Enable Agnostic Characterization and Real-Time Outbreak Investigations of Foodborne Pathogens

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Objectives: Approximately 48 million people get sick, 128,000 are hospitalized, and 3,000 die from foodborne diseases each year in the United States. The foodborne outbreaks are caused by bacteria such as Salmonella, Listeria, Shiga toxin-producing Escherichia and Vibrio. With the advancement of high-throughput sequencing technology, whole genome sequencing (WGS) of pathogens in public health laboratories has improved surveillance for foodborne disease outbreaks and antimicrobial resistance. WGS provides genome-wide high-resolution data for identifying and tracking outbreaks sooner and enables phylogenetic studies of bacterial genomes. Despite the growing demand and numerous benefits, the WGS library preparation workflow includes several critical steps that require careful human attention and labor, thereby limiting the widespread adoption.
Methodology: Clear Labs has successfully developed a fully automated WGS workflow on a completely integrated platform that includes a liquid handling robot, thermocyclers, plate racks, magnets and sequencers. The workflow is streamlined to include genomic DNA extraction, shotgun library preparation, amplification and purification of sequencing libraries, loading onto the sequencer and automatically uploading the sequencing data onto a cloud server. It is then followed by a host of bioinformatic processing, genome assembly and secondary analyses, which are performed using a modular bioinformatic pipeline. The workflow is also simplified to minimize hands-on time of sample loading at the beginning and also eliminate any human intervention after initiation of the run.
Significant Results: Hundreds of bacterial genomes of foodborne pathogens including top PulseNet species (Salmonella, Listeria, Escherichia, etc) have been processed through the fully automated workflows on many prototype platforms in-house. Clear Labs tested single-pathogen runs for throughputs ranging from 4 to 16 samples per run. More than 90% of the samples passed the PulseNet acceptance criteria of assembly metrics such as N50, total length, median insert size and mean coverage. The sequencing results of an experiment with pooling and cleaning up the PCR products of individually barcoded samples showed no difference compared to performing the clean-up on the individual samples for all the PulseNet criteria (P-Value >0.05, t-test). In addition, the variability in median insert size among the samples decreased significantly (P-value < 0.01, F-test) with the pooled processing.
Conclusions/Implication: With growing demand for WGS, timely processing of bacterial isolates on a large scale is critical. The traditional manual sequencing workflow requires technical expertise and is labor-intensive. In the manual library preparations, the libraries are pooled based on their individual DNA concentrations. Our automated platform uses bead-based tagmentation followed by PCR as the normalization mechanism to successfully remove the necessity to quantify the individual libraries prior to pooling. A fully automated WGS platform will enable many public health labs to achieve accurate and consistent results with less cost and hands-on time (from more than 6 hours down to less than 30 minutes). The power of this technology is not limited to only foodborne pathogens, but can also be applied to many other medical research including healthcare-associated infections (HAI) and pathogen surveillance programs.

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Poster 07T

Standardizing Sample Preparation and Clarification Methods for Wastewater Surveillance Monitoring of SARS-CoV-2

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Gaps exist in standardization of sample preparation and clarification activities to render an effluent wastewater sample suitable to quantify N1 and N2 targets of SARS-CoV-2 for wastewater surveillance monitoring. Pennsylvania Department of Health Bureau of Laboratories is conducting method optimization studies to evaluate effects of heat-inactivation and three different methods of sample clarification to determine impact on viral RNA using droplet digital polymerase chain reaction (ddPCR) to ascertain which is the preferred combination for routine wastewater processing. Sample aliquots that remain non-heated are being compared to heat-treated aliquots to explore the temporal relationship between heat-inactivation and viral RNA degradation. Additionally, sample clarification methods of sedimentation, centrifugation, and filtration are being compared to measure impact on quantities of viral RNA. Viral RNA degradation by heat-inactivation, which is suggested in literature, is being confirmed in this study. The study results must be tempered against other considerations for processing time, unit cost per test, and safety of laboratory personnel prior to final selection of sample preparation/clarification combination. Studies using wastewater effluent from partnering wastewater treatment plants across the Commonwealth of Pennsylvania are being conducted to evaluate each of these methods and confirm which combination of preparation and clarification is most suitable for routine wastewater surveillance monitoring.

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Poster 08T

Accessible, Effective, and Rapid: Online COVID-19 Education and Training for Laboratory Professionals

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Background: The COVID-19 pandemic shed light on the lack of easily accessible, customized education and training for laboratory professionals. With thousands of laboratory professionals, clinicians, and point of care testers being called upon for immediate, life-saving services, there was an obvious gap in resources available to them. Clinical laboratory professionals in local and remote areas across the country needed additional scientific, technical, and regulation trainings, and needed them to be easily accessible in the home or workplace.
The OneLab initiative includes seven elements, each designed to address the specific needs of laboratory professionals. This presentation will focus on OneLab's Rapid Education and Capacity-building Hub, or REACH. REACH launched in summer of 2022 and is the online platform that offers clinical and public health laboratory professionals access to diverse educational and capacity-building resources.
Method: In February 2021, CDC contacted over 1,000 laboratory professionals for an in-depth training needs assessment including a survey and focus group discussions. Three education/training themes were developed, and four cross-cutting themes identified, including the need for a collaborative environment, centralized resources, cohesive messaging for the public, and flexible learning modules. Laboratory professionals voiced needs for technical topics, preparedness and emergency operations, and crisis leadership.
CDC developed OneLab REACH as a customized learning management system (LMS) built on open-source software, in response to the need for a centralized resource for response-related training and education materials. It was designed and built in-house based on extensive requirement gathering and analysis of alternatives platforms.
In collaboration with public health professionals and educators, web developers, and laboratory clinicians, CDC's Division of Laboratory Systems created a centralized hub for COVID-19 related resources and trainings in the form of an open-source code-based LMS. This platform is free and available to anyone who has access to the internet. This site includes eLearning and job aids specifically relevant to the COVID-19 laboratory response.
Results: This LMS is built to be quick, flexible, and responsive. Learners can access short, self-paced trainings, job aids and other materials that often include continued educational credits (CEU's). The content, including courses on packing and shipping dangerous goods and testing best practices, is continually updated, built to be engaging, and structured on real-life scenarios.
Conclusion: During the COVID-19 pandemic and beyond, laboratory professionals have a demonstrated need for a learning platform that is easy to use, relevant, and accessible from wherever they are located. CDC developed an online platform that is agile, up-to-date, and in alignment with nationally recognized standards, customized specifically for these professionals. This platform is expected to reach over 10,000 online learners by the end of 2023 and will continue to positively impact the daily lives of laboratory professionals across the nation.
Expanded access to free, online public health courses and job aids will strengthen the existing public health infrastructure by improving the capacity for individual laboratories, especially those operating with less funding and fewer resources, to respond quickly and cohesively to public health threats.

