Poster 1
Implementing the New LN34 Assay to Keep Up with Rabies Cases in Allegheny County
H. Jones, Allegheny County Health Department

Rabies, a deadly zoonotic disease, poses a serious risk to public health because of its high death rate following the appearance of symptoms. Once the virus enters the central nervous system, it is fatal in nearly 100% of cases. In the United States, more than 90% of reported rabies cases come from wildlife with bats being the leading cause of human exposure and death. However, this can be entirely preventable though post-exposure prophylaxis (PEP) if administered before symptoms appear. By testing animal specimens for rabies, laboratories can make recommendations to exposed individuals for follow-up care as well as working to prevent the spread of the virus.

There are currently two CDC gold-standard ways to test animals for rabies: direct fluorescent antibody (DFA) testing and the newer LN34 real-time RT-PCR assay. The Allegheny County Health Department's Public Health Laboratory has employed DFA testing for years, but we are now turning our gaze towards implementing the LN34 assay to improve our rabies detection.

DFA testing works by fixing harvested brain tissues onto microscope slides and staining them with fluorescently labeled anti-rabies antibodies. These antibodies will bind to any rabies antigens that are present and flouresce under a UV fluorescent microscope. If there is no rabies present in the sample, the antibodies just wash away. This method has been used in laboratories across the country because of its high specificity and sensitivity. However, it requires special equipment, facilities, and expertly trained personnel to execute. This method also requires that the specimens are of a certain quality and condition: decomposed, dehydrated, or formalin-fixed samples are incompatible and can't be tested. Sample slides must also be fixed overnight, and turnaround time is currently 24 to 72 hours.

The LN34 assay fixes some of these downsides. This assay works by using the same brain tissues as DFA and homogenizing them so that they can be extracted and run via a single-tube reaction. The rabies virus genetic material is amplified and then detected by a fluorescent probe. The probe targets a highly conserved region of the genome that includes the nucleoprotein gene and the leader region. This allows it to detect all viruses that can induce rabies. This assay also has high specificity and sensitivity, but it has a more rapid turnaround time and higher versatility. The specimens used can be decomposed, dehydrated, or fixed with formalin and still produce accurate results. The LN34 assay can also be run with instruments commonly found among laboratories and doesn't require any special training to complete.

The ACHD PHL is planning on implementing this new assay side-by-side with our DFA testing. We are currently adapting our rabies necropsy suite to accommodate the machinery necessary for extraction and lysing. All equipment and primers/probes have been received. Validation with primers/probes is nearly complete with verification to start soon. We plan to run the same specimen samples through both DFA and the LN34 assay as a parallel study to ensure that we get accurate results.

Presenter: Hannah Jones, Allegheny County Health Department, hannah.jones@alleghenycounty.us

Poster 2
Development of an Avian Influenza H5N1, H7N9, H7N7, H5N6 and H9N2 Analytical Reference Material Set for Diagnostic Surveillance
H. Asbury, American Type Culture Collection (ATCC), J. Bose, American Type Culture Collection (ATCC), P. Gujjari, American Type Culture Collection (ATCC), L. Castro, American Type Culture Collection (ATCC), T. Correia, American Type Culture Collection (ATCC), L. Champ, American Type Culture Collection (ATCC), B. Chase, American Type Culture Collection (ATCC), S. Suh, American Type Culture Collection (ATCC), K. Young, American Type Culture Collection (ATCC), V. Knight-Connoni, American Type Culture Collection (ATCC), L. Papazisi, American Type Culture Collection (ATCC)

Highly pathogenic avian influenza (HPAI) viruses, particularly H5N1 clade 2.3.4.4b, have significantly impacted the poultry and dairy industries, becoming a major public health issue. In the US, the H5N1 virus has affected over 130 million birds since 2022. The culling of infected poultry flocks and large-scale bird deaths have resulted in significant economic losses for farmers due to reduced income from egg production and meat sales. The discovery of H5N1 traces in milk and incidences of mild infections among dairy workers since the summer of 2024 has affected the entire US dairy industry, raising significant public concern. These circumstances have prompted the USDA and FDA to conduct comprehensive surveillance testing.

Surveillance testing is vital for monitoring zoonotic HPAI strains and detecting early signs of pandemics. The reliable detection of new or emerging variant strains like H5N1, H5N6, H7N7, H7N9, H9N2, and H10N8 depends on using accurate analytical reference materials (ARMs) during diagnostics development. Without reliable ARMs, diagnostic tests may yield false results, undermining surveillance and public health efforts. As such, ARMs are crucial for calibrating diagnostic assays, ensuring they reliably detect these viruses in animal, human, and food samples and accurately measure viral load.

In response to the rapid global emergence of H5N1 and other serotype outbreaks in Asia, ATCC® has developed a comprehensive suite of quantitative synthetic RNA for HPAI virus serotypes H5N1, H5N6, H7N7, H7N9, and H9N2. These ARMs represent the most concerning and relevant influenza strains; for example, the H5N1 synthetic RNA product is based on a recent virus strain belonging to clade 2.3.4.4b. Each synthetic ARM contains the complete sequences from segments 4, 5, 6, 7, and 8, including the HA, NP, NA, M1, M2, NS1, and NEP/NS1 genes, covering 50% of the influenza genome. These segments are key diagnostic targets for molecular tests and provide sufficient genomic context for assessing assay specificity. These ARMs are manufactured using a highly reliable synthetic biology technology, verified through next-generation sequencing, and quantitated via Droplet Digital®PCR. Further, they do not contain any viable material and can be handled in BSL-1 settings. As such, they are intended to serve as safe and reliable positive controls for molecular tests for surveillance and diagnostics.

The usability of the synthetic RNA ARM panel for HPAI was experimentally evaluated using several published quantitative PCR assays, including those from the Centers for Disease Control and Prevention, the World Health Organization, the World Organization for Animal Health, and other highly cited sources. Additionally, we conducted an in-silico assessment of ARM compatibility with over 250 publicly available published assays. The data collectively demonstrate that all synthetic ARMs for HPAI are effectively designed and suitable for developing and validating molecular-based detection and quantification assays. Furthermore, our findings indicate that the synthetic RNA ARMs are equivalent to their corresponding native influenza RNA, making them a valuable BSL-1 alternative to BSL-3-derived materials. Our results suggest that these synthetic ARMs can serve as reliable and safe controls for molecular assays used in diagnostics and surveillance.

Presenter: Holly Asbury, American Type Culture Collection (ATCC), hasbury@atcc.org

Poster 3

Development and Validation of a Quantitative Synthetic Analytical Reference Material for the Monkeypox Virus

L. Papazisi, American Type Culture Collection (ATCC) , J. Bose, American Type Culture Collection (ATCC), B. Tang,, American Type Culture Collection (ATCC), S. Suh, American Type Culture Collection (ATCC), M. Tamhankar, American Type Culture Collection (ATCC), T. Correia, American Type Culture Collection (ATCC), S. Eskandarinezhad, American Type Culture Collection (ATCC), A. Wasko, American Type Culture Collection (ATCC), S. Rashid, American Type Culture Collection (ATCC), R. Bradford, American Type Culture Collection (ATCC), V. Knight-Connoni, American Type Culture Collection (ATCC), L. Papazisi, American Type Culture Collection (ATCC)

Mpox is a zoonotic viral disease caused by the human monkeypox virus (hMPXV), a double-stranded

DNA virus that belongs to the genus Orthopoxvirus and the family Poxviridae. hMPXV is endemic to several countries in Central and Western Africa and has been increasingly appearing in non-endemic areas. The virus causes a smallpox-like disease in humans and is characterized by two distinct genetic clades: Clade I and Clade II.
Rapid and accurate detection of this virus during the early stages of infection is essential for containment and timely treatment. While culture-based approaches can be used to detect hMPXV, they are typically time-consuming, labor-intensive, and require BSL-3 facilities. PCR-based methods provide a highly sensitive and rapid alternative screening approach. However, the development and validation of these assays depend on high-quality reference materials. To address this need, ATCC® designed and developed a quantitative synthetic molecular standard for hMPXV that contains specific hMPXV diagnostic biomarkers, including those characteristics of both clades. Here, we used a proprietary strategy to incorporate gene sequences typically targeted in various assays for viral detection to create a safe (BSL-1) and reliable positive analytical reference material (ARM).
The hMPXV ARM was authenticated via next-generation sequencing and quantified via Droplet Digital® PCR (ddPCR®; Bio-Rad®). The synthetic standard's functionality was tested via 13 published quantitative PCR (qPCR) assays, including those from the Centers for Disease Control and Prevention (CDC) that are specific for non-variola Orthopoxvirus and generic monkeypox. Serial ten-fold dilutions were used to create standard curves, with DNA concentrations ranging from 5.0 x 105 copies/µL to 5 copies/µL. Within this data set, the hMPXV standard had R2 values of 0.975 to 0.999, with slopes ranging from -3.291 to -3.477, displaying high efficiency and amplification.
The data collectively indicate that the hMPXV synthetic molecular standard is an effectively designed ARM suitable for developing and validating molecular-based detection and quantification assays. Our findings confirm the full compatibility of this novel ARM with 13 qPCR assays. Consequently, this ARM can function as a PCR template control, providing a safe and dependable positive control material for molecular assays employed in diagnostics and surveillance.

Presenter: Leka Papazisi, American Type Culture Collection (ATCC), lpapazisi@atcc.org

Poster 4
Rabies Virus Targeted Amplicon Sequencing and Metagenomic Whole Genome Sequencing Method Comparison and Optimization for Routine Surveillance
A. Rueda, Arizona State Public Health Laboratory, D. Francis, Arizona State Public Health Laboratory, K. Fitzpatrick, Arizona Department of Health Services

Rabies virus (RABV) kills approximately 70,000 people globally each year. While the infection and death rate have dramatically decreased in the US since the 1960s to less than 10 cases per year, approximately 4,000 animal cases are reported in the US annually. In the US Southwest, RABV is most commonly associated with bats, skunks, and mesocarnivores including coyotes and foxes. Rabies virus endemic to each host population have been classified into variants including Arizona Gray Fox, South Central Skunk and Bat variants. Spillover events in Arizona, including the introduction of Big Brown Bat EF-W1 variant to mesocarnivores in 2001-2009 and more recently in 2021-2023, as well as the first report of Mexican Skunk variant present in the state demonstrate the need for increased surveillance capabilities. Surveillance of the rabies virus and its variants via Next Generation Sequencing (NGS) is a valuable tool for tracking its distribution geographically and among host populations and provides greater resolution of variant typing compared to traditional antigenic methods. In this study we investigated two long-read sequencing methods to re-analyze archived RABV specimens at the Arizona State Public Health Laboratory; a CDC developed amplicon sequencing method targeting the nucleoprotein (N) and glycoprotein (G) genes and a metagenomic whole-genome sequencing (WGS) method based upon the SMART-9N approach. The SMART-9N method utilizes a random primed metagenomic WGS approach and has been shown to reliably and rapidly detect Zika, SARS-CoV-2 and yellow fever virus from clinical samples and may prove to be a reliable method for routine WGS surveillance of rabies virus. A simplified bioinformatics workflow is currently in development utilizing Reference Based Nanopore Amplicon Analysis Pipeline (RefNAAP) to produce QC reports and align reads to one of 14 reference sequences. The resulting FASTA sequences are then analyzed using RABV-GLUE, which utilizes RABV sequences and metadata from NCBI and is continually updated for the most up to date genotyping and analysis of the rabies virus. Variant typing with RABV-GLUE

increases the definition of RABV phylogenetics by utilizing a universal virus nomenclature first applied to SARS-CoV-2, providing increased capability over traditional antigenic methods.

Presenter: Austin Rueda, Arizona State Public Health Laboratory, austin.rueda@azdhs.gov

Poster 5
Impaired Driving Surveillance in Arkansas: A Collaboration Between the Arkansas State Crime Laboratory and Glen F. Baker Public Health Laboratory
K. Seely, Arkansas Department of Health, M. Heffington, Arkansas State Crime Laboratory, J. Edwards, Glen F. Baker Public Health Laboratory, K. Seely, Glen F. Baker Public Health Laboratory

Many forensic laboratories are limited in personnel, budget, and resources and are unable to undertake comprehensive drug testing for each impaired driving case. As a result, stop limit testing may be implemented as standard procedure if a driver's blood alcohol concentration (BAC) is measured above a specific cutoff, usually at or above the per se limit, and drug testing will not be performed for these cases as impairment could be demonstrated with the BAC result alone. Missing data on the driving population's drug usage has made it difficult to assess the prevalence of drugged driving and the crash risk associated with an individual that has drugs or combinations of drugs in their system.
To address the gaps in drug use data among impaired drivers, the Arkansas State Crime Laboratory (ASCL) and Glen F. Baker Public Health Laboratory (GFBPHL) have collaborated to begin an Impaired Driving Surveillance Project in the state of Arkansas. De-identified blood samples from suspected impaired drivers with a BAC ≥ .08 were provided to the GFBPHL by the ASCL for drug screening.

The primary objective of the Impaired Driving Surveillance Project is to provide data to determine the prevalence of polysubstance impaired driving in Arkansas. By using de-identified samples, the GFBPHL can expedite sample testing and reporting, which will provide up-to-date information on a rapidly shifting drug landscape to the ASCL, law enforcement, and healthcare providers. The study also aims to demonstrate a roadmap to successful and productive collaborations between state laboratories that further the mission of each's respective agency.

The ASCL provided the GFBPHL with de-identified blood samples from impaired driving casework. Samples were processed by supported liquid extraction (SLE) and analyzed via a Thermo Scientific Orbitrap Exploris 480, which was used to acquire full-scan, high-resolution MS and unit-resolution MS2 data from samples. Reference materials for each compound of interest were previously analyzed to create an in-house MS2 spectral library which was used to assist in identification and confirmation of compounds.

Sample testing commenced in June 2024. Thus far, 61 of 128 samples (48%) tested included drugs other than alcohol. Of those 61 drug positive samples, 28 samples tested positive for 2 or more drugs in addition to alcohol. CNS depressants have accounted for 43% of drug detections thus far, with benzodiazepines alone accounting for 56% of detected CNS depressants. Additionally, CNS stimulants, narcotic analgesics, cannabinoids, and dissociative anesthetics accounted for 19%, 18%, 12%, and 4% of drug detections, respectively. Unsurprisingly, delta-9 THC was the most detected compound overall.

The results of this project support the need to eliminate stop testing policies for DUID cases and further demonstrate that polysubstance drug use is pervasive among the population. By using stop testing policies, critical data is not gathered regarding drug use habits which inhibits law enforcement, healthcare professionals, and policy makers from taking the best actions to combat the issue. This project also demonstrates a successful collaboration between state partners to study a public safety and health issue that one department on its own cannot address sufficiently.

Presenter: Katie Seely, Arkansas Department of Health, kenton.leigh@arkansas.gov

Poster 6
Addressing Workforce Challenges in Public Health Laboratories Through ASCP BOC Credentialing
J. Baker, ASCP Board of Certification, J. Baker, ASCP BOC

The American Society for Clinical Pathology (ASCP), Board of Certification (BOC) plays a critical role in enhancing workforce competency and advancing public health outcomes by providing globally recognized, patient-centric credentials. Since ASCP BOC's inception in 1928, laboratory professionals from around the globe have attained more than 640,000 credentials in total. There are twenty-eight ASCP BOC certification and qualification examinations currently available that span the spectrum of medical laboratory and pathology specialties.
This poster explores the connection between ASCP BOC credentials and improved workforce performance in public health laboratories (PHLs), emphasizing key areas such as biosafety, environmental health, and infectious disease surveillance.
The ASCP BOC's rigorous credentialing process is rooted in meticulous examination development and maintenance practices, including regular practice analyses and computer-adaptive testing (CAT). These practices ensure that ASCP BOC credentials reflect current expertise, emerging concepts, and the evolving needs of laboratory medicine. The use of ISO 17024-accredited standards, validated by ANAB, guarantees psychometrically fair, legally defensible, and relevant credentialing processes. This robust framework equips credentialed professionals with the skills and knowledge necessary to address complex challenges in PHLs effectively.
In line with the ASCP BOC's value, this poster highlights how certifications such as Technologist in Molecular Biology, MB(ASCP) and Technologist in Microbiology, M(ASCP) enable professionals to excel in infectious disease surveillance, while qualifications in laboratory safety support critical biosafety protocols. Furthermore, the Credential Maintenance Program (CMP) fosters continuous professional development, ensuring that credentialed professionals stay current with technological advancements and best practices.
This poster presents data and correlations on ASCP BOC credentials and PHLs which demonstrate the tangible impact of ASCP BOC credentials on workforce competency and public health outcomes. Examples include improved diagnostic accuracy, enhanced safety compliance, and more efficient infectious disease monitoring. These findings underscore the ASCP BOC's mission to provide excellence in credentialing on behalf of patients worldwide and its vision to be the global standard in laboratory professional credentialing.
By aligning workforce development strategies with the ASCP BOC's proven credentialing framework, PHLs can recruit and retain highly competent professionals who contribute to better health outcomes. This alignment not only supports laboratory excellence but also advances the mission of PHLs to protect and promote public health on a broader scale.

Presenter: Joseph Brendan Baker, ASCP Board of Certification, joseph.baker@ascp.org

Poster 7
Connecting the Dots: APHL's Technical Assistance Expertise for Data Exchange Success
S. Abrams, Association of Public Health Laboratories, R. Abelman, APHL, B. Beaulieu, APHL, A. Mattson, APHL

APHL member laboratories and agencies are working to overcome an informatics deficit to make public health data more accessible and actionable. Serving as a centralized resource, APHL plays a crucial role in helping labs implement data exchange solutions swiftly by providing structure, guidance, and expertise.

APHL's Informatics program provides technical assistance (TA) to support a variety of national and global informatics initiatives that impact public health laboratories (PHLs), public health agencies (PHAs), and other partners, at both the national and global level. In collaboration with the project management office (PMO), TA teams assist each partner and their subject matter experts in implementing electronic data exchange projects that ensure secure and accessible data sharing among

federal, state, and other public health partners.

This poster will examine the important role of informatics TA in helping PHLs and PHAs address ongoing data needs while ensuring their capacity to respond to emerging public health challenges. It will demonstrate how APHL helps public health agencies leverage existing infrastructure to quickly meet new data-sharing demands, despite resource and funding constraints. Informatics solutions are essential for efficient data exchange and timely response, and APHL's TA is dedicated to supporting these efforts.

Presenter: Samuel Abrams, Association of Public Health Laboratories, samuel.abrams@aphl.org

Poster 8

Generational Shifts in Public Health Laboratories: Impacts on Workforce Composition, Satisfaction and Retention

S. Ahmadi, Association of Public Health Laboratories, S. Sarvarzade, Association of Public Health Laboratories, H. Ruitman, Association of Public Health Laboratories, L. Kurimski, Association of Public Health Laboratories, R. Bernard, Missouri Department of Health and Senior Services, D. Severson, Fairfax County Health Department, E. Levings, Fairfax County Health Department Laboratory, R. Zinner, Kentucky Division of Laboratory Services, R. Thomas, Louisiana Department of Health, V. Waddell, Arizona State Public Health Laboratory, S. Marine, State Hygienic Laboratory (Iowa), H. Wood, Michigan Department of Health and Human Services, K. Mitchell, Wadsworth Center, New York State Department of Health, S. Flowers, Association of Food and Drug Officials, K. Seiler, Vermont Dept of Health Laboratory

Background: Public health laboratories (PHLs) are undergoing significant generational shifts that affect workforce dynamics, job satisfaction, and employee retention. Since 2016, APHL has conducted periodic workforce surveys to monitor these changes. As Baby Boomers retire, Millennials and Generation Z are playing a larger role in shaping the workforce, bringing fresh perspectives and new priorities.

Methods: Data from APHL's workforce surveys conducted in 2016, 2022, and 2024 were analyzed to assess generational trends in workforce composition, satisfaction and retention intentions. Key performance indicators included work environment, engagement, development opportunities and leadership representation.

Results: The generational composition of the PHL workforce has shifted dramatically, with Baby Boomers declining from 36% in 2016 to 11% in 2024. Millennials now comprise 46% of the workforce, while Generation X remains stable at approximately one-third. Generation Z, although comprising only 10% of the workforce in 2024, showed the highest turnover intentions at 66%. Job satisfaction varies by generation: Baby Boomers improved steadily, Millennials remained lower, and Generation X saw gains by 2024. Female representation in supervisory roles has increased, achieving near gender parity among Millennials in 2024, a significant step toward equitable leadership as older generations retire. These findings illustrate the need for tailored strategies to address workforce retention and engagement across generations. Key findings include: gender parity in Millennial supervisory roles signals progress toward equitable leadership representation; generational shifts highlight the need for targeted retention strategies, particularly for Millennials and Generation Z, who report higher turnover intentions; workforce satisfaction and performance drivers differ across generations, necessitating tailored engagement and development opportunities; and Generational insights offer actionable strategies to foster collaboration and resilience in the PHL workforce.

Conclusion: Generational shifts in public health laboratories highlight the need to adapt workforce strategies to meet emerging challenges and seize new opportunities. By understanding generational dynamics and cultivating inclusive, supportive environments, PHLs can improve job satisfaction, enhance employee retention, and promote leadership equity ensuring a resilient, future-ready workforce.

Presenter: Sudaba Parnian Ahmadi, Association of Public Health Laboratories, sudaba.parnian@aphl.org

Poster 9
Supporting the Food Safety Workforce Through Collaborative Training, WGS and Enteric Workflow Efficiencies, and Retention Efforts
A. Bryant, Association of Public Health Laboratories, K. Larson, Association of Public Health Laboratories, A. Bryant, Association of Public Health Laboratories

Food Safety is a core function of public health laboratories. Foodborne outbreaks continue to be a significant source of illness in the US. As foodborne disease surveillance and testing methods rapidly evolve, it will be critical to regularly evaluate foodborne disease programs and ensure that the next generation of public health laboratory personnel are familiar and in line with current best practices. The Council to Improve Foodborne Outbreaks (CIFOR) plays a critical role in this process by actively identifying emerging barriers and developing guidelines and tools for public health professionals to address them. However, data from the 2024 Food Safety Survey showed limited awareness of the Council to Improve Foodborne Outbreaks (CIFOR) among APHL members.

CIFOR is a highly effective multidisciplinary collaboration among state, tribal, local, territorial (STLT) and federal agencies and public health associations. As a founding member of CIFOR, APHL works in concert with these partners to train and sustain public health laboratory professionals in team-based foodborne disease outbreak investigations. CIFOR offers many resources to public health laboratory professionals and supports several laboratory-focused projects including building data fields into PulseNet 2.0 to calculate the time to generate enteric WGS data, developing efficiencies in enteric WGS to speed cluster detection, cross-sector CIFOR Toolkit training opportunities, integrating laboratory results with epidemiology and environmental data and efforts to retain the food safety workforce.

Presenter: Amy Bryant, Association of Public Health Laboratories, amy.bryant@aphl.org

Poster 10
Building Jurisdictional Programs Will Improve Environmental Microbiology Outbreak Response Readiness
S. Comet, Association of Public Health Laboratories

Waterborne pathogens can cause massive disruption and fatal diseases, quickly impacting the health of large populations. Within the US, 7.15 million waterborne illnesses occur annually, resulting in greater than 100,000 hospitalizations, 6,630 deaths and $3.33 billion in healthcare costs1. Building and sustaining environmental microbiology response programs in states, territories, and local jurisdictions can help prevent these types of contamination events and provide the expertise, coordination, and training needed for rapid and effective response. This poster provides a summary of the importance and structure of environmental microbiology outbreak response (EMOR) programs and outlines their necessary capabilities and capacities, including response identification, environmental sampling, sample analysis, data interpretation/reporting and mitigating public health threats. This poster describes current resources provided by APHL and CDC to assist state and jurisdictions to build and sustain EMOR programs, such as the Environmental Microbiology Outbreak Response Community of Practice – an online forum and bi-monthly meeting for public health laboratory scientists to interact, share successes, ask questions and discuss challenges related to environmental microbiology outbreak response.

Presenter: Sydney Comet, Association of Public Health Laboratories, sydney.comet@aphl.org

Poster 11
Strengthening Public Health Laboratory Workforces: The Retention Scorecard
I. Eaves, Association of Public Health Laboratories, S. Sarvarzade, Association of Public Health

Laboratories, S. Ahmadi, Association of Public Health Laboratories, A. Raymer, APHL, L. Kurimski, Association of Public Health Laboratories, R. Bernard, Missouri Department of Health and Senior Services, D. Severson, Fairfax County Health Department, E. Levings, Fairfax County Health Department Laboratory, R. Zinner, Kentucky Division of Laboratory Services, R. Thomas, Louisiana Department of Health, V. Waddell, Arizona State Public Health Laboratory, S. Marine, State Hygienic Laboratory (Iowa), H. Wood, Michigan Department of Health and Human Services, K. Mitchell, Wadsworth Center, New York State Department of Health, S. Flowers, Association of Food and Drug Officials

Background: Workforce recruitment and retention challenges, as highlighted in APHL surveys and focus groups, have the potential to significantly impact laboratory knowledge retention. On average, 37% of respondents expressed intentions to leave their positions within the next four years. To address these challenges, APHL's Quality Systems and Analytics program, in collaboration with the Knowledge Management Committee (KMC), developed the Public Health Laboratory (PHL) Retention Scorecard. This tool enables laboratories to evaluate their practices, identify actionable strategies for improvement, and foster a more engaging workforce environment.

Methods: A comprehensive literature review helped highlight ten areas influencing workforce retention, such as Work-Life Balance, Communication, etc. They became the foundation for the scorecard. To ensure the tool reflected real-world laboratory experiences, members of the Knowledge Management Committee (KMC) provided input through detailed discussions and iterative reviews. This allowed the team to define subcategories to address the unique challenges PHLs face. The draft version of the scorecard underwent beta testing in late 2024, with KMC and Workforce Development Committee (WDC) members providing feedback on usability and relevance. A second round of testing was administered in early 2025, where KMC members implemented the tool within their laboratories. The final tool evaluates laboratory practices across ten areas using five development levels: None, Minimal, Moderate, Significant and Optimal. Designed in Microsoft Excel for accessibility, the scorecard includes embedded calculations to generate real-time insights, highlighting strengths and opportunities for improvement.

Results: The PHL Workforce Retention Scorecard's actionable design ensures laboratories can identify areas needing immediate attention while celebrating existing strengths. Using this tool, laboratories maintain full control of their data, with no sharing or external comparisons required. The tool also provides actionable insights, linking users to APHL resources such as the Recruitment and Retention Toolkit.

Conclusion: The PHL Retention Scorecard empowers labs to address workforce retention challenges with practical, data-driven solutions. By focusing on areas within their control, laboratories can foster a supportive environment where employees feel valued and engaged. This tool aligns with APHL's strategic workforce priorities, supporting sustainable recruitment and retention practices.

Presenter: Isaac Eaves, Association of Public Health Laboratories, isaac.eaves@aphl.org

Poster 12

The Evolution of the AIMS Platform as a Prominent Public Health Data Intermediary

E. Gonzalez-Loumiet, Association of Public Health Laboratories, K. Higginbotham, Association of Public Health Laboratories, V. Holley, Association of Public Health Laboratories, A. McShane, Association of Public Health Laboratories, M. Kourbage, Association of Public Health Laboratories, E. Sudduth, Association of Public Health Laboratories

Background: Originally conceived to maintain multiple point-to-point electronic interfaces and accommodate ever-changing transport mechanisms, the APHL Informatics Messaging Services (AIMS) Platform is now a trusted solution for public health data exchange across all 50 states. Methods: AIMS is a secure, cloud-based platform that accelerates the implementation of data exchange by providing shared services to aid in the visualization, interoperability, security and hosting of electronic data. The ability to visualize and share data efficiently, while hosting applications securely is a critical capability

for public health. The AIMS Platform solves each of these difficult challenges for the public health community. Results: The AIMS Platform connects all State Public Health Laboratories and Agencies in the country to hundreds of data exchange partners, including healthcare organizations, private laboratories, CDC, and HHS. AIMS securely transports millions of messages on a monthly basis across these partners; these messages span the full spectrum of public health reporting. In addition, AIMS provides translation and transformation services and hosts tools and infrastructure such as the COVID-19 ELR Data Lake, the IZ Gateway, CDC's Rabies Surveillance system, the ARLN's Lab Web Portal, eCR NOW, next-generation sequencing (NGS) tools, cloud-hosted State laboratory information management systems (LIMS), and the profile-driven NIST HL7 validator. AIMS went into production in 2024 with Detor, which enables public health laboratories to exchange electronic test orders and results with healthcare organizations using a scalable and LIMS-agnostic model. AIMS continues to push innovative approaches to public health informatics, with eCR data from TEFCA QHIN partners to more than 57 jurisdictions. By serving as an intermediary, the AIMS Platform takes the burden of interoperable data exchange off of public health practitioners and lets them focus on what's important, while also ensuring that decision-makers have access to better, more complete, higher quality data. Conclusion: In addition to current functionality, APHL and CDC are planning ambitious new services on the AIMS Platform to modernize the nation's public health system.