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Outbreaks and Surveillance

Poster 09T

Withdrawn

Poster 10T

Withdrawn

Poster 11T

SARS-CoV-2 and Monkeypox Next-Generation Sequencing: Technical Workflow Development, Analysis and Reporting from Dallas County Public Health Laboratory

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Introduction: Next-generation sequencing (NGS) of SARS-CoV-2 and emerging pathogens e.g. Monkeypox virus has been a core Public Health initiatives for the last several years. Identification of pathogen clades and variants highly facilitates surveillance and epidemiological investigations supporting a robust local outbreak response.
Objective: The objective of the Dallas County Health and Human Services (DCHHS) Public Health Laboratory (PHL) NGS program is to develop and establish sequencing workflows and analysis pipelines for SARS-CoV-2, Monkeypox and other emerging and ongoing infectious microorganisms including Syphilis, HSV and HIV.
Methods and Materials: Presented here is the deployment of Illumina sequencing platform that includes MiSeq sequencing device, reagents, Sequencing Hub (BaseSpace) and then a third-party analysis software (CLC Genomics Workbench) for sample processing, DNA library preparation, generating sequencing data and Bioinformatics analysis.
Technical workflow: Based on current Illumina COVIDSeq protocol, the sequencing workflow starts with total nucleic acid (RNA/DNA) extraction and quantitation from the patient specimens (e.g. NP-swab). Going forward, ~1-10 ng of the extracted RNA/DNA material is processed to make sequencing libraries. The pooled library is then properly denatured and loaded onto a MiSeq sequencer. After about 12 hours of runtime, the sequencing data (generating Fastq files) are then imported to cloud-based analysis platforms.
Results: A total of about 70 COVID specimens have been successfully sequenced and a further 200 and more Monkeypox specimens are in process to be sequenced at the DCHHS PHL. Among COVID cases, 20 (sub-)variants of Omicron have been identified with majority found to be within the clade 22B (July through September 2022). Additionally, a Monkeypox sequencing workflow has been developed and adopted on COVIDSeq assay which is able to detect Clade IIb (West African Clade) as part of the ongoing sequencing process. Next step will include submission of sequences to national and global databases (NCBI, GISAID etc).
Conclusions: The DCHHS PHL next-generation sequencing program will continue to provide essential educational and technical tools for public health response and public health institutions to assist in genomic epidemiology, real-time surveillance and outbreak investigation of SARS-CoV-2 and Monkeypox and other pathogens of public health concerns.

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Poster 12T

Incidence of Co-infections of SARS-CoV-2, Influenza A and Other Respiratory Pathogens from 2021-2022

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Background: Due to the occurrence of the COVID-19 pandemic, many of the routine testing procedures at the New Hampshire Public Health Laboratories (NH PHL) shifted drastically. In order to perform influenza surveillance while maintaining SARS-CoV-2 testing demands, the NH PHL performed the CDC Influenza SARS-CoV-2 Multiplex assay for all influenza and SARS-CoV-2 PCR testing during the peak respiratory seasons of 2020 - 2022. Routine use of the assay allowed the NH PHL to detect co-infections of SARS-CoV-2 and influenza viruses. Additionally, the NH PHL implemented the Biofire RP2.1 before the 2021-2022 season, increasing the capacity to detect co-infections with SARS-CoV-2 and influenza viruses. Utilizing both assays, the frequency of positive SARS-CoV-2 and influenza samples, as well as any co-infections that occurred amongst them, was observed.
Methods: The CDC Influenza-SARS-CoV-2 Multiplex PCR assay utilizes real time PCR to simultaneously detect the presence of RNA from Influenza A, Influenza B and SARS-CoV-2 viruses. This assay was used from February – April 2021, and was implemented permanently starting in October 2021. The Biofire RP2.1 utilizes film array technology to test for 23 different respiratory pathogens simultaneously. Sample data was pulled from the 2021-2022 season, specifically January 1, 2021-October 10, 2022 using the NH PHL's Laboratory Information Management System.
Results: A total of 181 influenza positives were detected using the CDC Multiplex assay. During this time, only 3 co-infections of SARS-CoV-2 and Influenza A were detected, accounting for just 2% of Influenza A positives. Three Influenza A positives were found using the Biofire, and only 1 was a co-infection with SARS-CoV-2. A total of 30 SARS-CoV-2 positives were found using the Biofire panel, and 5 were co-infections with non-influenza respiratory pathogens.
Co-infections were detected on both assays, with the majority found on the Biofire RP2.1. Use of the RP2.1 on select specimens revealed several instances of SARS-CoV-2 co-infections with seven different respiratory pathogens: adenovirus, human rhinovirus, human enterovirus, metapneumovirus, parainfluenzavirus 3, coronavirus NL63, and respiratory syncytial virus.
Discussion: Numerous challenges were presented by the COVID-19 pandemic, one of which was allocating resources for diagnostic and surveillance testing other than SARS-CoV-2. By using the CDC Influenza SARS-CoV-2 Multiplex assay, the NH PHL was able to pool resources to perform both influenza surveillance and SARS-CoV-2 testing. In addition, the Biofire RP2.1 was effective at detecting these viruses, as well as other respiratory pathogens. Co-infections were rare compared to the number of samples that tested positive solely for SARS-CoV-2 or influenza. Future research could include investigating the potential relationship between SARS-CoV-2 variants and the occurrence of co-infections utilizing sequencing data generated at the NH PHL.

Presenter: Jessica Alexander, New Hampshire, Public Health Laboratories, Concord, NH, jessica.l.alexander@dhhs.nh.gov

Poster 13T

Utilizing Long-Read Sequencing to Examine blaNDM-5 Plasmid Transmission Dynamics in a Congregate Companion Animal Facilities

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Background: Conjugative plasmids carrying antibiotic resistance genes such as carbapenemase genes (CG) are driving the global spread of carbapenem resistance. The MN Department of Health and University of MN Veterinary Medical Center collaborate to detect CG in companion animals. In 2022, a carbapenem-resistant Escherichia coli was isolated from a post-surgical wound in a canine patient originating from the Middle East; blaNDM CG was detected. The dog resided in a congregate animal facility containing animals of domestic U.S. and international origin, prompting screening of additional dogs for CG. Bioinformatics methods were used to identify potential plasmids and understand blaNDM transmission potential in this population.
Methods: Screening (real-time PCR, Cepheid GeneXpert Carba-R) was performed on rectal swab specimens from dogs in the congregate animal facility; blaNDM-positive specimens underwent bacterial culture and isolation. Short-read (Illumina MiSeq) and long-read (Oxford Nanopore GridION) sequencing were performed on isolates to examine strain relatedness, identify AR genes, and confirm plasmid presence. Several bioinformatics tools were used to assess long-reads and better understand transmission dynamics among dogs.
Results: Of 72 dogs screened, 27 (38%) were positive for blaNDM-5. Twenty-seven E. coli isolates were recovered from rectal swabs of 24 dogs. Isolates were of 3 sequence types (ST): ST361 (n=20), ST167 (n=6), ST1163 (n=1); 2 dogs harbored both ST361 and ST167. GridION sequencing was performed on 1-3 isolates from each ST. Conjugative and/or mobilizable plasmids within different incompatibility (Inc) group families were predicted in each ST. ST361 isolates contained 2 predicted plasmids: IncF harboring blaNDM-5 and IncY (no AR genes). ST167 isolates harbored 3 predicted plasmids: IncF carrying blaNDM-5 and blaCTX-M-15, IncC harboring blaSHV-1, and a replicon cluster 2335 element. ST1163 isolates contained 4 predicted plasmids: IncF carrying blaNDM-5, IncX1 harboring blaTEM, IncX4 (no AR genes), and IncI (OmpC). An IncF plasmid harboring blaNDM-5, which is predicted to be conjugative, was found in all ST. Predicted plasmids harboring blaNDM-5 were 99.9% identical within ST, but varied significantly between ST. A conserved integron carrying blaNDM-5 and other AR genes was present in all isolates.
Conclusions: Companion animal importation is an understudied component of CG transmission dynamics in the U.S. Here, IncF heterogeneity across ST suggests multiple importations of blaNDM-5-producing E. coli strains prior to further transmission within the canine population of the facility. No human cases were detected during this investigation. Long-read sequencing can be used to identify mobile genetic elements in bacterial isolates and to explore their potential role in CG transmission. These methods can meaningfully contribute to outbreak investigation and development of risk-based prevention practices.