Presenter: Eduardo Gonzalez-Loumiet, Association of Public Health Laboratories, eduardo.gonzalez@aphl.org

Poster 13
Considerations for the Implementation of Wastewater Surveillance for Drugs in Public Health Laboratories
K. Granger, Association of Public Health Laboratories, E. Morin, Association of Public Health Laboratories

Wastewater surveillance (WWS) has been used for decades outside the United States to detect targets of public health concern[1] but gained traction in the US in 2020 as a surveillance system for SARS-CoV-2 during the COVID-19 pandemic. While wastewater surveillance has mostly been utilized for pathogenic targets, there is increasing interest in leveraging WWS for chemical targets, including licit and illicit drugs due to its ability to capture data beyond traditional methods like surveys and healthcare data, as substance use, substance use disorder (SUD) and overdose are prevalent throughout the US. In 2022, 24.9% of the US population reported having used a substance within the past year and in 2023, CDC reported 107,543 fatal overdoses. In response to a growing need for innovative surveillance mechanisms to monitor drug use and overdose, jurisdictions may consider implementing a WWS for drugs program in adjunct to existing surveillance systems to obtain useful data that is not currently captured. This poster will provide recommendations for public health laboratories, describe benefits and limitations, discuss ethical considerations including risks to vulnerable populations and concerns of punitive use of data, highlight opportunities for collaboration, outline examples of data-driven public health action, and emphasize the need for extreme care in ensuring data obtained is used solely for public health benefit.

1. Manor Y, Handsher R, Halmut T, et al. Detection of Poliovirus Circulation by Environmental Surveillance in the Absence of Clinical Cases in Israel and the Palestinian Authority. J Clin Microbiol. 1999;37(6):1670-1675.

Presenter: Kelsey Granger, Association of Public Health Laboratories, kelsey.granger@aphl.org

Poster 14
Tracking Phenazine Production in Bacterial Biofilms of *Pseudomonas aeruginosa*
L. Kemper, Association of Public Health Laboratories, E. Key, APHL, E. Knorr, APHL, J. Paczkowski, Wadsworth Center

Pseudomonas aeruginosa is an opportunistic pathogen that is one of the leading causes of antibiotic-

resistance infections in hospitalized immunocompromised patients because it forms biofilms on medical implants and catheters in hospitalized patients. *P. aeruginosa* relies on quorum sensing (QS), a mechanism of bacterial cell-cell communication, to drive expression of collective behaviors, such as biofilm formation and toxin production, during infection of a host. QS relies on the production, secretion, and group-wide detection of signaling molecules called autoinducers to regulate hundreds of genes involved in virulence and pathogenesis. Biofilms are a community of bacteria that produce extracellular polysaccharides (EPS), proteins, DNA, and other molecules to form a matrix barrier they encapsulate themselves within to protect themselves from the environment. Antibiotics and host defense mechanisms are unable to effectively kill the bacteria within the biofilm because the EPS that embodies the biofilm prevents or slows the movement of antibiotics through them, thus decreasing the effectiveness of these drugs. Due to the complexity of the QS network of *P. aeruginosa* and its profound effect on regulating biofilm formation, it is important that we better understand how QS works in biofilms and other virulence mechanisms employed by bacteria so that therapeutics can be developed to decrease antibiotic-resistant infections in hospitalized patients.

Pyocyanin (PYO) is a phenazine toxin that is secreted by *P. aeruginosa* during host infections. PYO supports colonization in patients by facilitating intracellular signaling, redox balance, and iron acquisition (Recinos et al., 2012). PYO's redox functions are toxic to the host through generation of reactive oxygen species that destroy host defensive cells. Additionally, PYO can act as an electron shuttle to promote anaerobic respiration within the biofilm (Okegbe et al., 2017).

Two redundant operons, phz1 and phz2, are responsible for PYO production in *P. aeruginosa*. Our lab has shown that the RhlR QS system, which drives community behavior in chronic, hyper-biofilm forming clinical isolates, also regulates PYO production (Simanek KA 2022). RhlR, a transcription factor, directly binds to the promoters of phz1 and phz2, resulting in PYO production and changes the biofilm phenotypes (Recinos et al., 2012). It is not yet understood how the Rhl QS system influences biofilm maturation and toxin production over time or location within the biofilm. We hypothesize that PYO production will activate in the center, where anaerobic respiration is starting, and at the edges, where the bacteria are the most metabolically active and influenced by environmental signals. To achieve this, we tagged the promoters of the PYO synthesis operons, phz1 and phz2, with mScarlet and luciferase reporters to track transcriptional activation of the operons during biofilm growth in wild type, Rhl QS deletion strains, and clinical-relevant Rhl mutant strains to determine the effect of the Rhl system on biofilm formation and pyocyanin production overtime. We have identified where PYO production is localized and when it onsets during biofilm maturation in one of our clinical isolates. Knowledge of where important QS signaling molecules are localized and when they are secreted in biofilms will aid in the future development of treatments to improve the health of patients infected with antibiotic-resistant microbes.

Presenter: Leah Kemper, Association of Public Health Laboratories, leah.kemper@health.ny.gov

Poster 15
Biorisk Management: Piloting ISO 35001 Across Public Health Laboratories
M. Marsico, Association of Public Health Laboratories, J. Baron, Science and Safety Consulting

Released in 2019, the ISO 35001 standard provides a framework for developing and implementing a biorisk management program. APHL, in collaboration with the CDC have developed a strategy to provide guidance for public health laboratories (PHLs) interested in ISO 35001 implementation and have successfully piloted the implementation of ISO 35001 at four PHLs through the winter of 2024. This poster will outline the value of the International Organisations for Standardization (ISO) 35001: Biorisk Management for Laboratories and Other Related Organisations to attendees along with highlighting the APHL/CDC ISO 35001 Implementation Project across the initial pilot laboratories and future pilot laboratories.

Presenter: Michael Marsico, Association of Public Health Laboratories, michael.marsico@aphl.org

Poster 16
Advancing Public Health Laboratories Technical and Leadership Skills: APHL's Training Needs Assessments
L. Natiq, Association of Public Health Laboratories, L. Kurimski, Association of Public Health Laboratories, A. Raymer, APHL, R. Bernard, Missouri Department of Health and Senior Services, D. Severson, Fairfax County Health Department, E. Levings, Fairfax County Health Department Laboratory, R. Zinner, Kentucky Division of Laboratory Services, R. Thomas, Louisiana Department of Health, V. Waddell, Arizona State Public Health Laboratory, S. Marine, State Hygienic Laboratory (Iowa), H. Wood, Michigan Department of Health and Human Services, C. Findley, Louisiana Department of Health, J. Fesler, PA Department of Environmental Protection - Bureau of Laboratories, R. Allman, Delaware Public Health Laboratory, K. Seiler, Vermont Dept of Health Laboratory

Training Needs Assessments (TNAs) systematically identify gaps in knowledge, skills, and competencies, ensuring training programs effectively address workforce needs. Between September 2023 and May 2024, APHL conducted a series of TNAs to assess the laboratory professionals' technical and scientific skills. These assessments, designed as brief and user-friendly surveys for program managers and supervisors, prioritized urgent training needs, identified essential resources, and established pathways for delivery across key program areas, including:

- Environmental Health
- Infectious Diseases
- Food Safety
- Public Health Preparedness and Response
- Quality and Safety Systems.

In January 2024, APHL expanded its focus to leadership development within public health laboratories, targeting individual laboratory staff through a new survey promoted via the Laboratory Leaders of Today, Emerging Leaders Program, and Regional Consortia programs.

Key findings from the TNAs highlighted training needs in the following areas:

- Method validation, validation studies
- Next-generation sequencing
- Quality systems and continuous quality improvement
- Root cause analysis
- Designing exercises and drills

Most respondents expressed a preference for virtual sessions and courses, in-person workshops and conferences, and practical resources such as guidance documents, tools and templates, and case studies. These insights help APHL programs and committees in refining training offerings, developing targeted resources, and enhancing member support.

The results, available via summary reports on the APHL website, contribute to workforce development, improved laboratory performance, and greater resilience within the U.S. public health laboratory system.

Presenter: Laila Natiq, Association of Public Health Laboratories, Laila.natiq@aphl.org

Poster 17
One Health Partnerships and Communication Strategies
R. Nickla, Association of Public Health Laboratories, J. Breher, County of San Diego, S. Shea, Association of Public Health Laboratories, A. Dickey, New York State Department of Agriculture and Markets

According to the Centers for Disease Control and Prevention (CDC), One Health is an approach

recognizing the health of people is closely connected to the health of animals and our shared environment. While the concept of One Health is not new, it has become more important in recent years due to changing factors and interactions between people, animals, plants and our environment. There is a recognized need for multidiscipline and cross agency collaborations to maintain effective public health systems due to increasing environmental contaminants and emerging zoonotic diseases. Successful public health interventions require the cooperation of human, animal, and environmental health partners to communicate and coordinate activities. By promoting collaboration across all sectors, the One Health approach can achieve the best health outcomes for people, animals, and plants in a shared environment.

A One Health focused pre-conference workshop was presented for the APHL Annual Conference 2025. The interactive workshop provided participants with tools and strategies for developing and enhancing public health laboratory (PHL) One Health programs and communications with One Health considerations. A summary of the workshop design and resources provided will be described further within this poster presentation for how PHLs can enhance their own efforts toward integrating a One Health perspective.

Presenter: Robert Nickla, Association of Public Health Laboratories, robert.nickla@aphl.org

Poster 18
Framework Design Study: Nursing Care Facility Wastewater Testing of *Candida auris*
P. Saingam, Association of Public Health Laboratories, M. Steadmon, Hawaii Department of Health, D. Di, Hawaii Department of Health, D. Ornellas, Hawaii Department of Health, E. Desmond, Hawaii Department of Health

Candida auris, a pathogenic yeast surviving on skin and contaminated surfaces, can cause multi-drug resistant infections and life-threatening illnesses among immunocompromised individuals. The number of *C. auris* infections has been rising with reported outbreaks among residents at care facilities in multiple states but not yet in Hawaii. As of January 2025, there are 45 care facilities in Hawaii. Early detection of *C. auris* infection would help prevent the outbreak and challenges to the care facilities. Detection of *C. auris* in wastewater was previously demonstrated as tool to monitor infection in the community, including previous positive detection from a wastewater treatment plant in Hawaii. In this study, a framework for the *C. auris* infection detection was designed to guide the integration with the care facility wastewater testing program at the Hawaii State Laboratories Division (SLD), which routinely monitors COVID-19, Flu, and respiratory viruses. The framework consisted of three stages, (1) identifying goals of *C. auris* wastewater testing, (2) literature review of wastewater *C. auris* detection, identification, and antifungal resistance tests, (3) assessment of SLD routine wastewater testing program limitations and pinpoint test candidates, and (4) candidate test validation through spiking genetic materials or live cells of *C. auris*. This study provided an updated review of available methods of wastewater *C. auris* detection and a case study of integrating *C. auris* monitoring into the established wastewater testing for viral diseases with the goal to prevent and control *C. auris* outbreaks at the care facility.

Presenter: Prakit Saingam, Association of Public Health Laboratories, prakit.saingam@gmail.com

Poster 19
A ‘Detor’ for Electronic Test Orders and Results
R. Shepherd, Association of Public Health Laboratories

Presenter: Rachel Shepherd, Association of Public Health Laboratories, rachel.shepherd@aphl.org

Poster 20
Public Health Laboratory Fellowship and Internship Programs: An APHL-CDC Initiative
K. Solis, Association of Public Health Laboratories

The Association of Public Health Laboratories (APHL) works to strengthen laboratory systems serving the public's health in the US and globally. The Public Health Laboratory Fellowship Program: an APHL-CDC Initiative, administered by APHL and funded by the Centers for Disease Control and Prevention (CDC), strengthens laboratory systems and workforce by developing the next generation of public health scientists and mentors. The Public Health Laboratory Fellowship Program offers experiential learning opportunities across nine laboratory science focus areas including bioinformatics, biosafety, environmental health, infectious disease and others. Each fellow is trained in alignment with established laboratory core competencies and functions and works within host laboratories on designated projects that support public health initiatives, including the One Health initiative. Host laboratories include state and local public health laboratories and non-federal academic, agricultural, chemical, environmental, food safety and veterinary laboratories.

The fellowship program is well established and since 2021, the program has had 74 host laboratories participate across 41 states, 260 fellows have been placed, and 182 mentors have supported fellowships.

Presenter: Kristen Solis, Association of Public Health Laboratories, kristen.solis@aphl.org

Poster 21
The Value of Regional Consortia Stands the Test of Time
B. Su, Association of Public Health Laboratories

There are seven regional consortia that have been in existence for 5 to 50 years and continue to find the value of building relationships and strengthening capabilities through sharing activities and best practices. Regional consortia continue to collaborate and work towards a common goal that benefits the participating laboratories and provide an opportunity to share knowledge and contribute to the larger public health laboratory community. Driven by state, local, and territorial laboratory engagement by public health and environmental laboratories, regional consortia encourage partnerships that build consensus and collectively share strategies, technical resources and training. The poster will highlight recent activities and accomplishments from each of the seven regional consortia, all with the outcome of improving laboratory service delivery.

Presenter: Bertina Su, Association of Public Health Laboratories, bertina.su@aphl.org

Poster 22
Cell Cycle Modulation as a Strategy to Prevent Coronavirus-mediated Syncytia: From Mechanism to Therapeutic Intervention
A. Calloway, Association of Public Health Laboratories, H. Ullah, Howard University

The emergence of SARS-CoV-2 variants necessitates therapeutic strategies targeting conserved viral elements. The 5'-polyU tract of the coronavirus antigenome, uniquely absent in host cell transcripts, represents such a target. Our previous research demonstrated that DNA oligonucleotides containing polyA sequences effectively inhibit viral replication and syncytium formation in the mouse coronavirus (MHV-A59) model. Specifically, oligonucleotide treatment disrupted key viral processes including double-stranded RNA (dsRNA) synthesis and virion infectivity, while leaving host cells unaffected. This establishes a novel antiviral mechanism potentially applicable across RNA viruses, including coronaviruses.

Coronavirus infection typically results in multinucleated syncytia formation, suggesting significant host cell cycle regulation perturbation. However, the molecular mechanisms linking 5'-polyU targeting to prevent syncytium formation and potential cell cycle effects remain unclear. We hypothesize that our oligonucleotide approach may restore normal host cell cycle progression while preventing syncytium formation.

To test this hypothesis, we propose to: (1) characterize cell cycle checkpoint modifications during infection with and without polyA treatment; (2) map the molecular pathways connecting 5'-polyU

targeting to syncytium formation inhibition; and (3) analyze the relationship between cell cycle restoration and reduced viral spread. This research will advance our understanding of viral pathogenesis and validate the therapeutic potential of targeting the 5'-polyU tract across coronavirus variants. The strategy's specificity for viral sequences ensures minimal impact on host cells while effectively limiting viral spread and tissue damage.

Presenter: Amaya Calloway, Association of Public Health Laboratories, amayacc01@gmail.com

Poster 23
A Movel Use of a BIOFIRE® FILMARRAY® Research Configuration Panel for Real-time Surveillance of Respiratory Analytes in Influent Wastewater
C. Firmage, bioMérieux, F. Chatigny, bioMérieux, N. LaCross, Utah Department of Health and Human Services

Wastewater-based surveillance is the intentional and consistent measurement of health markers in a wastewater or wastewater-derived sample. The BIOFIRE® FILMARRAY® qPCR system uses diagnostic panels designed to detect many human pathogens from a single patient sample of various sample types. The system includes integrated sample preparation, nucleic acid extraction, nested multiplex PCR, and melt curve analysis. Furthermore, crossing point (Cp) values are now available through the BIOFIRE® FIREWORKS™ software.

To assess the performance of the BIOFIRE® FILMARRAY® system, a respiratory research configuration of existing BIOFIRE® assays were used to monitor influent wastewater in a yearlong study. This study was performed in partnership with the Utah Department of Health and Human Services (UDHHS) Wastewater Division. This study was performed from August 2023 to August 2024. Testing was performed twice weekly on influent wastewater samples collected from 5 testing sites in or near Salt Lake County, Utah. The research configuration assays were chosen to assess their ability to detect the presence of analytes such as SARS-CoV-2, Influenza A/B, Respiratory Syncytial Virus (RSV), Adenovirus, seasonal Coronaviruses (non-SARS-CoV-2), Parainfluenza virus (PIV), Human Rhinovirus/Enterovirus, Human Metapneumovirus (hMPV), *Mycoplasma pneumoniae*, *Bordetella pertussis*, *Bordetella parapertussis*, and *Chlamydia pneumoniae* in wastewater. Testing was performed with concentrated and unconcentrated influent wastewater samples. Concentration was performed using the Promega Environ Total Nucleic Acid Kits, following the recommended preparation method. Crossing point (Cp) analysis was performed for SARS-CoV-2 using the BIOFIRE® FIREWORKS™ software, to generate trend results which were then compared to the laboratory comparator (qPCR / ddPCR) and to clinical incidence rates in Utah. Further Cp analysis was performed for seasonal viruses and compared to clinical incidence rates when available.

For influent wastewater samples across all testing sites, SARS-CoV-2 was detected in approximately 97% of concentrated samples and in 76% of unconcentrated samples tested. Influenza, RSV, PIV, hMPV, and Seasonal Coronaviruses were consistently detected throughout respiratory season. Increasing wastewater signal was observed for Influenza A/B and RSV from mid-October peaking on January 5th, corresponding with clinical incident rates for the state. The environmental presence of these viral analytes continued into the summer months. Adenovirus and HRV/EV was detected in the majority of samples for the duration of this study. A single positive sample was observed for *Bordetella pertussis* on July 10th, 2024. *B. parapertussis* was detected in 5% of all concentrated samples, with near zero detections for the remaining bacterial assays.
The BIOFIRE® respiratory research configuration showed robust analyte detection for influent wastewater samples. These feasibility results suggest that the BIOFIRE® assays can be used for the intended purpose of wastewater-based surveillance. The performance of the research configurations used in this study have not been evaluated by any regulatory agency.

Presenter: Cody Firmage, bioMérieux, cody.firmage@biomerieux.com

Poster 24
Advancements in High Matrix Neutralization for Sample Preparation of Surface, Ground and

Wastewater Samples
D. Bissonnette, Biotage

Environmental laboratories routinely follow the Environmental Protection Agency (EPA) 1600, 600, and 8000 series methods to analyze contaminants in surface water, groundwater, and wastewater. However, the physical and chemical complexity of these samples present significant challenges during sample preparation, including high particulate loads and matrix interferences. To address these challenges, many laboratories have adopted methods to reduce sample preparation volumes from the traditional 1L samples. While this approach minimizes handling difficulties, it can negatively impact laboratory reporting limits by increasing the detection limits for target analytes.

To overcome these limitations, we introduce a novel 47mm depth filtration disk holder incorporating the ISOLUTE® HM-N filtration product. This system effectively neutralizes high-matrix interferences and integrates seamlessly with solid-phase extraction (SPE) techniques. Laboratories can process 1L wastewater samples in under 10 minutes with unprecedented ease and yield a 35% reduction in solvent use. This innovative sample matrix filtration approach enhances performance criteria by reducing detection limits, increasing accuracy, and improving precision when compared to traditional liquid-liquid extraction and micro-extraction techniques. This work highlights the transformative potential of this new technology to simplify and optimize sample preparation workflows. Environmental laboratories that implement the 47mm depth filtration disk holder for EPA 1600, 600, and 8000 series methods will maximize sample processing efficiency, improve sustainability, and produce reliable data.

Presenter: Deanna Bissonnette, Biotage, deanna.bissonnette@biotage.com

Poster 25
Comparing Methods for Testing PFAS in Solid Matrices: USEPA 1633, USFDA C-010.03, & European Union Reference Laboratory
D. Bissonnette, Biotage

Extraction protocols and analytical methods have been utilized and revised for detecting Per-and Polyfluoroalkyl Substances (PFAS) in aqueous matrices since 2013. The toxicity and persistence of PFAS drove the evolution of drinking water methods and initiated the process to regulate PFAS in solid matrices. The US Environmental Protection Agency (USEPA) published a single lab validation study in 2021 for Method 1633 and released 1633A in December 2024. This method monitors 40 PFAS targets in fish tissues, soils, biosolids, and wastewater. During that time, the US Food and Drug Administration (USFDA) released a single lab validation in April 2024. This study monitors 30 PFAS targets in different foodstuffs. In April 2023, the European Commission published Regulation (EU) 2023/915, which sets guidelines on maximum levels for 4 target PFAS analytes in food. In response to these guidelines, the European Union Reference Laboratory (EURL) developed 2 separate sample preparation methods for the determination of 33 PFAS analytes in food of animal and plant origin.

This comparison study outlines techniques developed by the USEPA, USFDA, and EURL for testing PFAS in solid matrices. The various sample matrices, preparation procedures, target analytes, and analytical parameters within each technique are detailed. Through a simplified and automated workflow, the Biotage® Lysera, TurboVap® LV, and Extrahera HV-5000 provide efficient sample homogenization, liquid extraction, solid phase extraction, and solvent concentration that can be applied to all three techniques. The results from our evaluation demonstrate the importance of effectively removing matrix interferences without negatively impacting precision, accuracy, and detection levels of target analytes. This work demonstrates how understanding the similarities and differences between each technique can drive improvements for sample processing and analytical performance of PFAS in a wide variety of solid matrices.

Presenter: Deanna Bissonnette, Biotage, deanna.bissonnette@biotage.com

Poster 26
Seroprevalence of Mpox Infection in Urban Male STI Clinic Clients After a Major Outbreak

A. Jassem, British Columbia Centre for Disease Control, G. Cortez, University of British Columbia, I. Enriquez, University of British Columbia, D. Luk, University of British Columbia, T. Valadbeigy, University of British Columbia, D. Tan, Unity Health, M. Singal, British Columbia Centre for Disease Control, A. Jassem, British Columbia Centre for Disease Control

Background: In British Columbia (BC), Canada, cases of mpox virus (MPXV) infection continue to be sporadically diagnosed following a major outbreak in 2022. The burden of mpox disease may be higher than estimated by confirmed case counts owing to mild or non-specific presentations that do not get tested. Our objective was to determine the seroprevalence of MPXV infection and infection-induced immunity in a population at high risk a year after the outbreak.
Methods: The quantitative Meso Scale Discovery V-Plex Orthopoxvirus Panel of vaccinia and MPXV virus antigens was first evaluated using residual serum samples from uninfected and infected individuals, including participants likely vaccinated against smallpox (born before 1972) and samples predating 2022. Modified vaccinia Ankara-Bavarian Nordic (MVA-BN) vaccination status, day of symptom onset, number of lesions at presentation, and HIV status was considered.
For subsequent serosurveys, we chose 1812 residual sera from males seeking syphilis testing at four urban STI clinics in BC; 596 from July 2023 (Period 1), 562 January-March 2024 (Period 2), and 654 August-September 2024 (Period 3).
Results: A reactivity algorithm was developed based on the detection of anti-E8L antibodies, along with additional antigens (either A35R or B6R). This algorithm demonstrates a sensitivity of 88% (95% CI [79-94%]) and a specificity of 96% (95% CI [90-98%]). MVA-BN vaccination status, the number of lesions, and HIV status were not associated with antibody level. To date, 596 and 562 serosurvey samples were tested from periods 1 and 2, respectively, with results pending for period 3. Overall, 15% of males were positive for anti-MPXV antibodies, with 18% in period 1 and 13% in period 2. Positivity rate did not differ by syphilis status (15.6% in positives, 18.8% in negatives, p=0.46), but was higher in those born before 1972 (46% vs 23%, p=< 0.0001). For MVA-BN-unvaccinated males born after 1972, 7% were positive for anti-MPXV antibodies.
Conclusions: We validated a specific serological test for measuring previous MPXV infection. Mpox seroprevalence estimates over a year after a major outbreak in a non-endemic region revealed that a high proportion of males seeking syphilis testing in urban STI clinics have been infected to date. These findings highlight the importance of serosurveys for assessing population health risk and potential health impacts in the event of disease reemergence.

Presenter: Agatha Jassem, British Columbia Centre for Disease Control, agatha.jassem@bccdc.ca

Poster 27
Trends in Design, Construction and Operation of Public Health Laboratories
N. Roisen, BWBR, K. Lauer, BWBR, A. Taylor, Cornerstone Commissioning, T. Norton, Kraus Anderson Construction

Building or renovating public health laboratory facilities is a challenging process with difficult decisions at every turn. Laboratory professionals considering construction projects of any size will be better equipped to execute their goals successfully if they have a plan in place to navigate the latest trends impacting the design and construction industries:

Safety & Security
Flexibility and Adaptability
Staff Satisfaction
Sustainability, Energy Use
Sensitive Testing Environments
Site Selection
Public Engagement

Preconstruction Activities
Bidding Environment
Construction Cost Trends

Schedule Planning
Working with Public Agencies
Laboratory Construction Trends
BSL-3 Construction

Systems Commissioning and Operations
System Selections
Energy Use Optimization
Building Automation
BSL-3 Commissioning
Equipment Certification/Testing
Trends and Alarms
Ongoing/Preventive Maintenance

Our poster will share lessons learned and examples from recent project experience so attendees can better understand today's design and construction environment.

Presenter: Nathan Roisen, BWBR, nroisen@bwbr.com

Poster 28
Comparison of Portable XRF and Single Quadrupole ICP-MS for ppm-Level Pb Analysis: Feasibility Study of XRF for High-Concentration Pb Analysis
D. Adhikari, California Department of Public Health, L. Nguyen, Biochemistry Section, Environmental Health and Laboratory, DELH/CLS, California Department of Public Health, E. Carlos, Biochemistry Section, Environmental Health and Laboratory, DELH/CLS, California Department of Public Health, N. Nguyen, Biochemistry Section, Environmental Health and Laboratory, DELH/CLS, California Department of Public Health, J. DeGuzman, Biochemistry Section, Environmental Health and Laboratory, DELH/CLS, California Department of Public Health, K. Choe, Biochemistry Section, Environmental Health and Laboratory, DELH/CLS, California Department of Public Health, W. Song, Biochemistry Section, Environmental Health and Laboratory, DELH/CLS, California Department of Public Health, J. She, California Department of Health, Center for Laboratory Sciences

Inductively Coupled Plasma Mass Spectrometry (ICP-MS) provides sub-parts per trillion (ppt) detection capabilities for elemental analysis and when coupled with liquid chromatography it can be used in complex speciation studies. However, due to its high sensitivity, ICP-MS may not be most suitable for analyzing high-concentration samples without extensive sequential dilution prior to analysis. Injection of highly concentrated samples can necessitate substantial instrument cleanup before further use. In contrast, X-ray fluorescence (XRF) offers a non-destructive, rapid, and cost-effective method for elemental analysis at the parts per million (ppm) level. Furthermore, XRF can be utilized to identify and quantify elemental composition in samples suspected to have high concentrations, thus reducing the need for extensive sample preparation and mitigating the risk of contamination in ICP-MS analysis.
In this study, diverse non-housing samples were analyzed for their lead (Pb) concentration using both a portable XRF and a single quadrupole ICP-MS. Calibration of both instruments was conducted using industry-standard calibration standards. The ICP-MS was recalibrated daily, while XRF calibration was verified through triplicate measurements of a Pb standard prior to each use and recalibrated if the precision and accuracy of the daily calibration checks deviated beyond 100 ± 10%. The objectives of this study were to (i) establish a correlation between ICP-MS and XRF measurements of Pb at ppm-level concentrations in both solid and liquid matrices, and (ii) determine the reporting limit and method detection limit for Pb analysis in solid and liquid matrices using an XRF.
Our comparison of ICP-MS and XRF results revealed a strong correlation ($R^2 > 0.90$) for solid samples with Pb concentrations greater than 20 ppm. Pb concentrations below 20 ppm were either undetectable by XRF or resulted in a weak correlation ($R^2 < 0.2$) with ICP-MS results. Additionally, a small fraction (< 1%) of samples with Pb concentrations below 20 ppm, as measured by ICP-MS, exhibited significantly elevated false-positive Pb concentrations when analyzed by XRF, likely due to interference from other elements such as Fe, As, Cr, and Hg.
These findings demonstrate that a portable XRF can serve as a reliable instrument for analyzing Pb

content in non-housing samples suspected to have high concentrations, which minimizes risks of ICP-MS contamination. However, our study highlights the necessity of developing a standardized operating procedure and establishing an appropriate method detection limit for XRF analyses and address potential interferences to minimize false positives.