Presenter: Melissa Anacker, Minnesota Department of Health, St. Paul, MN, melissa.anacker@state.mn.us

Poster 14T

First Report on the Emergence of Carbapenamase-Producing Enterobacterales (CRE) Harboring blaNDM-7 in the State of Georgia

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Background: New Delhi metallo-β-lactamase (NDM) is a plasmid-encoded class B carbapenamase found in gram-negative bacteria capable of hydrolyzing a wide variety of drugs such as penicillins, cephalosporins, monobactams, and carbapenems. Though uncommon in the United States, NDM-producing Enterobacterales are more common in healthcare settings in other countries and are an emerging public health threat. The blaNDM-7 gene variant was first described in an E. coli strain from Germany and subsequently reported sporadically in many countries. In the United States, NDM-7-producing Enterobacterales have been reported in Minnesota and Nebraska. As part of the Antimicrobial Resistance Laboratory Network, antimicrobial surveillance and outbreak investigations are being performed in Georgia to detect the emergence and spread of organisms harboring plasmid and chromosomal genes conferring carbapenem resistance mechanisms.
Methods: Between January 2020 and May 2022, a total of 2570 CRE isolates were submitted to the Georgia Public Health Laboratory (GA-PHL) for phenotypic (MALDI-TOF and mCIM) and genotypic characterization (β-Lactamase Streck ARM-D Kit). 99 isolates were tested positive for blaNDM. Whole genome sequencing (WGS) libraries were prepared with Illumina DNA Prep and Nextera XT kits (Illumina Inc) and sequenced on MiSeq. Paired-end raw reads were assembled using standard bacterial genome assembly and antimicrobial resistance (AMR) was characterized using the flaq_amr pipeline. MLST subtypes (ST) were identified with PubMLST typing schemes. PlasmidFinder was used for the detection and characterization of plasmid sequences in each assembled genome. Core genome single nucleotide polymorphisms (SNPs) were calculated with Snippy using the highest N50 genome assembly as the reference. Epidemiological metadata was mined from Electronic Laboratory Reports (ELR) and the State Electronic Notifiable Disease Surveillance System (SENDSS).
Results: Five isolates (3 isolates of Klebsiella pneumoniae, 1 isolate each of Enterobacter cloacae complex and K. variicola) from 4 patients harbor blaNDM-7. These isolates were cultured from multiple specimen types including endocervix, blood, and sputum. The IncX3 plasmid was detected in all five isolates. Based on the SNP analysis, 38707 base pairs were aligned among the five isolates and the SNPs were calculated at nucleotide positions shared across this core genome alignment. E. cloacae complex harboring both blaNDM-7 and blaKPC-3 and K. variicola harboring blaNDM-7 were isolated from endocervical and blood specimens collected from the same patient at two different facilities. Two closely related strains (4 SNPs) of K. pneumoniae harboring blaNDM-7 were isolated from two patients within the same facility. One K. pneumoniae ST37 isolate harboring blaNDM-7 was more divergent (1288 and 1286 SNPs) compared to the other two K. pneumoniae isolates.
Conclusions: To the best of our knowledge, this is the first report of blaNDM-7 from Southeastern United States. It is crucial to keep monitoring the emergence of blaNDM-7-containing CREs. WGS provides valuable, real-time, epidemiologic-informed genomic subtyping that can enhance the detection of potential transmission of the blaNDM gene. It supports the implementation of public health action and targeted infection prevention interventions such as containment strategy in healthcare settings to prevent the transmission of blaNDM-containing CRE.

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Poster 15T

Genomic Diversity of New Delhi Metallo-β-lactamase (NDM)-Producing Carbapenem-Resistant Enterobacterales in Georgia

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Background: New Delhi metallo-β-lactamase (NDM) is a plasmid-encoded class B carbapenamase found in gram-negative bacteria capable of hydrolyzing a wide variety of drugs such as penicillins, cephalosporins, monobactams, and carbapenems. Though uncommon in the United States, NDM-producing Enterobacterales are more common in healthcare settings in other countries and are an emerging public health threat. As part of the Antimicrobial Resistance Laboratory Network, antimicrobial surveillance and outbreak investigations are being performed in Georgia to detect the emergence and spread of organisms harboring plasmid and chromosomal genes conferring carbapenem resistance mechanisms. Due to the increase in epidemiologically linked NDM-producing carbapenem-resistant Enterobacterales (CRE) in Georgia, the main objective of this study is to characterize the genomic diversity of CRE isolates containing the blaNDM gene by whole genome sequencing (WGS).
Methods: Between January 2020 and May 2022, a total of 2570 CRE isolates were submitted to the Georgia Public Health Laboratory (GA-PHL) for phenotypic (MALDI-TOF and mCIM) and genotypic characterization (β-Lactamase Streck ARM-D Kit). 99 isolates were tested positive for blaNDM. WGS libraries were prepared with Illumina DNA Prep and Nextera XT kits (Illumina Inc) and sequenced on MiSeq. Paired-end raw reads were assembled using standard bacterial genome assembly and antimicrobial resistance (AMR) was characterized using the flaq_amr pipeline. MLST subtypes (ST) were identified with PubMLST typing schemes. To determine the genetic relatedness of blaNDM-harboring isolates, core genome single nucleotide polymorphisms (SNPs) were calculated with Snippy and neighbor-joining phylogenetic trees were constructed in Jalview.
Results: Based on WGS and bioinformatics analysis, blaNDM variants namely blaNDM-1 (n=49), blaNDM-5 (n=45) and blaNDM-7 (n=5) were identified. Within Enterobacterales genera, Escherichia coli (n=38), Klebsiella spp. (n=32), and Enterobacter spp. (n=18) strains harbor blaNDM genes. Predominantly, K. pneumoniae (n=28) and Enterobacter cloacae complex (n=6) strains harbor blaNDM-1, blaNDM-5, and blaNDM-7, whereas E. coli (n=38), E. cloacae (n=12), and Citrobacter freundii (n=4) harbor blaNDM-1 and blaNDM-5 variants. Based on the phylogenetic analysis, multiple clusters of closely related isolates within the same species have been identified. E. cloacae has two clusters, one predominated by ST78 and one unidentified. E. coli clusters with more than two isolates include ST127, ST405, and ST410. However, E. coli ST167 is documented in three different clades. Major K. pneumoniae clusters include ST16, ST147, and ST307.
Conclusion: Based on blaNDM genomic characterization, three variants were documented including blaNDM-1, blaNDM-5, and blaNDM-7. Most within-species clusters of closely related genomes are predominated by one subtype. These clusters could be of potential interest for assisting outbreak investigation and understanding the clonal dissemination within the facilities. WGS provides valuable, real-time, epidemiologic-informed genomic subtyping that can enhance the detection of potential transmission of the blaNDM gene. It supports the implementation of public health action and targeted infection prevention interventions such as containment strategy in healthcare settings to prevent the transmission of blaNDM-containing CRE.

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Poster 16T

Public Health Laboratory Covid-19 Response: Development of Rapid COVID Testing Across New York City

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The sudden onset of the COVID-19 pandemic in March 2020 in New York City (NYC) resulted in significant challenges to existing laboratory preparedness plans and initially required rapid implementation of testing capability and capacity. In order to meet increased testing demands for the expected second wave of infections in the fall of 2020, the NYC Public Health Laboratory (PHL) planned for a significant expansion in staffing and testing infrastructure. This included the establishment of nine new COVID Express laboratory facilities modeled after the PHL Quickie at the Chelsea Sexual Health Clinic, a clinic-based system that provides rapid testing for chlamydia and gonorrhea in a matter of hours rather than days. Existing sexual health clinics across NYC were leveraged using the Chelsea model for COVID-19 testing. To allow for this rapid expansion, a planning committee developed testing capacity objectives of 500 clients per day per laboratory as well as determining clinic and laboratory staffing requirements to facilitate patient enrollment/check-in, specimen collection and transport, and laboratory testing. In addition, testing instrumentation was selected based on performance specifications, run capacity, and turnaround time. To overcome delays in the hiring process, an emergency contract was established with a temp agency to hire 75 testing staff. Nasopharyngeal swabs were found to be the preferred specimen source and as a result, self-collection was not an option and over 100 health professionals within NYC Department of Health and Mental Hygiene were activated as part of our incident command system activation protocol to staff the testing clinics. The purchasing of testing instrumentation, test kits and supplies was expedited through emergency contracts. Laboratory permits were acquired in close collaboration with state regulators with the assistance from both newly hired and existing staff. PHL's quality management team and agency leadership coordinated the training and staffing of clinical staff as per regulatory requirements. Within 4 months from inception, the nine laboratories began and continue to provide free testing for SARS-CoV-2 with mostly same day resulting (up to a maximum turnaround time of 24 hours). This highlights the success of rapidly expanding testing capacity expansion during a public health response, which could be used as a model for other jurisdictions to expand testing for other emerging infectious diseases.