Presenter: Dinesh Adhikari, California Department of Public Health, Dinesh.adhikari@cdph.ca.gov

Poster 29

Isotope Dilution UPLC-ESI-MS/MS Method for the Determination of Urinary VOC Metabolites

P. Behniwal, California Department of Public Health, J. Gallardo, CDPH, J. DeGuzman, Biochemistry Section, Environmental Health and Laboratory, DELH/CLS, California Department of Public Health, S. patton, Biomonitoring Resource Center, Commonweal, J. She, California Department of Health, Center for Laboratory Sciences

Volatile organic compounds (VOCs) are a group of volatile chemicals originating from both natural and anthropogenic sources. Wildfires, cattle farms, tobacco smoke, paints, solvents, and gas-burning stoves are all examples of environmental sources and anthropogenic sources of VOCs. Exposure to VOCs occurs mainly through inhalation, ingestion, and skin contact. Long-term exposure to VOCs has been associated with asthma, birth defects, certain cancers, and damage to the central nervous system. Measurement of urinary VOC metabolites provides a relatively easy and non-invasive sampling benefit and data complementary to blood and serum measurements.
This method describes the urinary measurement of stable VOC mercapturic acid metabolites, formed with a high degree of specificity via the glutathione pathway, making them unique exposure biomarkers. We have adopted and modified a VOC measurement method (1) to simultaneously identify and quantify 25 urinary VOC metabolites of acrolein, acrylamide, acrylonitrile, benzene, 1-bromopropane, 1,3-butadiene, carbon-disulfide, ethylbenzene, ethylene oxide, isoprene, N,N-dimethylsulfide, propylene oxide, styrene, tetrachloroethylene, toluene, trichloroethylene, vinyl chloride and xylene in urine.
Analysis and quantitation are conducted using reversed phase liquid chromatography–negative electrospray ionization tandem mass spectrometry (LC–ESI-MS/MS) with isotope dilution. Method accuracy and precision was evaluated using standard reference and quality control materials, which demonstrated good accuracy, precision, and sensitivity for assessment of VOC exposure in a general population. The method was applied for the analysis of samples from a study of active-duty firefighters from California's 2018 Camp Fire, the Camp Fire Firefighter (CFF) study. Statistical analysis of results of the CFF study is underway and findings will be presented at the conference.
Reference:
1) K. Udeni Alwis, Benjamin C. Blount, April S. Britt, Dhrusti Patel. David L. Ashley, Simultaneous analysis of 28 urinary VOC metabolites using ultra high-performance liquid chromatography coupled with electrospray ionization tandem mass spectrometry (UPLC-ESI/MSMS), 2012, Analytica Chimica Acta, (750), 152 -160,

Presenter: Paramjit Behniwal, California Department of Public Health, paramjit.behniwal@cdph.ca.gov

Poster 30

Multizone Modeling of a K-5 School Building Using CONTAM: Integrated Ventilation and Air Cleaning Strategies for Wildfire Events

Y. Won, California Department of Public Health, K. Kumagai, California Department of Public Health, Z. Wang, California Department of Public Health, W. Chen, California Department of Public Health

Abstract: The frequency and intensity of extreme wildfires have doubled over the past twenty years due to climate change, posing significant health risks to vulnerable populations, including K-5 school students. Indoor air quality (IAQ) during wildfires is influenced by ventilation and filtration systems and their operational conditions. This study evaluates PM2.5 and CO2 concentrations under representative ventilation scenarios with portable air cleaners (PACs) in a school setting using a multizone model (CONTAM). The floorplan of a K-5 school building for which we had measured PM2.5 and CO2

concentrations before was used to set up the simulated environment. It includes one hallway and eight classrooms, each accommodating approximately 20 students. Each classroom had an air change rate of 12 h-1 (including air recirculation), with 18% outdoor air intake. After validating the model by comparing simulation results with the previous measurements (Chen et al., 2024) , 50 more scenarios with assumed varying ventilation conditions (mechanical vs. natural ventilations) and PAC operations were analyzed for a three-day wildfire event period with an average outdoor PM2.5 concentration of 30 µg/m3 for 8 a.m. to 4 p.m. Results show that CO2 concentrations can reach up to 2000 ± 1000 ppm when all outdoor air dampers and openings are closed. Under the mechanical ventilation condition, using a MERV 13 filter with an air change rate of 12 h-1 and 20% outdoor air can reduce concentrations to 700 ppm for CO2 and 5 µg/m3 (16.7% of outdoor levels) for PM2.5. Under natural ventilation with closed openings, PM2.5 concentrations can increase to 50% of outdoor levels within two hours, exposing students to high concentrations for the remainder of the class hour. PAC operation at air change rates of 2 h-1 and 5 h-1 can decrease PM2.5 concentrations to 7.5 µg/m3 (25% of outdoor levels) and 4 µg/m3 (13.3% of outdoor levels), respectively. These findings highlight the effective filtration strategies to mitigate wildfire-related IAQ impacts in schools.

Presenter: Youngbo Won, California Department of Public Health, youngbo.won@cdph.ca.gov

Poster 31
Radiological Emergency Preparedness at California Department of Public Health
L. Yang, California Department of Public Health, T. Shvareva-Piekarz, Center for Laboratory Sciences, California Department of Public Health

Radiochemistry Unit (RCU) at Drinking Water and Radiation Laboratory / Center for Laboratory Sciences is the only state government laboratory capable of performing radiochemical analysis in California - one of the largest states with two Nuclear Power Plants and many facilities using radioactive materials. During a radiological emergency, RCU will receive hundreds to thousands of samples with a short turnaround time to provide California public with information of surrounding radiation exposure levels. This poster overviews the development of RCU emergency response capabilities within the last three years.

In 2022 RCU, along with other state public health laboratories, participated in U.S. Environmental Protection Agency - organized Radiochemical Analytical preparedness full scale exercise, based on simulated Sr-90 water system contamination incident. The exercise involved full method development within 2 months, communication with stakeholders, sample analysis and data reporting, and had numerous swift emergencies injects. It served as an excellent learning platform for RCU emergency response efforts, leading to the development of several rapid testing methods, new data report protocol and overall improvement of laboratory coordination in response to various emergency scenarios.

In 2023 RCU joined Food Emergency Response network and successfully participated in two emergency exercises investigating the presence of gamma-emitting contaminants in food samples. The laboratory has built a procedure for authenticating unexpected radionuclides, assessing the extent of contamination, and reporting the results in short timeframe; and is ready to receive food samples during a radiological incident.

In 2024, as a member of California Nuclear Power Plant Emergency Response Program, RCU prepared and participated in Diablo Canyon Nuclear Power Plant Plum Phase and Ingestion Pathway drill. This exercise, involving many federal, state, and local jurisdiction partners, helped RCU to understand and assess the state of laboratory preparedness and communication skills and learn the ability to coordinate the response as a part of the state-wide team. U.S. Federal Emergency Management Agency auditors observed, evaluated, and provided valuable feedback on laboratory safety, sample receiving and accessioning, preparation, and testing protocols, as well as state of instruments and data processing.

In addition, RCU has initiated an effort to establish a clinical testing program, with urine as a matrix of choice. Sample collection, preparation, and testing protocols, as well as waste disposal program

development are underway.

In conclusion, over the last three years the RCU has learned many new emergency procedures and methods and gained a valuable experience allowing to advance the radiological emergency preparedness in California.

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Poster 32
Enhancing Antimicrobial Resistance Testing in California
A. Murshed, California Department of Public Health - Microbial Diseases Laboratory Branch, A. Murshed, California Department of Public Health Microbial Diseases Laboratory Branch, R. Azurin, California Department of Public Health Microbial Diseases Laboratory Branch, Z. Berrada, California Department of Public Health

The CDC established the Antimicrobial Resistance Laboratory Network (ARLN) in 2016 to address emerging antimicrobial-resistant pathogens, including carbapenemase-producing organisms (CPOs), Candida auris, and Neisseria gonorrhoea. These organisms can cause serious healthcare-associated infections (HAI) or sexually transmitted infections and are rapidly developing antimicrobial resistance (AR). Through Epidemiology and Laboratory Capacity (ELC) and Strengthening HAI/AR Program (SHARP) funding support, CDPH's Microbial Diseases Laboratory (MDL) and 14 local public health labs (LPHLs) in California have collaborated to increase test capacity for antimicrobial resistant (AR) pathogens in the state. This presentation summarizes the increased testing capacity for AR pathogens from August 2022 to July 2024 in California through the joint efforts of the California AR Laboratory Network, MDL's AR Coordinators, CDPH's HAI program, local health jurisdictions, West Regional ARLN in Washington (WA ARLN), and the CDC.

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Poster 33
Implementation and Validation of a HPLC-PCOX Method for the Determination of PSTs in Shellfish
T. Stepps, CDPH, T. Stepps, APHL, M. Benitez, CDPH, R. Ravi, CDPH, E. Chandrasena, CDPH, S. Ruberu, CDPH

Paralytic shellfish toxins (PSTs) are marine biotoxins that can induce paralytic shellfish poisoning (PSP). PSP is a deadly illness with 39 reported deaths in California since 1903. PSP toxins are produced by a specific type of single-celled algae that can be found in the marine environment year-round. Bivalve shellfish (such as mussels, oysters, and clams) filter phytoplankton, leading to the accumulation of PSP toxins. These toxins can then be transmitted to humans when contaminated shellfish are consumed. Paralytic shellfish toxins reversibly bind to receptors on the voltage gated sodium-channel, blocking neural transmission, resulting in motor and sensory irregularities. The large socio-economic impact of these naturally occurring biotoxins has led to extensive monitoring, testing, and the creation of new methods to identify lower concentrations of PSTs. Two methods approved by the Food and Drug Administration (FDA) for PSP testing in all species of shellfish are the Mouse Bioassay (MBA) and the High-Performance Liquid Chromatography (HPLC)-Post Column Oxidation (PCOX) with fluorescence detection. Currently, at the California Department of Public Health (CDPH) Center for Laboratory Sciences (CLS), PSP toxin monitoring in shellfish is performed using the MBA method. The goal of this project is to move from the MBA animal testing method to the HPLC-PCOX chemical testing method. There were various challenges when implementing this methodology, such as frequent instrument washing and maintenance requirements, retention time shifts for the C-toxins due to column equilibration, standards purchased in pairs, and a lack of negative mussel matrix. The limit of detection (LOD) and limit of qualification (LOQ) was calculated for GTX1 (Calibrator 1) and C-1 (Calibrator 5) which are specific types of PSTs in the saxitoxin family. The LOD for GTX1 Cal 1 was 0.020 ugSTXdiHCleq/100g and the LOQ was 0.061 ugSTXdiHCleq/100g. The LOD for C-1 Cal 5 was

0.0072 ugSTXdiHCleq/100g and the LOQ was 0.022 ugSTXdiHCleq/100g. The largest total toxicity by PCOX was determined to be 76.9 ugSTXdiHCleq/100g while the lowest total toxicity was 0.0 ugSTXdiHCleq/100g. The PCOX method was found to show higher accuracy than the MBA method which had highest total toxicity of 64 ugSTXdiHCleq/100g and the lowest total toxicity of 34 ugSTXdiHCleq/100g.

This presentation aims to discuss the challenges of implementing the PCOX method and the method verification processes conducted in the laboratory.

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Poster 34
Modeling the Potential Spread of Japanese Encephalitis Virus in California via Port Introductions
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Japanese encephalitis virus (JEV), the leading cause of viral encephalitis in many Asian countries, is a vector-borne virus transmitted by infected Culex spp. mosquitoes. As a member of the Flavivirus genus, alongside dengue and West Nile Virus (WNV), JEV infections are predominantly asymptomatic, though severe cases can result in rapid-onset fever, neurological complications, and even death. Transmissions intensify in warmer and rainy seasons, and diagnosis typically relies on serological testing of cerebrospinal fluid. As of October 30th, 2024, California has confirmed seven autochthonous dengue cases, a virus exotic to the state. This highlights the growing risk of introductions of other exotic arboviruses. California's extensive trade with Asia and the established presence of Culex spp. mosquitoes make the region highly vulnerable to JEV introduction. Heightening this risk, JEV's nonspecific symptoms and serological cross-reactivity with other flaviviruses may delay its diagnosis and impede timely control efforts. This study aims to estimate the transmission potential of JEV in California using a deterministic compartmental model that incorporates interactions between birds, mosquitoes, and humans. The model is validated against human WNV infection data from 2021-2023 and examines scenarios of delayed diagnosis, climate change effects, increasing pig populations, and port-based introductions. Preliminary results suggest that JEV could spread, and replicate WNV transmission dynamics observed in California, emphasizing the importance of proactive surveillance and control measures for potential JEV incursions.

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Poster 35
Genomic Sequencing of novel Arenaviruses: Witsand, Bobomene, Omdraaivlei and Mopeia Viruses
J. Arcilla, Centers for Disease Control and Prevention, A. Schuh, Center for Disease Control and Prevention, J. Montgomery, Centers for Disease Control and Prevention, S. Whitmer, Centers for Disease Control and Prevention

Arenaviridae are a family of negative-sense RNA viruses that infect Piscine, Mammalian, and Reptilian hosts. The Mammarenavirus genus predominantly infects rodent hosts and zoonotic spillover to humans can cause disease. The Mammarenavirus genome consists of a single S (small) and L (large) segment; the S segment encodes the viral nucleoprotein (NP) and glycoprotein precursors (GPC), whereas the L segment encodes the viral polymerase (L) and the RING-finger domain (Z). Mammarenavirus are split into Old World and New World serogroups, which are generally separated geographically into the Americas (New World) and Africa/Eurasia (Old World). The Old World serogroup includes Lymphocytic Choriomeningitis virus (LCMV), Lassa virus (LASV), Mopeia, Mobala,

and Ippy viruses, whereas the New World serogroup is further broken separated into clades A-D and include human pathogenic viruses, such as Junin, Machupo, Guanarito, Sabia and Chapare viruses. While much is known about New World Mammarenaviruses that cause human disease, little is known about Old World Mammarenaviruses and their distribution on the African continent. Here, we present 5 novel Arenavirus genomes that were sequenced from archived viral stocks, originally classified as "South African Arenaviruses." Viral isolates were deep-sequenced and complete genomes were built using de novo assembly.
Phylogenetic analysis identified that the 5 novel viral genomes separated into two distinct clades. Witsand (collected in north/central South Africa) and Omdraaivlei (collected in western South Africa) viruses are closely related to each other and most closely related to Okahandja and Bitu viruses collected in Namibia and Angola, respectively. Witsand, Okahandja, and Bitu viruses were originally collected from Micaelamys namaquensis, whereas Omdraaivlei virus was collected from Otomys unisulcatus. The three additional novel genomes, Bobomene (collected from the South Africa-Zimbabwe border) and Mopeia SPU 30/82/1 (collected from south/central Zimbabwe) and Mopeia SPU 491/84/73 (collected from the South Africa-Mozambique border) are closely related and share a most recent common ancestor with Mopeia viruses collected in Mozambique. The Mopeia viruses from Zimbabwe and South Africa were collected from Mastomys natalensis (these rodents also carry Lassa virus), whereas Bobomene virus was collected from Aethomys chrysophilus.
It is currently unknown whether the novel Arenaviruses Witsand, Omdraaivlei, Bobomeme and Mopeia cause human disease. With the additional complete genomes, pan-OW Arenavirus primer/probe sets will be developed to investigate whether additional novel Arenaviruses can be detected in rodents and humans that exhibit viral hemorrhagic fever disease.

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Poster 36
Evaluation of False-negative β-Lactamase qPCR Results in Carbapenemase-producing Organisms
B. Bertrand, Centers for Disease Control and Prevention, T. Kiryutina, Georgia Public Health Laboratory, I. Sawh, Georgia Public Health Laboratory, G. Tiga, Georgia Public Health Laboratory, L. Burns, Georgia Public Health Laboratory, A. LaCrue, Georgia Public Health Laboratory, T. Parrott, Georgia Public Health Laboratory, A. Ramaiah, Georgia Public Health Laboratory

Carbapenemase-producing organisms (CPOs) pose a significant public health threat because of their resistance to most antibiotics. Effective efforts to combat CPOs rely on detection initiatives, like the Centers for Disease Control and Prevention's (CDC) Antimicrobial Resistance Laboratory Network (AR Lab Network). As a part of AR Lab Network, Georgia Public Health Laboratory (GPHL) performs routine testing on bacterial isolates for the detection of CPOs. We identified discordant results between methods used to detect resistance in GPHL's CPO workflow, so we investigated these results to ensure quality laboratory testing. We compared results from CPO isolates reported by GPHL during January–October 2024 and analyzed real-time PCR amplification plots for signs of potential false-negatives. According to GPHL's CPO testing algorithm, bacterial isolates were initially tested for carbapenemase (CP) production using the modified carbapenem inactivation method (mCIM). CP-producing isolates were further tested for the presence of β-lactamase genes by Streck ARM-D β-lactamase real-time polymerase chain reaction (qPCR). Isolates positive for β-lactamase genes, such as the New Delhi Metallo-β-lactamase (NDM) gene, were further characterized by short-read Illumina whole genome sequencing (WGS) and analyzed using CDC's PHoeNIx pipeline for antimicrobial resistance gene detection. We identified isolates that had qPCR results discordant with either mCIM or WGS. Discordant results might be a result of multiple factors, including the presence of other carbapenemase genes not detected by this qPCR panel, false-negative qPCR results, serial subculturing, or other technical concerns. GPHL uses a fixed threshold based on a normalized fluorescence value of 40,000 to determine if gene targets were detected for the CPO qPCR assay. To determine if discrepancies were attributable to false negative qPCR results, we reanalyzed amplification plots for all isolates with discordant qPCR results to see if amplification existed below this threshold level. We tested 285 mCIM+ isolates by qPCR. Of these, β-lactamase genes were not

detected in 21 (7.4%) isolates; these were identified as Enterobacter cloacae complex (n=16), Serratia marcescens (2), Klebsiella aerogenes (1), Aeromonas hydrophila (1), and Klebsiella pneumoniae (1). Two mCIM+/qPCR- isolates had notable amplification of the blaNDM gene target that did not pass the normalized fluorescence threshold, indicating potential false negatives. WGS was performed on 100 CPOs during this time frame. Of these, six had discrepant results, three qPCR-WGS+ and three qPCR+WGS-. Two of the qPCR-WGS+ isolates showed late amplification of the blaNDM gene target by qPCR, which did not pass the standardized threshold limit set for this assay; they were determined to be potential false-negative qPCR results. This investigation revealed multiple discrepant results between CPO identification methods. Four potential false-negative CPO isolates (2 mCIM+qPCR- and 2 qPCR-WGS+) were identified by late amplification of the blaNDM gene target on qPCR below the standardized threshold limit. Although the set threshold is used for the current GPHL qPCR assay, we are exploring alternatives to decrease false negatives. Accurate gene detection is crucial for CPO surveillance, determining CPO sequencing priorities, and early outbreak detection.

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Poster 37
Repurposing Rapid Antigen Tests to Sequence Circulating SARS-CoV-2 in Milwaukee, Wisconsin, 2024–2026
D. Dang, Centers for Disease Control and Prevention

Background: To characterize local SARS-CoV-2 subvariants, the City of Milwaukee Health Department Laboratory (MHDL) will perform phylodynamic analysis on locally obtained samples. Phylodynamics integrates genomic and epidemiologic data to characterize viral transmission patterns, enabling MHDL to assess the associations of socioeconomic status, vaccination uptake, and racial disparities regarding SARS-CoV-2 transmission and variant diversity in Milwaukee. During peak pandemic years (2020–2023), samples primarily came from clinical testing. Because clinical testing has subsequently declined, MHDL is establishing a novel program to repurpose rapid antigen tests (RATs) to identify new sources of viral samples for variant surveillance.

Methods: During the 2024–2025 respiratory illness season, MHDL will distribute 5,000 SARS-CoV-2 RATs to Milwaukeeans free of charge. RAT kits will be strategically distributed at free health clinics, community centers, and senior housing facilities located throughout the city. Each RAT kit will include an instructional flyer and a quick-read (QR) code directing people to mail SARS-CoV-2-positive RAT cartridges to the laboratory. Participants will scan the QR code to record census block group and sample collection date to establish location and time metadata that will be used for further epidemiological analysis. No personal or other demographic data will be gathered. MHDL will use established viral extraction and sequencing protocols and metadata analysis tools to perform phylodynamic analysis on SARS-CoV-2 from these positive samples. To eliminate language barriers, MHDL will work with health department communication experts to effectively explain the study purpose and provide clear written instructions in English, Spanish, and Hmong. MHDL will also clearly communicate our confidentiality practices to gain trust with wary communities that have lower vaccination rates. Drop boxes and prepaid envelopes will be provided to cover mailing costs and reduce financial and time barriers to participation. Geographic distribution of returned samples will be closely monitored, and MHDL will modify distribution and communication strategies as needed. These methodologies will ensure inclusive participation and capture samples representing Milwaukee's racial and socioeconomic diversity.

Results: We anticipate that these strategies will provide hundreds of SARS-CoV-2 samples for sequencing and subsequent phylodynamic analysis. We also expect that our inclusive communication and outreach methods will increase participation from historically underrepresented areas of Milwaukee.

Conclusions: We will outline MHDL's strategies for effective implementation of our SARS-CoV-2 RAT repurposing program and describe lessons learned. Successful project execution would result in the collection and sequencing of positive RATs from a representative cohort of participants in Milwaukee.

We anticipate that the SARS-CoV-2 diversity and transmission data gathered from this project will help us to implement community-specific mitigations and messaging to prevent COVID-19 and improve health outcomes for all Milwaukeeans.

Presenter: Derek Dang, Centers for Disease Control and Prevention, xtl5@cdc.gov

Poster 38
Reassessment and Optimization of an LC-MS/MS Method for Detection of Chlorine Exposure Biomarkers in Preparation for an LRN-C Pilot Study
D. Davis, Centers for Disease Control and Prevention, T. Blake, Centers for Disease Control and Prevention

Chlorine is a widely distributed and highly produced toxic industrial chemical (TIC) with a history of synthetic utility and use as a chemical weapon. It is a public health concern due to its wide availability and the potential consequences of an accidental or intentional release. In the event of such an incident, it is critical to have the ability to detect and measure biomarkers indicative of acute chlorine exposure. Previously, our laboratory developed a clinical method using liquid chromatography tandem mass spectrometry (LC-MS/MS) which considered two known biomarkers for chlorine exposure, 3-chlorotyrosine (Cl-Tyr) and 3,5-dichlorotyrosine (Cl2-Tyr). It is important to note that Cl-Tyr can be found in samples from individuals with a variety of inflammatory diseases because of the endogenous enzyme myeloperoxidase and may also potentially be detected due to occupational or recreational exposures to chlorinating agents. Data from our analysis of surplus serum samples (n=1780) from the National Health and Nutrition Examination Survey (NHANES) 2015-2016 cycle has provided additional insight into the concentrations of both chlorinated tyrosine biomarkers and their baseline prevalence in the general population with Cl2-Tyr only being detected at low levels in 7/1780 samples tested. Our laboratory now aims to reassess and update our current method by incorporating the NHANES findings and improving the sample preparation and chromatography to optimize for detection of the more specific biomarker Cl2-Tyr. The updated method will also undergo ruggedization in preparation for a planned method transfer pilot study targeting three state public health laboratories from the Laboratory Response Network for Chemical Threats (LRN-C). Upon a successful completion of the pilot study, the method could be transferred more broadly to help build chlorine exposure testing capability across the network.

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Poster 39
Undetermined Outbreaks (UnO) Phase One: Developing Metagenomics to Detect Foodborne Illness Outbreaks of Undetermined Etiology Using Outbreaks of Known Etiology
A. Huang, Centers for Disease Control and Prevention, C. Cole, CDC/DDID/NCEZID/DFWED/EDLB, A. Huang, CDC/DDID/NCEZID/DFWED/EDLB

The national foodborne pathogen surveillance system, known as PulseNet, relies on bacterial isolates for genomic subtyping and must adapt to the declining availability of isolates due to the rise in culture-independent diagnostic tests (CIDTs). We are developing methods for metagenomic sequencing of stool samples to obtain pathogen subtyping data to perform direct-from-stool foodborne illness surveillance without a microbial isolation step. This represents an opportunity to solve Undetermined Outbreaks (UnOs), where no etiology is confirmed. UnOs comprise over 25% of foodborne disease outbreaks, a significant public health blind spot. Additionally, this can help us understand why the CIDTs used by clinical labs fail or show off-target reactions. Our UnO project involves developing a two-tiered pipeline to discover novel foodborne pathogens and changes to known pathogens. It also engages partner public health agencies and laboratories to help test and deploy these methods.

Development of the two-tiered UnO pipeline (formerly known as UNDIs) involves testing with sets of outbreak stool specimens, HMAS panel development, and bioinformatic pipeline development. The first tier involves screening outbreak samples with a targeted HMAS panel for identifying known and suspected diarrheal pathogens. If the UnO panel does not identify a diarrheal pathogen in common

across most of the outbreak specimens, we then proceed to the second tier where metagenomic shotgun sequencing is used with binning bioinformatics to identify novel pathogens or changed known pathogens. We are testing primers for the UnO known and suspected pathogen HMAS panel, and have developed a binning bioinformatics pipeline, called nf-UnO, in Nextflow for binning and analyzing shotgun metagenomic sequencing of diarrheal stools for pathogen discovery.

In Phase One of the UnO project, ten state public health laboratory and epidemiology partners are collecting stools specimens from diarrheal outbreaks of mostly known etiology. A collaboration with the Minnesota Department of Health and the Association of Public Health Laboratories, this phase provides sets of outbreak specimens to challenge our UnO pipeline, allowing us to “discover” the known agent. We describe results from a variety of foodborne illness outbreaks collected by Alabama, Colorado, Connecticut, and Utah. Finally, we provide a roadmap for future deployment of the UnO pipeline to partner public health agencies and laboratories.

Presenter: Andrew Huang, Centers for Disease Control and Prevention, wwm8@cdc.gov

Poster 40
Lessons Learned from a Chemical Safety Risk Assessment of Whole Genome Sequencing Workflow in a Public Health Laboratory
F. Knight, Centers for Disease Control and Prevention, T. Johnson, CDC, M. Keckler, CDC

Background: Safety risk assessments are a critical component of public health laboratory work. They ensure that the safety of laboratorians is prioritized, and that the quality of results meets regulatory standards. This is particularly important for testing performed under the Clinical Laboratory Improvement Amendments of 1988 (CLIA). Under CLIA, a breach of safety or quality could potentially necessitate halting clinical testing. This may result in patient harm if labs are not able to meet turnaround time requirements for reporting test results to medical providers. The safety risks for whole genome sequencing (WGS) are primarily chemical in nature, due to the reagents used for each workflow step and because bacterial DNA is not infectious. This abstract presents the results of our WGS safety risk assessment, as well as lessons learned from evaluating the sequencing needs of CDC’s Clinical and Environmental Microbiology Branch (CEMB).

Methods: Working within functional domains across CEMB (e.g., Genomics, Safety, Surveillance, Environmental Microbiology), we performed a holistic review of all WGS standard operating procedures (SOPs) and determined whether documentation should be updated or revised. Safety data sheets (SDSs) for chemical reagents used in our sequencing SOPs were reviewed to identify health and environmental hazards, and a risk mitigation plan was created to ensure safety of laboratory staff performing WGS. Finally, needs assessment meetings were conducted with personnel to discuss gaps in safety/training resources.

Results: Eight chemical hazards were identified during our SOP/SDS review, including formamide, a carcinogen and teratogen that can also cause organ toxicity in the liver, kidney, and blood. The primary potential exposure to this chemical occurs when staff discard chemical waste (~100-500 ml) from the sequencing cartridge after its removal from the instrument. We ensured proper waste disposal techniques and use of personal protective equipment were in place to minimize the risk of exposure to this chemical.

Our needs assessment resulted in a CEMB-wide effort to unify WGS SOPs and training resources. We developed harmonized sequencing SOPs using standardized language and formatting, allowing for better communication and collaboration between the teams performing WGS and eliminating redundancy in branch documentation. This effort also led to the creation of a workflow job-aid detailing all steps involved in our sequencing process, which will be used as a training and refresher tool going forward.

Finally, it was determined that CEMB should restructure organization of its electronic SDS documentation to improve ease of use—for example, by using filenames that are easy to interpret so

that the necessary safety documents can be located quickly in the case of a chemical exposure.