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Poster 17T

Genomic Surveillance of Antimicrobial Resistant Pathogens in a Non-Regional ARLN Laboratory

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To help address the emergence of carbapenem-resistant healthcare-associated pathogens, the Michigan Department of Health and Human Services (MDHHS) implemented genomic surveillance of select pathogen-resistant combinations, including Enterobacterales, Pseudomonas aeruginosa, and Acinetobacter baumannii. Whole genome sequencing (WGS) is used with phenotypic and PCR methods to characterize resistance determinants and elucidate genomic markers of interest. Phylogenetic analysis of WGS data and genomic epidemiology are used to monitor for outbreaks, transmission dynamics, and emergence of novel resistance markers.

After identification of mCIM positive isolates or potential epidemiological clusters, WGS is performed on Illumina platforms alongside foodborne and other bacterial organisms that are part of routine surveillance in Michigan. Initial quality metrics have been established for antibiotic resistant organisms prior to performing phylogenetic analyses. Reports are generated to share with epidemiology counterparts for data sharing and situational awareness of surveillance.

Since surveillance began in 2019, 357 isolates have been sequenced representing 17 different genus/species. Phenotypic and PCR testing identified 6 resistance types in addition to pan-resistant or pan-non-susceptible. The utilization of WGS, has allowed for further discrimination and typing of resistance genes, while also the identification of novel resistance markers that are not captured by the current PCR assay. The most common pathogens found are NDM-resistant K. pneumonia (40% of sequenced isolates) and NDM-resistant E. coli (20%).

The application of AR surveillance has been leveraged to identify clusters of related bacterial isolates. These include an outbreak of NDM+ E. coli linked to a contaminated endoscope (6 patients) and persistent transmission of NDM+ K. pneumoniae within a hospital and long-term care facility (60 patients). WGS surveillance combined with epidemiology has already proven to be useful for detecting and investigating outbreaks.

Presenter: Stephen Dietrich, Michigan Department of Health and Human Services, Lansing, MI, dietrichs@michigan.gov

Poster 18T

Related *K. pneumoniae* Infections Exhibiting Distinct NDM Variants

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The Virginia Division of Consolidated Laboratory Services (DCLS) provides quality testing services to aid Virginia Department of Health (VDH) investigations of suspected infectious disease outbreaks. Next-generation sequencing and genome analyses are increasingly being applied to better understand transmission in congregate-setting, communicable disease outbreaks. In this presentation, DCLS highlights a recent suspected skilled nursing facility outbreak investigation of two residents with Carbapenem-resistant Klebsiella pneumoniae harboring New Delhi metallo-beta-lactamase (NDM) genes.

The two patients under investigation were residents of the same facility for an overlapping time period, and both were located in the same care wing. DCLS received the cultures from both patients in compliance with the Code of Virginia and the Board of Health Regulations for Disease Reporting and Control (Sections 32.1-36 and 32.1-37 of the Code of Virginia and 12 VAC 5-90-80 of the Board of Health Regulations for Disease Reporting and Control). Phenotypic and molecular characterization of the two K. pneumonia cultures established nearly identical antimicrobial susceptibility testing profiles - Sensititre GN7F, NDM genes detected (Streck ARM-D β-lactamase PCR kit), and carbapenemase production by the modified Carbapenem Inactivation Method (mCIM). DCLS performed whole genome sequencing on the isolates using the Illumina DNA Prep kit on the MiSeq (2 x 250 paired end) and the reads were analyzed with a series of pipelines available in the STaPHB-toolkit, Tredegar and Dryad. Antibiotic resistance determinations from Dryad were cross-compared to NCBI AMRFinderPlus, and also PHoeNIx.

Dryad comparison indicated that the two isolates (referred to as Case A and Case B) were closely related, with less than 5 distinguishing single nucleotide variants (SNVs); however, the Dryad AMR profile for Case A did not include any NDM-prediction, whereas NCBI AMRFinderPlus and PHoeNIx predicted the presence of NDM-7 in Case A. Dryad predicted the presence of NDM-19 in Case B. NDM-19 has not previously been seen in the U.S., and differs from NDM-7 by only one SNV, A233V. No travel history was reported in either of the patients. Case A with the discrepant NDM results was re-sequenced, as it was thought that perhaps the result was an artifact of sequence assembly differences between Dryad, NCBI Pathogen Detection and PHoeNIx. Combining the results from the first and second sequencing attempts on Case A, the laboratory corroborated the findings of the presence of NDM-7 using Dryad. Since NDM-7 and NDM-19 are closely related, it was determined that one of the SNV differences identified between Case A and Case B was in the NDM gene, distinguishing Case A's NDM-7 from Case B's NDM-19. Further characterization of the isolates using long-read sequencing is projected to better elucidate the nature of these carbapenemases in the two related isolates. The genomic analysis of these cultures presents a unique case-study of likely intra-facility disease transmission of K. pneumoniae with distinct NDM variants.

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Poster 19T

Enabling Confident Healthcare Decisions for SARS-CoV-2 Patients by Bridging Roche Antigen, Viral Load (PCR), and Antibody Tests (BRAVA): An Interim Analysis

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Introduction: The goal of this study was to describe viral and immune kinetics throughout SARS-CoV-2 infection as determined by the Antigen (Ag), Antibody (Ab) and PCR biomarkers, detected by the Elecsys® SARS-CoV-2 Antigen, the cobas® SARS-CoV-2, the Liat® SARS-CoV-2 & influenza A/B, the Rapid Anti-SARS-CoV-2 antibody (Ab), and the Elecsys Anti-SARS-COV-2 tests, as predictors of infectivity (as determined by positive viral culture).
Methods: Index patients (IPs) with SARS-CoV-2 (confirmed by PCR) and their Household contacts (HHCs), were enrolled at Frankfurt University Hospital, Germany. Patient samples were collected and tested across an average of ten longitudinal visits. Viral and immune patterns were evaluated throughout SARS-CoV-2 infection as determined by Ag, Ab, PCR, and culture. Appropriate statistical analysis were employed using SAS v.9.4.
Analysis: As of August 2022, data from14 households (30 persons) were analyzed, including 17 IPs and 13 HHCs. The viral loads in nasal and saliva samples obtained from 17 symptomatic IPs were analyzed at their first visit. Higher viral loads (inversely related to Cts) were detected at visit one in the nasal swabs (NS) as compared with the saliva (S) (Visit 1: Nasal Ct=24.38, Saliva Ct=29.67). NS stayed positive for up to 16 days. Earlier Ct values (cobas nasal swab orf-1), on average, of < 34.6 were associated with positive Antigen test and on average, < 30.56 were associated with positive viral culture. Antigen assay results were reactive for 100% (27/27) of positive viral cultures. For vaccinated persons, Immunoglobulin G (IgG) antibodies were present from day 1 until study end. Immunoglobulin M (IgM) antibodies were not detected. For the unvaccinated (HH1), IgG antibodies were detected at day 7 until study end. IgM antibodies were detected at day 5 until study end. Five (38.5%) HHC's became positive by PCR at ~7 days post-enrollment and remained PCR positive for an average of 17 days, representing five characterized household transmission events to date within the interim timeframe of the study. Ct values < 31 and/or a positive Ag test were parameters most strongly related to infectivity at study interim.
Conclusion: To conclude, preliminary data suggest that kinetic analysis across the patient journey with multiple biomarkers and sample types, provides a fuller context of infectivity; this may be approximated by Ct values < 31 and/or by a positive Roche Antigen test. Kinetic modeling may also allow for greater understanding of the temporal relationship between the waning viral load (acute diagnostic markers) and onset of immunity (antibody production). This is an interim analysis. Ongoing analysis includes temporospatial biomarker positivity for IP-to-HHC transmission events, stratified by vaccination status and time to seroconversion.