Conclusions: WGS is increasingly a major public health activity. The assessment described here resulted in several improvements to our laboratory work, including identification of chemical risks encountered in WGS, updated SOPs and trainings, new job-aid resources, and recommendations to streamline the chemical exposure response. Because laboratory research and diagnostics are always evolving, risk assessments must remain integral and continuous aspects of public health laboratory operations, ensuring both personnel and environmental safety and test result accuracy.

Presenter: Frances Knight, Centers for Disease Control and Prevention, uqo5@cdc.gov

Poster 41
Leveraging a Memorandum of Understanding (MOU) for Increasing Surge Testing Capacity When Responding to Public Health Threats
E. Lopez, Centers for Disease Control and Prevention, S. Courtney, CDC, J. Chaitram, Centers for Disease Control and Prevention

Introduction: In 2018, a Memorandum of Understanding (MOU) was signed by CDC, APHL, ACLA, and CSTE to better prepare for public health threats (PHTs) that require a high volume of laboratory diagnostic testing. While public health laboratories (PHLs) have the expertise to characterize infectious organisms, handle samples, and scale up routine operations, PHL systems are not designed to execute diagnostic testing at a large scale beyond the initial phases of public health emergencies. This MOU serves as framework for collaboration with public and private partners to increase laboratory surge testing capacity as well as nationwide access to testing for PHTs.
Methods: In response to the COVID-19 response, partners participated in monthly meetings to discuss response plans and addressed gaps in response efforts by establishing roles and responsibilities for responding to present and future PHTs and by improving coordination and communication efforts. Engaging partners early in the response helped maximize resources, disseminate standardized guidance, and ensured laboratories had access to appropriate testing supplies and relevant trainings. The partners also facilitated information sharing and technology transfer between CDC and laboratories to reduce start-up time. MOU membership is regularly assessed and expansion to additional partners is under continuous consideration.
Results: Through outreach the MOU membership has grown from 4 to now 12 member agencies and organizations that span the public-private landscape. The COVID-19 response leveraged existing public-private partnerships to coordinate diagnostic testing support nationwide and introduced the unprecedented use non-traditional testing and collection sites. This response highlighted the urgency of information sharing at an unprecedented level, and the MOU created a forum for efficient and effective information sharing to critical public and private testing partners at near real-time. Furthermore, use of the MOU contributed to an improved PHT response early in the 2022 Mpox response. CDC collaborated with MOU partners to identify commercial laboratories that could rapidly onboard testing to increase surge testing capacity and significantly improve nationwide access to testing within two months. And most recently, MOU partners contributed to the development of the National Response Testing Framework (NRTF), which aims to distinguish the roles and responsibilities of both public and private partners during all phases of a response.
Conclusion: Effective communication and coordination before, during, and after PHTs allows laboratories to share vital information and enhances readiness. The MOU provides a mechanism to leverage public-private partnerships to improve responses to PHTs and its goals have been effectively accomplished throughout multiple responses. These efforts enable broader engagement, incorporate lessons learned, and provide flexibility to address new and emerging challenges in response readiness. As we learn more from other responses, we will continue to review and improve the MOU.

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Poster 42
Building a Risk Assessment Tracker for Public Health Laboratories
M. Pitts, Centers for Disease Control and Prevention, N. Wiese, Centers for Disease Control and

Prevention, B. Freeman, Centers for Disease Control and Prevention, B. Mainou, Centers for Disease Control and Prevention

Background: The purpose of the risk assessment process is to ensure laboratory scientists consider the biological, chemical, physical, and radiologic risks posed by the work that will be performed. There are five essential components of risk assessments: risk identification, risk analysis, risk evaluation, risk mitigation and management, and monitoring and review. Tracking the completion, approval, and implementation of risk assessments is crucial for maintaining a safe work environment, while also ensuring compliance with regulatory requirements as a public health laboratory. To improve administration and workflows surrounding the development of risk assessments, we developed a Risk Assessment Tracker to organize and effectively manage various components of risk assessments from concept to completion.

Methods: To bridge the capacity for risk assessments, we developed a Microsoft 365 Teams List to manage and track the processing and review of risk assessments. Microsoft 365 Teams List allows users to create manual and automated lists to manage data, making it a powerful tool for project management. Microsoft 365 Teams List can handle various project management tasks including creating data columns and forms, sorting, grouping, filtering and creating visual representations of data, sharing files, and highlighting information while tracking changes over time.

A Microsoft Teams List was used as a key tool to manage and improve the workflow around risk assessments across our program of subject matter experts. Risk assessments were submitted via an intake form capturing key details including location, biosafety risk, and teams responsible for conducting the assessment. After submission, the assessments were systematically categorized and tracked based on attributes such as methods, procedures, and status (ongoing or completed). Completed risk assessments and related documents were attached for review, streamlining the review and management of this process within a single platform. Upon completion, a data dashboard offered visual analytics to evaluate the tool's effectiveness in managing and tracking the number of risk assessments completed, average completion time, distribution by category, and number of assessments per location or method.

Results: Our Risk Assessment Tracker enhanced the management of both completed and ongoing risk assessments by streamlining input, organization, categorization, sorting, and tracking. With real-time status updates, the tracker improved our ability to efficiently manage and monitor assessments in the Polio and Picornavirus Branch. Our Risk Assessment Tracker was used as a project management and data visualization tool to organize, manage, and monitor risk assessments to ensure operations meet regulatory requirements and maintain a safe and secure workplace. This tool demonstrates efficiency improvement in our risk assessment workflows, clear oversight of the risk assessments being managed, and valuable insights for leadership to make faster, informed decisions on where to focus efforts to optimize risk assessment workflow capacity.

Presenter: Mareena Pitts, Centers for Disease Control and Prevention, xtm3@cdc.gov

Poster 43

Assessing QuantiFERON-TB Gold Specimen Integrity During Courier Transport to the Laboratory in Nashville, Tennessee

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Introduction:
Public health laboratories maintain specimen integrity and ensure accurate, reliable test results by establishing preanalytical policies and procedures for specimen handling and transport. In July 2023, the Tennessee Department of Health, Division of Laboratory Services (TDHDLS) implemented multiple

changes as to how specimen temperatures are monitored and recorded at laboratory arrival to improve preanalytical system quality. Temperature monitoring has identified multiple specimens received outside their acceptable temperature range for testing. This problem has disproportionately affected samples for QuantiFERON-TB Gold (QFT) testing. During July 2023–July 2024, TDHDLS rejected approximately double the percentage of QFT specimens, compared with the previous year. Specimens arriving outside their required temperature ranges are rejected for testing, resulting in an undue burden on patients and submitters. To understand the role of specimen temperature during transport has in rejection of specimens, we assessed courier shipping conditions and identified steps to improve specimen integrity.
Methods:
We partnered with three health departments (HDs) to monitor QFT specimen temperatures throughout the shipping process, including at laboratory arrival. HDs were asked to place digital data loggers (DDLs) inside QFT specimen bags to collect longitudinal temperature data during courier shipments. DDLs record the temperature every five minutes. After specimen delivery, data were downloaded from DDLs and analyzed using Fridge-Tag 2L software. Transport logs were reviewed to align DDL data with shipping process steps.
Results:
All nine QFT specimen shipments monitored during October–November 2024 exceeded the laboratories' recommended shipping temperature range of 4°C–8°C during ≥1 step of the shipping process. In one shipment, QFT specimen temperatures exceeded the allowable range of 4°C–27°C for uncentrifuged QFT specimens. Specimen temperatures were recorded within range at laboratory arrival but had deviated outside the acceptable temperature range for three hours during transport. Three QFT shipments were packed in lockboxes with coolants according to protocol, whereas the other six lacked appropriate coolant. The temperature range of specimens packed in lockboxes without coolants (11°C–33°C) varied greater than specimens packed with coolants (5°C–8°C).
Conclusions:
We observed variability in the courier's shipping conditions across and within the three HD routes. Additionally, we observed differences in how specimens were packaged for courier transport. Monitoring specimen temperature during transport revealed deficiencies with the courier transport procedures that might have gone undetected if temperature was only checked at laboratory arrival. DDL data highlight temperature as a crucial factor to address in the laboratories' preanalytical system. These findings underscore the need for standardized packaging and shipping procedures to maintain specimen integrity. TDHDLS is developing and implementing standardized protocols to ensure QFT specimens remain within the 4°C–8°C range during each step of the shipping process. These efforts will ensure cold chain maintains specimen integrity and ensure accuracy of test results that TDHDLS provides to patients and their care teams.

Presenter: Mitchell Ramuta, Centers for Disease Control and Prevention, xtm5@cdc.gov

Poster 44
Detection and Localization of Influenza A and B RNA in Formalin-fixed, Paraffin-embedded Lung and Trachea Tissues by RT-PCR/In Situ Hybridization
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Background
Influenza viruses are a leading cause of respiratory infections globally, ranging from mild symptoms such as cough and fever to severe complications like pneumonia and myocarditis, which can be fatal. Seasonal influenza and influenza pandemics continue to pose significant public health challenges. Evaluation of tissues from fatal cases can provide important insight into disease pathogenesis and viral tissue tropisms and sites of viral replication. Additionally, tissue-based assays can be the valuable diagnostic tools, particularly for cases in which formalin-fixed, paraffin-embedded (FFPE) tissues are the only specimen type available. We developed two in situ hybridization (ISH) assays for influenza A and B viruses and compared them with other tissue-based diagnostic assays, including real time reverse transcription-polymerase chain reaction (rRT-PCR) and immunohistochemistry (IHC).

Methods
Two separate RNAscope-based ISH assays for the specific detection and localization of influenza A (H1N1 and H3N2) and B were designed and developed by the CDC's Infectious Diseases Pathology Branch (IDPB). FFPE lung and trachea autopsy tissues from 50 cases with clinical suspicion of influenza infection and positive by rRT-PCR were evaluated using the influenza A and B ISH assays. The cases were received in IDPB between 2009-2022 and age range of cases was from 1 to 60 years. ISH results were compared with the rRT-PCR and IHC and correlated with other patient data such as duration of illness, comorbidities (e.g., lupus, obesity) and bacterial coinfections.

Results
ISH detected influenza A in 16 of 19 (84.2%) influenza A rRT-PCR confirmed samples and 20 of 31 (64.5%) influenza B rRT-PCR confirmed samples, while IHC detected 10 of 19 (52.6%) influenza A rRT-PCR confirmed samples and 19 of 31 (61.3%) influenza B rRT-PCR confirmed samples. Viral RNA was localized by ISH in bronchial epithelial cells, bronchial gland, tracheal epithelium, alveolar pneumocytes and lymphoid tissues. The duration of illness prior to death ranged from 1 to 10 days for ISH positive cases and 1 to 5 days for IHC positive cases. Bacterial coinfection or comorbidity was present in 9 of 19 (47.3%) and 10 of 19 (52.6%) of influenza A cases, respectively, and in 14 of 31 (45.1%) and 8 of 31 (25.8%) of influenza B cases, respectively. In comparison to IHC, ISH assays more frequently detected influenza A and B viruses in tissues from cases with bacterial coinfection or comorbidity (bacterial coinfection - ISH: 77.8% for influenza A and 50% for influenza B, IHC: 77.8% for influenza A and 35.7% for influenza B; comorbidity- ISH: 70% for influenza A and 50% for influenza B, IHC: 50% for influenza A and 37.5% for influenza B). The rRT-PCR Ct ranges for influenza A were 16.6–30.0 for ISH and 16.9–29.6 for IHC while the rRT-PCR Ct ranges for influenza B IHC-positive samples were 19.8–32.3 for ISH and 23.3–32.2 for IHC.

Conclusion
These novel ISH assays can serve as a valuable tool for detecting and localizing influenza A and B viral RNA in FFPE tissues. Additionally, ISH is more sensitive then IHC for detection of influenza viruses in autopsy tissues, identified these viruses more frequently in cases with bacterial coinfections or co-morbidities, and cases with longer duration of illness. These findings suggest that application of tissue-based influenza A and B rRT-PCR and ISH assays may enhance sensitivity of viral detection, help to differentiate between influenza A and B viruses and provide valuable insights into pathogenesis.

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Poster 45
Preparing for PulseNet HMAS Implementation in 2026
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Culture-independent diagnostic tests (CIDTs) for foodborne diarrheal bacterial pathogens are a boon to the clinician, providing rapid, sensitive, and affordable testing for multiple pathogens using minimally processed primary specimens. However, these methods do not produce the isolates used by many surveillance programs, like PulseNet and NARMS, to detect potential outbreaks. As the availability of clinical isolates declines, the burden of isolation in support of surveillance is falling on public health labs (PHLs). To address this challenge, EDLB is developing highly multiplexed amplicon sequencing (HMAS) panels targeting regions of pathogen genomes important to surveillance in stool-derived DNA. Sequencing the amplicons from these panels will allow PHLs to obtain genomic subtyping data with a resolution like that of isolate whole genome sequencing.

Using this workflow, laboratorians extract DNA from CIDT-positive stool specimens directly or from stool enriched in a pathogen-specific broth, amplify subtyping data from the DNA using the appropriate

pathogen-specific HMAS panel, and sequence the resulting amplicons on the Illumina MiSeq. Each panel will amplify core genome multi-locus sequence typing (cgMLST) targets like those currently used in PulseNet-BioNumerics cgMLST subtyping schemes, serotyping targets, and virulence targets. A typical panel may include >2000 primer pairs; EDLB is using the Standard BioTools Juno and X9 platforms to execute panels currently. Data analysis is performed using the custom Nextflow pipeline Step-mothur, which will become available through the PulseNet 2.0 platform in the future.

This poster will provide an overview of data from the nine-state Salmonella HMAS subtyping panel pilot and the standardized protocol EDLB will release to all PulseNet participating labs in 2026. The Salmonella HMAS panel consists of 2461 cgMLST primer pairs designed from 19 reference genomes, 9 Salmonella genus-specific primer pairs, and 2 amplification control primer pairs. Additional serotyping markers are under development to be added to the panel.

To support PulseNet labs in strategizing for future implementation of the Salmonella HMAS panel, support materials will be available which detail laboratory space considerations as well as projected start-up and long-term costs. Current pilot participants will be present at the meeting to speak to their hands on experience, as well.

HMAS as a strategy for replacing isolate-dependent surveillance workflows has many potential benefits for PHLs and leverages existing infrastructure. Additional panel development for other PulseNet pathogens is planned, with STEC panel testing scheduled to begin in 2026.