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Poster 20T

Unveiling the Causes of False Positive Non-Variola Orthopoxvirus PCR Results

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Background: On June 18, 2022, Indiana Department of Health Laboratory (IDOHL) detected the first Monkeypox (MPX) case using CDC's Non-Variola Orthopoxvirus (NVO) PCR assay with a CT value of 36.3 for VAC1 target. The return of a negative MPX specific PCR result from CDC cast some doubt on the performance of the NVO assay. With the increased testing volume, more discordant results between the IDOHL and CDC Lab were observed, with many NVO positive results not supported by epidemiological data. The false positive NVO results impacted patient treatment, contact tracing, and prevention measures. Identifying the causes of the false positive NVO results and a solution to the issue became urgent.

Methods: To identify the causes of these false positive NVO results, the IDOHL examined every step from specimen receiving to PCR threshold setting and result interpretation. This included examining the plate locations of specimens during the extraction steps and PCR assay setup. Assured by staff members' high-quality performance at IDOHL and results from an environmental swab test, we focused on how the data was analyzed per the LRN SOP, the amplification raw data, the component view of each specimen, the normalization of background florescence, and the correlations between patients' risk/clinical information and the NOV VAC1 Ct values. Two different software packages, the ABI interpretive software (v.1.4.1) and vendor provided software Designer Analysis Software 2.5 (DA2), were evaluated on their consistency of result interpretation.

Results: During the root cause investigation, a Ct value of 34 and above was indicative of a higher false positive rate; ten specimens that were designated as NVO Detected by the software v.1.4.1 showed no amplification when examined through the component view of that same software. This discrepancy can be overcome by DA2, a software having better background fluorescence normalization algorithm and automatic threshold setting. Data from 45 specimens that were tested at IDOHL and had CDC testing results was reanalyzed using the DA2 software; the DA2 results correlated better with risk/clinical information. Since the IDOHL implemented the DA2 software for data analysis and result interpretation in late August, no false positive result has been observed.

Conclusions: The IDOHL worked closely with its epidemiology partners, other public health labs, and the instrument vendor on identifying the causes of false positive NVO results. The effort unveiled the causes and resulted in a reliable solution. The IDOHL also proactively communicated and shared with CDC the findings and solution, which may have contributed to best practices that were related to repeat testing and reviewing with component view when using ABI interpretive software v.1.4.1.

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Poster 21T

Wastewater Surveillance of 180+ Bacteria, 50+ Fungi, 40+ Viruses and ~1200 Antimicrobial Resistance Markers in Northern Nevada

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Since the beginning of the SARS-CoV-2 pandemic, wastewater surveillance has gained an increased importance as a public health tool and has greatly contributed to public health decision making and policy. Previous articles have shown that other pathogens, antimicrobial resistance (AMR), and illicit/prescription drug use trends can also be elucidated by wastewater surveillance. With Illumina's Respiratory Pathogen Infectious Disease/Antimicrobial Resistance Enrichment Panel Kit, the Nevada State Public Health Laboratory (NSPHL) adapted this method to test twenty-seven wastewater samples (mid-march to early April of 2022) from two wastewater reclamation facilities and four different University of Nevada Reno buildings. This experiment identified forty pathogenic microbes (bacteria, viruses, and fungi) known to cause human disease and thirty antimicrobial resistance markers (and their corresponding alleles) present in communal wastewater. Identified pathogens are quantified (copies of pathogen per liter) to analyze prevalence trends within the area. This method can potentially detect over 180 bacteria, 40+ viruses, 50+ fungi and approximately 1200 antimicrobial resistance markers.

Through this method, our laboratory was able to identify bacteria with known resistance such as Enterobacter cloacae complex (20/27 samples), Klebsiella pneumoniae (20/27), Escherichia coli (20/27), Pseudomonas aeruginosa (2/27), and Acinetobacter baumannii (2/27). Also, we were able to pinpoint many AMR genes that yield resistance such as GES (21/27), OXA (23/27), KPC (18/27), CTX-M (13/27), and VIM (3/27). From the period of mid-March to early April, NSPHL received and sequenced 22 AMR resistant specimens of P. aeruginosa, A. baumannii, and K. pneumoniae. Through AMR finder plus we were able to detect OXA (22/22), KPC (3/22), and CTX-M (2/22) but were not able to find GES and VIM. Further research is needed to establish if there is a high concordance of wastewater surveillance with communal presence of certain bacteria and true AMR burden. But potentially, this early knowledge from wastewater surveillance can identify likely outbreaks in buildings and communities as well as advise health care professionals how best to treat a particular infection based on the resistance present in the area. Future work for this project is to set up further opportunities for comparison as well as expand this wastewater surveillance system to encompass pharmaceuticals and illegal substances.

Presenter: Lauryn Massic, Nevada State Public Health Laboratory, Reno, NV, lmassic@unr.edu

Poster 22T

Using Unique Molecular Identifiers to Increase the Accuracy of Oxford Nanopore Technologies' MinION Sequencing for Foodborne Enteric Surveillance

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Background: Public health laboratories (PHLs) routinely sequence enteric bacterial isolates for surveillance to detect foodborne outbreaks. PHLs use Illumina short-read sequencing that produce fragmented assemblies and can be expensive. The Oxford Nanopore Technologies' (ONT) MinION is a small, affordable long-read sequencer that lacks the accuracy of short-read sequencers. Ligating unique molecular identifiers (UMIs) to library template molecules before amplification may improve MinION accuracy. We tested Karst et al.'s (2021) UMIs method for suitability for enteric surveillance by comparing core genome multi-locus sequence typing (cgMLST) calls from 50 isolates sequenced with Illumina, ONT native, and ONT-UMI methods.
Methods: 25 Shiga toxin-producing Escherichia coli and 25 Salmonella isolates were used to compare ONT native and ONT-UMI to Illumina sequencing. For ONT methods, isolates were cultured using standard methods and extracted using Promega's Wizard High Molecular Weight kit. The ONT native workflow followed ONT protocols (Ligation Sequencing Kit (SQK-LSK109), Native Barcoding Expansion kit (EXP-NBD104/114) and R9.4.1 flowcell). The ONT-UMI workflow followed the Karst et al. (2021) shotgun protocol for sequencing and assembly. The ONT native library reads were base called, demultiplexed, and trimmed using Guppy, quality checked (QC) with NanoPlot, assembled with Flye and polished using Racon and Medaka. Illumina data was generated using DNA Prep libraries (PulseNet SOP PNL35) and sequenced on a MiSeq using 500 cycle chemistry (PulseNet SOP PNL38). FastQ files were analyzed in BioNumerics v 7.6.3 including assembly, identification using average nucleotide identity, and organism specific databases for QC and genotyping. Assemblies were compared by calling cgMLST alleles from each data set in BioNumerics, using Illumina data as the reference.
Results: We were able to successfully sequence and assemble all 50 isolates using the native ONT method. Using BioNumerics, we compared native ONT data to Illumina and identified cgMLST alleles for STEC & Salmonella isolates. Using a modified ONT protocol, we are working on ONT-UMI proof of concept development.
Conclusions: ONT's MinION may provide PHLs a tool for foodborne outbreak surveillance, providing a cheaper option compared with Illumina sequencing while upholding data quality. A smaller footprint and lower cost could reduce resource demand by PHLs participating in foodborne enteric outbreak surveillance.

Reference: Karst et al. Nat Methods 18, 165–169 (2021).

Presenter: Ryan Paradis, Centers for Disease Control and Prevention, Atlanta, GA, qxy2@cdc.gov

Poster 23T

Automated Genomic Surveillance in Wyoming with Microsoft Azure and Nextflow

R.A. Petit, T. Fearing, C. Rowley, A. Norberg, J. Mildenberger, R. Christensen and J.M. Reed, Wyoming Public Health Laboratory, Cheyenne, WY

Over the course of the SARS-CoV-2 pandemic, genomic surveillance became a critical tool for tracking infections across populations. At the Wyoming Public Health Laboratory (WPHL), genomic sequencing capacity was well established, but the bioinformatic capacity was not robust enough to accommodate high throughput data generation and analysis. In order to address this issue, the WPHL grew its bioinformatics capacity by leveraging the cloud to respond to the pandemic. WPHL genomic surveillance has now expanded into other microbial pathogens as well as wastewater. To improve future preparedness, the WPHL continues to use the experience gained over the last few years to build robust bioinformatics infrastructure.

The WPHL uses the Microsoft Azure platform and Nextflow pipelines for analysis. Based on previous experience, the WPHL built the Azure infrastructure with the understanding that our IT department may require platform changes in the future due to contractual or security needs. To provide flexibility for cross platform use, the WPHL used Terraform for “infrastructure as code”, which allows us to easily rebuild on other platforms if necessary. This potential for change also led us to adopt Nextflow as our primary workflow manager. Nextflow has built in support for many platforms and only requires a simple parameter change to switch platforms. Using these platforms has positioned the WPHL to rapidly adapt to future changes.

During setup and implementation, WPHL bioinformatics specialists recognized a need to reduce staff analytical input while continuing to make efficient use of genomic surveillance data. Previously, our pipelines were manually executed and interpreted. In the height of the pandemic this worked and kept things moving, but as we expand to more organisms it has become more difficult and time consuming. To address this growing burden, we automated our bioinformatic pipelines with Azure services for event triggered analyses. These triggers automatically take sequenced samples through a set of bioinformatic pipelines and can be monitored by members of WPHL. The results of these analyses are then seamlessly integrated into Microreact, a tool for genomic epidemiology that is utilized by the lab and epidemiologists at WPHL. Through this automation, we have reduced the data analytics burden for ongoing genomic surveillance efforts, allowing for laboratory scientists to increase sequencing throughput.

Presenter: Robert Petit, Wyoming Public Health Laboratory, Cheyenne, WY, robert.petit@wyo.gov

Poster 24T

Scaling Pathogen Genomics and Automated Reporting in California with Terra.bio and Looker

E. Smith[1], K. Libuit[1], F. Ambrosio[1], C. Kapsak[1], M. Scribner[1], S. Wright[1], J. Bell[2], E. Baylis[2], S. Messenger[2], J. Hacker[2], C. Morales[2], D. Wadford[2], J. Sevensky[1]; [1]Theiagen Genomics, Highlands Ranch, CO, [2]California Department of Public Health, Richmond, CA

The SARS-CoV-2 pandemic placed unprecedented demands on public health laboratories to scale genomic surveillance efforts to inform epidemiological investigations, monitor lineage trends, and identify new and emerging variants before the onset of community transmission. To address this challenge in California, public health officials established COVIDNet, a collaboration between the California Department of Public Health (CDPH), local public health labs, academia, and industry which has resulted in hundreds of thousands of SARS-CoV-2 genomes from more than thirty labs throughout California. The process of data upload, analysis, and reporting of SARS-CoV-2 genomic data is nearly fully automated, as is the consolidation of results across many different labs that use different sequencing methods. This was enabled through the integration of three modular platforms hosted on the Google Cloud (GCP): Terra.bio, Big Query, and Looker. Importantly, security of this GCP infrastructure is maintained through the use of Google Cloud Identities that are mapped to state or local government credentials. Raw sequence data are uploaded to Terra.bio, a browser-based GUI application for launching scalable, reproducible, portable, and standardized workflows. The resulting data are automatically exported from Terra.bio workspaces into Big Query, a data management tool that enables the consolidation of many different data tables and data types into a single, simplified data source. This data source serves as the model for Looker, a HIPAA-compliant visualization and reporting platform that generates automated queries that alert public health officials 24/7 when new data of public health importance are generated. For example, an email alert based on a selected lineage or mutation of significance will automatically be sent to authorized state and local public health officials, including laboratory directors, microbiologists, bioinformaticians, and genomic epidemiologists when identified in the data set. The alerts allow automated and prompt communication of significant traits to inform public health officials for decisions and action. Looker also has the ability to limit user access to specific rows or columns within the entire dataset, circumventing the need to maintain separate data sources based on sensitive metadata. Looker functions are now being applied to other pathogens in California where alerts are triggered for select deletions or detection of certain antimicrobial resistance genes. The flexibility, security, and scalability of this model make it an ideal solution to meet the bioinformatics needs of any public health lab performing pathogen genomics.

Presenter: Emily Smith, Theiagen Genomics, Highlands Ranch, CO, emily.smith@theiagen.com

Poster 25T

Development of a Rapid. Uniform, State-wide Surveillance Program for COVID Variants in New York State Wastewater

K. St. George[1], D. Lamson[1], J. Plitnick[1], V. Schnurr[1], L. Rickerman[1], S. Griesemer[1], Y. Ahmed[2], C. Durham[2], D. Hill[2], D. Lang[1], L. David[2]; [1]Wadsworth Center, New York State Department of Health, Albany, NY, [2]Syracuse University, Syracuse, NY

Sequencing of SARS-CoV-2 positive clinical samples from throughout New York State was initiated at the Wadsworth Center, New York State Department of Health (NYSDOH), in March 2020 and expanded in December that year in response to the emergence of variants. In 2021, a consortium of four academic sequencing laboratories was established to perform most of the NYS surveillance sequencing for SARS-CoV-2 variants. In 2022, the work of the consortium has been further expanded to include the sequencing of SARS-CoV-2 positive wastewater samples from throughout the state. The expansion to wastewater monitoring has been prompted by the successful detection of the virus in wastewater samples, and the decreased availability of community samples due to increased point of care and home testing.

To assure the most rapid, sensitive, and uniform results in this new program, ThermoFisher Ion Torrent Genexus instruments were installed at all consortium sites. Data from clinical testing demonstrated sequences to be obtainable with AmpliSeq chemistry from samples at lower viral loads and to a greater depth of coverage, compared to other methods (ARTIC/Illumina, ClearLabs Dx, and AmpliSeq Ion Torrent S5), and results were available within 25 hours of initiation of testing.

An evaluation panel comprised of 16 samples was prepared at the Wadsworth Center for distribution to the five sites (4 consortium plus Wadsworth) and contained samples of total nucleic acid from multiple SARS-COV-2 isolates, representing both currently circulating variants and ancestral lineages. Each sample contained varying percentages (from 5-75%) of up to six different lineages. To confirm purity of the isolates used in the panel, they were verified by Whole Genome Sequencing (WGS) using the Genexus instrument followed by mixed-lineage analysis using Freyja. Absolute quantification of the N1 target was performed with the Qiagen QIAcuity Digital PCR system for each isolate (viral copy number per reaction ranged from 1,550 to 16,800) and by real-time RT-PCR using the New York State SARS-CoV-2 assay of the N1 target. The Cycle Threshold (CT) values determined the proper dilution needed for optimal sequencing: final CTs ranged from 23.19 to 25.85. After the panel was verified internally, it was distributed to each of the laboratories to perform WGS and upload the data to the centralized cloud. This process was carried out to assess the performance and consistency of the sequencing, data analysis and data transfer pipelines, prior to going live the following month.

This network will be performing sequencing of positive wastewater samples collected from more than 200 sites throughout New York State. The trimmed files are to be pushed to a centralized cloud computing hub where bioinformatic variant analysis is performed. Analyzed results are discussed and shared with NYSDOH prior to upload to the New York State SARS-CoV-2 wastewater dashboard and the Centers for Disease Control and Prevention National Wastewater Surveillance System via DCIPHER.

Presenter: Kirsten St. George, Wadsworth Center, New York State Department of Health, Albany, NY, kirsten.st.george@health.ny.gov

Poster 26T

Expanding Laboratory Surveillance of Enterotoxigenic *Escherichia coli* in Minnesota

E.R. Verbrugge, E. Cebelinski and J. Haan, Minnesota Department of Health, St. Paul, MN

Enterotoxigenic *Escherichia coli* (ETEC) is a common cause of traveler's diarrhea and can cause foodborne disease outbreaks. ETEC virulence is determined by the presence of genes that encode heat-labile (LT) or heat-stable (ST) enterotoxins. Increasingly, ETEC is being diagnosed in clinical laboratories through culture-independent diagnostic tests (CIDT) like the FilmArray® GI Panel (Biofire Diagnostics). In Minnesota, ETEC is a reportable disease and culture confirmation is imperative for public health surveillance and identification of ETEC outbreaks. To improve ETEC laboratory surveillance, The Minnesota Department of Health Public Health Laboratory (MDH-PHL) is evaluating new culture-dependent real-time PCR assays. MDH-PHL tested 58 CIDT-positive ETEC samples received from July to October 2022. All samples were cultured on Sorbitol MacConkey agar and culture sweeps were used to create crude extracts for PCR. Extracts were tested for the presence of LT and ST using a CLIA-validated SYBR Green multiplex PCR. Published TaqMan PCR methods that can detect LT and ST were evaluated. The TaqMan methods can further distinguish ST-encoding strains by detecting ST gene variants, STh and STp. STh has been described in human derived isolates and STp is mainly associated with porcine or animal isolates. Since January 2022, concordance between the FilmArray® GI Panel and the current SYBR Green PCR method has been 49% at MDH-PHL. 29% of samples (17 of 58) were positive for ST on both assays and were able to be characterized as either STh (65%, 11 of 17) or STp (35%, 6 of 17) on the new TaqMan PCR method. 5 (9%) additional LT positive samples and 2 (3.5%) additional ST positives were found by the TaqMan PCR method, amounting to a 10% increase in overall confirmation rate. MDH-PHL plans to continue testing all ETEC positive specimens identified by CIDT platform and monitoring the confirmation rate of ETEC. This study has demonstrated that laboratory surveillance in Minnesota can be improved through the implementation of more sensitive, accurate, and descriptive PCR assays. The results of this study also add important details about potential source attribution, which can be combined with epidemiological data to further improve our understanding of the dynamic population of ETEC in Minnesota. Denotation of ST attribution could be used to expand ETEC outbreak surveillance efforts in Minnesota and aid in epidemiological investigations.

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Poster 27T

A Simple and Robust Workflow for Detecting Monkeypox Virus from Skin Swabs and Wastewater

S. Teter and B. Hook, Promega Corporation, Madison, WI

Monkeypox emerged as a public health concern in early 2022, and since, public health agencies have been working diligently to develop strategies to diagnose infections and understand the prevalence of the infection within the population at large. Detecting the virus from swabs of suspected lesions is one of the primary methods of confirming a monkeypox infection, while community spread can be tracked by detecting the virus shed from infected individuals in wastewater samples. In both cases, the general workflow for detecting the virus involves concentration and purification of nucleic acid from the sample, then detection of the monkeypox viral DNA by polymerase chain reaction (PCR) based techniques. This work demonstrates an automated means of purifying monkeypox DNA from both swabs and large volumes of wastewater using the Maxwell® RSC Instrument. An automated method was selected for sample processing to minimize both hands-on time and potential for human errors during sample processing. The monkeypox viral DNA present in the purified sample was then detected in an optimized quantitative PCR (qPCR) assay using the GoTaq® Enviro qPCR System with multiplexed detection of monkeypox DNA and an RNase P assay control target based on the Centers for Disease Control (CDC) recommended primers and probes. The efficacy of this workflow was confirmed by spiking the starting sample matrices, lesion swabs or wastewater, with synthetic monkeypox viral DNA and/or inactivated monkeypox viral particles followed by detection of the monkeypox target using the multiplex monkeypox and RNase P qPCR assay. This workflow offered robust performance for sensitive detection of the viral DNA even with difficult sample types, such as nucleic acid originating from wastewater samples. Overall, this work offers a ready-to-use solution for monkeypox viral detection from sample preparation to viral detection by amplification.

Presenter: Melanie Preston, Promega Corporation, Madison, WI, melanie.preston@promega.com

Poster 28T

Long-read Sequencing Enables In-depth Characterization of Plasmids Encoding KPC-2 in *Citrobacter freundii* Outbreak

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Antimicrobial resistance among bacteria such as Citrobacter freundii is a public health concern. Sequencing bacterial isolates to identify genes associated with antimicrobial resistance enhances the capacity to understand and minimize outbreaks. Twelve isolates for C. freundii obtained from patients in Utah exhibited reduced susceptibility to antimicrobials. Nanopore sequencing revealed that eleven isolates harbored a plasmid encoding KPC-2 and one with KPC-3, which are class A carbapenem-hydrolyzing enzymes associated with β-lactam resistance. Phylogenetic analysis was used to compare the core genome of chromosomal sequences (2,822 genes), resulting in two distinct clusters of isolates containing eight and three isolates, respectively. The similarity of the core genomes was suggestive of two concurrent outbreaks. During the process of characterizing the plasmids encoding KPC-2 of the two clusters, a surprising amount of variation was observed in the plasmids of the eight-isolate cluster, with plasmid sizes ranging from 57kb to 208kb (median = 70kb) with large rearrangements observed in three of the eight plasmids.

Presenter: Erin Young, Utah Public Health Laboratory, Taylorsville, UT, eriny@utah.gov

Poster 29T

Genomic Surveillance Identifies SARS-CoV-2 Transmission Patterns in Local University Populations, Wisconsin, USA, 2020–2022

A. Ramaiah, M. Khubbar, A. Bauer, K. Akinyemi, J. Weiner and S. Bhattacharyya, City of Milwaukee Health Department, Milwaukee, WI

Novel variants of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) continue to evolve as the current coronavirus disease 2019 (COVID-19) pandemic extends into its third year. Understanding SARS-CoV-2 circulation in university populations is vital for effective interventions in a higher education setting that will inform public health policy during pandemics. In this study, we performed whole-genome sequencing of 537 of 1,717 SARS-CoV-2 positive nasopharyngeal/nasal swab samples collected between September 2020-April 2022 from two university populations in Wisconsin, United States. Extensive bioinformatic analysis discovered that these sequences were distributed into 57 lineages/sub-lineages belonging to 15 clades of which the majority were from 21K (Omicron, 36.13%) and 21J (Delta, 30.91%). Nearly 40% (213) of the sequences were Omicron of which BA.1 and its eight descendent lineages account for 91%, while the remaining belong to BA.2 and its six descendent lineages. The independent analysis of these two universities' sequences revealed significant differences in the circulating SARS-CoV-2 variants. Phylogenetic analysis of university sequences with a global sub-dataset demonstrates that the sequences of the same lineages from the university populations were more closely related. Genome-based analysis of closely-related strains along with phylogenetic clusters and mutational differences identified that potential virus transmission occurred within as well as between universities, and between the university and local community. Although this study improves our understanding of distinct transmission patterns of circulating variants in local universities, expanding real-time sequencing and bioinformatics capacity will aid local jurisdictions not only in identifying emerging SARS-CoV-2 variants like BA.4 and BA.5, but improve data-driven public health mitigation and policy efforts.

Presenter: Arunachalam Ramaiah, City of Milwaukee Health Department, Milwaukee, WI, arama@milwaukee.gov

Poster 30T

Broad, Genomic AMR Surveillance with an Efficient, Probe-capture NGS Panel

J. Bierstedt, N. Shah, M. Roos, S. Kuersten, K. Broadbent and R. Schlaberg, Illumina, San Diego, CA

Antimicrobial resistance (AMR) represents a critical public health threat. Screening of environmental sources (e.g., surfaces in health care settings, wastewater) allows for spatial and temporal monitoring. Targeted next-generation sequencing (NGS) can be used to detect and genetically characterize thousands of AMR markers in a single test combined with identification of relevant bacteria and fungi. The urinary pathogen ID/AMR panel (UPIP) detects 3700+ AMR markers covering the vast majority of AMR threats on the CDC and WHO priority lists combined with the genitourinary infectome (170+ pathogens). We compared the UPIP workflow (UPIP capture probes, Illumina DNA library prep with enrichment, Explify UPIP BaseSpace app) and shotgun sequencing for AMR detection in wastewater. AMR sequences represented 0.02% of sequencing reads in shotgun metagenomic libraries compared to 49% of sequencing reads with UPIP. UPIP detected 3-4X more AMR markers (incl. carbapenemase and ESBL genes) at 280X greater coverage depth and 5X more priority pathogens with 10X fewer reads (UPIP: 5M reads/sample, shotgun metagenomics: 50M reads/sample). Targeted NGS with UPIP unlocks broad, efficient, high-resolution resistome sequencing of complex environmental samples such as wastewater.

Presenter: Robert Schlaberg, Illumina, San Diego, CA, schlaberg@gmail.com

Poster 31T

Pennsylvania Department of Health Bureau of Laboratories Mobilization of Resources to Respond to the COVID-19 Pandemics

L. Dettinger, T. Bannon, C. Branton, J. Brooks, K. Premkumar, K. Xia, Pennsylvania Department of Health, Bureau of Laboratories, Exton, PA

The COVID-19 pandemic constitutes the largest global public health crisis in the past century. As the state public health laboratory in Pennsylvania (PA), the PA Department of Health Bureau of Laboratories (BOL) mobilized a variety of resources to respond to the pandemic and ensure the quality and efficiency of laboratory services.

During the early months of the pandemic, existing BOL staff worked overtime to cover COVID-19 testing seven days a week. Staff from all areas of the BOL were cross-trained to perform sample reception, demographic entry, and testing. All laboratory supervisors were trained to review and approve test results. No other testing programs were shut down during the response. Contract employees were hired to increase testing capacity by extending operating hours.

A biosafety risk assessment was conducted with the aid of Association of Public Health Laboratories tools, Centers for Disease Control and Prevention (CDC) COVID-19 guidance, and laboratory staff input. Safety practices were incorporated into test standard operating procedures and safety training was provided. Modifications to the risk assessment, procedures, and training were made as more information on the disease became available.

Real-time polymerase chain reaction (PCR) testing was performed using CDC protocols and Applied Biosystems 7500 Fast Dx thermocyclers. Multiple nucleic acid extraction systems were used, including the Biomerieux NucliSENS easyMAG, Roche MagNA Pure 96, Qiagen EZ1 XL, and Thermo Fisher KingFisher Flex. Validation of the test procedures on multiple systems increased overall testing capacity and ensured that testing was not entirely dependent upon the availability of supplies or service from one manufacturer. A Roche cobas® 8800 system was added to further diversify PCR testing. Serology, whole genome sequencing, and variant identification procedures were also adopted.

A lab web portal was implemented to allow for electronic test order submission and resulting. Manual results transcription was eliminated by expanding instrument interfacing with the laboratory information management system (LIMS) by direct interface or electronic data transfer. HL7 messaging was implemented to report influenza data from the LIMS.

Laboratory facilities were reorganized and renovated to provide additional workspace. The specimen receiving laboratory was expanded into a second room. Instruments and equipment were relocated to centralize extraction, testing and specimen storage as much as possible. Acquisition of the cobas® 8800 required conversion of the employee breakroom into a laboratory large enough to hold the instrument. Training lab space was converted to working lab space for the waste water surveillance testing.

Mobilization of resources requires significant collaboration and teamwork among internal and external partners. The successes achieved and lessons learned are invaluable in preparedness for and response to future public health crisis or emergencies.

Presenter: Dongxiang Xia, Pennsylvania Department of Health, Bureau of Laboratories, Exton, PA, dxia@pa.gov

Poster 32T

Broad Viral Genomic Surveillance using a Single Hybrid-capture Enrichment Panel

N. Soffer, J. Koble and Y. Lin, Illumina, San Diego, CA

A majority of viral outbreaks over the last few decades have been caused by re-emerging known viruses. There are approximately 66 viruses that have been identified as high concern for outbreak potential. The Viral Surveillance Panel (VSP) was designed to enrich for whole genomes of 66 viruses of high public health impact. The workflow includes the VSP hybridization-capture panel, Illumina RNA Prep with Enrichment and the DRAGEN™ Microbial Enrichment BaseSpace™ app. VSP allows for detection and complete genome assembly of viruses in the panel and related strains. Multiple users from around the world were provided early use of the VSP Panel. Here we report the broad sample types and genome coverage achieved from samples ranging from residual medical waste skin lesion samples, to wastewater. VSP users were able to generate whole genomes (coverage >99%) for Monkeypox, Norovirus, Hepatitis C, Western and Eastern Equine Encephalitis and many more. This collective data demonstrates how VSP can be used for both environmental and bio-banked remnants genomic surveillance of viruses.

Presenter: Nitzan Soffer, Illumina, Inc., San Diego, CA, nsoffer@Illumina.com

Poster 33T

Integrating Next Generation Sequencing Results into the Laboratory Information System to Improve Reporting for Surveillance and Outbreak Investigation

K. Musser, T. Halse, M-C. Rowlinson, K. Mitchell, E. Nazarian, P. Lapierre, W. Haas and C. Prussing, Wadsworth Center, New York State Department of Health, Albany, NY

Next generation sequencing (NGS) technology has been widely implemented in public health laboratories and has improved clinical, surveillance and outbreak investigation testing and reporting. NGS testing presents new challenges for the laboratory, including complicated analyses, data storage requirements, reporting and implementation in line with regulatory compliance. Wadsworth Center (WC) has implemented NGS testing for a variety of applications over the last 10 years. However, as with many laboratories performing NGS, WC has been constrained by the large amount of data generated by this test methodology, integration of this data into the LIMS, and reporting in a digestible format. During the COVID-19 pandemic, a new module was created to allow multiple sample accession numbers to be grouped together when pooling of samples was performed. Over the past year, WC has amended this module to group accession numbers together that are samples associated with the same outbreak, in a new investigations module.

This module includes the ability to create investigation accession numbers that allows any number of sample accession numbers to be added. Each original sample accession contains the bioinformatic pipeline outputs, quality control data associated with NGS metrics, pipeline details including version, and NCBI sequence identifiers. When an investigation accession number is created a Department of Health epidemiologist is added as the requestor/submitter, and a description and summary of the investigation is included. A list of included accessions with preselected fields for demographic information consistent with prior NGS reporting for each type of investigation was developed. Attachments can also be added to the report such as a SNP matrix, phylogenetic tree and any additional interpretations. The report can continue to be manipulated when new accessions are added over time. All reporting is electronic from the LIMS and can be securely accessed through web browser and downloaded by the requesting epidemiologist.

Several of our NGS tests are utilized for clinical reporting in addition to surveillance and investigation reporting for relatedness. These include carbapenemase-producing organisms received as part of the AR Lab Network, Mycobacterium tuberculosis complex, and other bacterial organisms including healthcare associated, vaccine preventable disease associated, Legionella, and foodborne associated. The additional functionality with the “investigation” module has enhanced our reporting for these areas. The long-term impact of having NGS reporting consistently maintained, audited and available in our LIMS with direct connection to patient demographics represents a major improvement, particularly as WC plans to continue to expand its NGS offerings for both clinical and surveillance applications. These updates are critically important to ensure NGS data integration into our reports in order to provide the most actionable data to our epidemiologists and clinicians.

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Note: M denotes the poster was posted/presented on Monday, T that it was posted/presented on Tuesday.