Presenter: Amanda (Jo) Williams-Newkirk, Centers for Disease Control and Prevention, igy7@cdc.gov

Poster 46
Whole-Genome Sequencing of Influenza A and RSV from Wastewater Using Nanotrap Microbiome A Particles and NEBNext DNA Library Preparation
P. Acer, Ceres Nanosciences, L. Saunders, Ceres Nanosciences, G. Pricer, Ceres Nanosciences, P. Andersen, Ceres Nanosciences, B. Lepene, Ceres Nanosciences

Wastewater-based epidemiology (WBE) has gained prominence as a tool for tracking viral pathogens during the COVID-19 pandemic. Influenza A viruses, which periodically emerge from animal reservoirs, pose a continual threat to global health. Similarly, respiratory syncytial virus (RSV), a single-stranded RNA virus, has persisted as a leading cause of respiratory illness in infants and young children. Rapid genomic analysis of these viruses is essential for vaccine development, therapeutic strategies, and public health preparedness.
Each Influenza A virus contains eight RNA genome segments, and sequencing the entire genome is critical for understanding viral evolution and transmission. RSV, while not segmented, requires comprehensive genomic analysis to monitor strain variations, emergent subtypes (A and B), and vaccine or therapeutic evasion mutations. Detection and sequencing of these viruses from wastewater remain challenging due to low viral titers and matrix inhibitors, often resulting in incomplete genomes. Here, we applied the Nanotrap® Microbiome A Particle wastewater enrichment workflow to NEBNext® library prep protocols. Specifically, the NEBNextiiMS Influenza A DNA Library Prep and NEBNext RSV UltraExpress FS Library Prep protocols. The Nanotrap Microbiome A particles enriched Influenza A virus and RSV from contrived wastewater samples. Nucleic acids were extracted using the Monarch® Mag Viral DNA/RNA Extraction Kit in under 2 hours. RNA served as a template for targeted cDNA synthesis and amplification. NEBNext® Primers and LunaScript® Multiplex One-Step RT-PCR Kit generated amplicons, optimized for both RSV subtypes and Influenza A genome coverage. Sequencing was performed using Oxford Nanopore Technologies for Influenza A and Illumina platforms for RSV.
This method provides a same-day scalable, high-throughput solution for large-scale wastewater surveillance. By integrating Nanotrap Microbiome A Particles with targeted library preparation protocols, we developed an end-to-end workflow for whole-genome sequencing of Influenza A and RSV. This approach addresses key challenges in detecting these viruses, supporting real-time monitoring of viral transmission, evolution, and vaccine efficacy.

Presenter: Patrick Acer, Ceres Nanosciences, pacer@ceresnano.com

Poster 47
Enhanced Detection of Influenza A Virus in Milk Using Nanotrap® Microbiome A Particles
P. Acer, Ceres Nanosciences, D. Goldfarb, Ceres Nanosciences, L. Saunders, Ceres Nanosciences, S. Mosavi, Ceres Nanosciences, P. Andersen, Ceres Nanosciences, D. Gratalo, Gingko Biosecurity

Milk, particularly raw or low-pasteurized forms, has been identified as a potential vector for zoonotic influenza viruses, including highly pathogenic avian influenza A (H5N1) virus. This presents significant public health concerns, especially in regions where raw milk consumption is common. Current published methods use small sample volumes, which limits detection in cases where viral concentration is low. To address this challenge, we evaluated a novel workflow designed to improve the sensitivity and efficiency of influenza A virus detection in milk using Nanotrap® Microbiome Particles. H1N1 was selected as a proxy for H5N1 in this study, allowing us to optimize the method prior to validation on real-world samples.
The workflow captures and concentrates viral particles from 35 mL of milk using Nanotrap Microbiome A Particles, followed by nucleic acid extraction with the MagMAX Wastewater Ultra Kit on the KingFisher Apex system. This automated process is efficient, enabling the preparation, concentration, and extraction of up to 24 samples in just two hours. When compared to a published competitor method that uses only 25 µL of milk, the optimized workflow demonstrated a significant improvement in sensitivity, achieving a reduction of more than 6 Ct in qPCR threshold cycles. Sequencing data generated by Ginkgo Biosecurity confirmed the presence of H1N1 influenza virus in the contrived samples. Additionally, data showed high coverage and depth across all 8 gene segments of the H1N1 influenza A virus, validating the Nanotrap method's ability to preserve and detect viral genomic material and demonstrating Ginkgo Biosecurity's methods for sequencing and characterizing genomic data. This study establishes a reliable methodology for detecting influenza A virus in milk, with strong potential for application to native H5N1-positive samples. The method's automation and sensitivity not only minimize hands-on time but also enhance throughput, making it well-suited for large-scale monitoring programs. By offering a cost-effective, scalable solution, this workflow addresses critical gaps in public health surveillance and provides a promising tool for mitigating the risks associated with influenza viruses in milk.

Presenter: Patrick Acer, Ceres Nanosciences, pacer@ceresnano.com

Poster 48
A Scalable Method to Concentrate and Culture *Candida auris* from Wastewater Using Nanotrap Microbiome Particles
D. Goldfarb, Ceres Nanosciences, L. Saunders, Ceres Nanosciences

The adoption of wastewater-based epidemiology (WBE) for monitoring pathogens has rapidly expanded worldwide, driven by strong evidence linking WBE and clinical case trends. The success of WBE for monitoring of SARS-CoV-2 prevalence in specific regions has led to interest in monitoring additional microorganisms, including yeast, such as Candida auris (C. auris). Transmission of Candida species is oftentimes linked to physical contact with an infected host or through direct contact with a contaminated surface particularly in hospitals and healthcare facilities. C. auris has also been observed to contain genes which confer antimicrobial resistance (AMR), leading to further need for broad surveillance of infectivity rates due to the limited treatment options available. Detecting these microbes also presents challenges; they contain stronger cell structures and are difficult to lyse using common nucleic acid extraction workflows, and their concentrations in wastewater is often very low due to infection control measures in healthcare settings. Culture-based methods have proven effective for detecting and characterizing C. auris in wastewater, improving the ability to study its infectivity at the community level.

This study presents a wastewater testing method that utilizes Nanotrap® Microbiome A and B Particles to capture and concentrate C. auris prior to culture. Nanotrap Microbiome A and B Particles were added to 10 mL and 35 mL wastewater samples that were contrived with C. auris to concentrate the

yeast, then added to 2 mL of S2 Media salt Sabouraud dulcitol broth, containing 8 µg/mL fluconazole to inhibit other Candida species from growing. A protocol for culture of C. auris from wastewater published by Rossi et al. in 2023 was then followed to successfully enrich and culture the concentrated microbes on solid phase media. Briefly, the Nanotrap Particle suspensions were enriched at 42°C for 5 days, agitating at 250 rpm. Following this period, the suspensions were separated, and 1 µL of the supernatant was plated on HardyCHROM Candida medium. Plates were further incubated and observed after 48 hours. Colonies were removed and resuspended in 1x PBS, then nucleic acids were extracted using the NucleoMag DNA/RNA Water Extraction Kit (MACHEREY NAGEL; REF 744220.1). Presence of C. auris was confirmed through digital PCR on the QIAcuity instrument with the GT-Digital C. auris Wastewater Surveillance Assay Kit (GT Molecular; Cat# 100355).

The Nanotrap Particle method was capable of concentrating C. auris for enrichment using the established culture method, and colonies were individually extracted and detected using a specific primer and probe set. Both 10 mL and 35 mL wastewater samples were successfully cultured after concentration, allowing larger sample volumes to be used in the event of low concentrations of C. auris within the wastewater. The use of centrifugation or Nanotrap Particles for concentration prior to culture were compared through plating studies, showcasing similar colony formation between the two methods across multiple sample dilutions, ranging down to 1:1,000 of the initial C. auris pathogen load. The Nanotrap Particle workflow is also scalable, as it is compatible with the KingFisher™ Automation System, offering a higher throughput sample concentration process, which enables more individual samples to be tested within a single workflow.

Presenter: Daniel Goldfarb, Ceres Nanosciences, dgoldfarb@Ceresnano.com

Poster 49
High-throughput Mpox Clade Ib Detection in Wastewater
L. Saunders, Ceres Nanosciences, D. Goldfarb, Ceres Nanosciences, P. Acer, Ceres Nanosciences, D. Gratalo, Gingko Biosecurity

The adoption of wastewater-based epidemiology (WBE) for monitoring viral pathogens has rapidly expanded worldwide, driven by strong evidence linking WBE and clinical case trends. WBE measures aggregate disease prevalence in a sewer shed, independent of individual health care behaviors or clinical symptom presentations. Mpox virus is an emerging threat to global health, and is comprised of two distinct viral clades, named clade I and II. Each clade has individual subclade variants, which exhibit different levels of virulence. Transmission of mpox is often linked to close physical contact, including intimate contact, with an infected human, or through direct contact with infected animals or contaminated materials. Outbreaks of mpox have occurred since 2022, commonly characterized within clade II, but the recent evolution to the clade Ib variant has led to the WHO declaration that mpox is a Public Health Emergency of International Concern. Existing surveillance assays are capable of detecting Mpox clade Ia and II, but these measures fail to identify the clade Ib variant, leading to a need for an expanded panel to ensure rapid detection of the pathogen so proper response can be taken by public health officials.
An end-to-end sample processing workflow was developed to meet these needs, with aims to provide a turnkey solution for detection of mpox clade Ib within wastewater samples. First, grab or composite samples of raw wastewater, as well as composite aircraft wastewater samples, are collected. Nanotrap Microbiome A Particles are then employed to concentrate the mpox virus from these samples, and an extraction kit is used to purify nucleic acids for downstream detection. This workflow can be performed manually or automated. With the extracted sample, Gingko Biosecurity's proprietary MPOX Pan-clade I assay as well as the CDC MPOX clade Ia and II assay are used to perform dPCR on the QIAcuity instrument to detect viral presence. Following positive detection, sequencing is performed using either Illumina enrichment sequencing or amplicon sequencing performed on Illumina or Oxford Nanopore Technology platforms This allows confirmation and further characterization of mpox subtypes within the initial wastewater sample.
Through use of these methods, organizations can establish a high throughput workflow for detection and analysis of mpox clade Ib. The method can be optimized for multiple environments including low resource settings where manual sample processing is required. The Nanotrap Particle technology is

capable of being utilized with a wide range of wastewater sample matrices, allowing for efficient concentration of target microbes. Ginkgo Biosecurity's dPCR and sequencing methods have been highly optimized to improve sensitivity for low abundance
pathogens in wastewater and can be combined with Ginkgo's bioinformatics expertise for a complete data solution. Nanotrap Particles integrated with Ginkgo's novel assay and bioinformatics approach offer a solution for rapid detection of new public health threats.

Presenter: Lauren Saunders, Ceres Nanosciences, lsaunders@ceresnano.com

Poster 50

Wastewater Surveillance During a Large-scale multi-Day National Special Security Event

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Chicago hosted the 2024 Democratic National Convention (DNC) from August 19-22, 2024. Given the event's national significance, threat potential, and numerous dignitaries' attendance, it was designated a National Special Security Event. The Chicago Department of Public Health (CDPH) provided public health surveillance for the event within the Public Health Emergency Operations Center (PHEOC); CDPH consolidated and summarized multiple public health surveillance systems to provide actionable intelligence for local, state, and federal partners.

Wastewater concentration levels were monitored before, during, and after the DNC to supplement traditional public health surveillance data (e.g., hospital visits). If an unexpected increase in a pathogen's concentration level were seen, further epidemiologic investigation would be initiated to corroborate the signal, assess the context of the elevated level, and evaluate the potential need for public health action.

Using Chicago's existing wastewater surveillance (WS) program, we expanded surveillance to three enteric pathogens (norovirus, Salmonella spp., and Shiga-toxin-producing E. coli) given their association with previous outbreaks at mass gathering events. We added two sampling sites which included effluent from the two primary DNC venues but were outside of the security parameter.

To establish a pre-event comparison period, samples were collected twice weekly for a month leading up to the DNC; daily the week before and then during the DNC; and twice weekly for two weeks following the DNC. All WS samples were collected passively using Moore swabs. Pathogens were quantified using dPCR.

We received WS data within 12-24 hours of collection. The data were normalized to PMMoV, cleaned using R (version 4.3), and uploaded into CDPH's internal DNC data dashboard. WS data from the event were compared to pre-event WS data alongside other real-time surveillance data systems, including medical aid station reports, 311 complaints, and ER visits tagged as DNC-related.

During the four-day event, CDPH found no concerning trends or signals from WS that suggested a public health issue of concern. These findings align with other syndromic surveillance data systems. We detected an increase in salmonella concentration levels at one DNC site, which increased from the start to the end of the event before dropping back to pre-DNC levels on the day after the event concluded. Subsequent epidemiologic investigation of the salmonella signal involved a review of contemporaneous data from the other data systems; we found no evidence of an increase in gastrointestinal illness in our clinical surveillance systems through ED visits and syndromic surveillance system.

CDPH demonstrated that incorporating WS as a supplemental surveillance tool is feasible during large-scale events. With the same-day analysis, epidemiologists could quickly assess and report to partners. Additionally, incorporating three enteric pathogens and two new venue-associated sampling sites accomplished and improved the mass-event utility of the WS data. Limitations include the short timeframe of the event, i.e., attendees might have departed before becoming sick, the inability to fully isolate DNC venues for WS due to the security parameter, and Moore Swab saturation time. WS proved to be a valuable complement to traditional surveillance data sources, providing insights into areas that may have gone undetected otherwise.

Presenter: Dorothy Foulkes, Chicago Department of Public Health, dorothy.foulkes@cityofchicago.org

Poster 51

Investigation of an Increase in *Salmonella* in Wastewater During the 2024 Democratic National Convention

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Background

For the 2024 Democratic National Convention (DNC) (8/19-22), the Chicago Department of Public Health (CDPH) established enhanced surveillance activities to facilitate rapid detection and response to potential public health threats. We leveraged multiple surveillance data, including expanded wastewater surveillance (WS) for gastrointestinal (GI) pathogens. During the DNC, WS detected a sharp increase in Salmonella at a sampling site downstream of a primary DNC venue.

Methods

Enhanced WS was conducted from August 5 to September 6, 2024, including PCR-quantification of multiple pathogen biomarkers in WS which were analyzed alongside traditional clinical surveillance data. WS samples were collected from 2 distinct “DNC-associated” sewersheds, which captured wastewater from the 2 primary DNC venues. Using 24h passive collection, WS samples were collected twice weekly leading up to the DNC (7/2–8/11) to establish a run-in comparison period; daily the week before and the week of the DNC (8/12–23) and twice weekly for two weeks following the DNC (8/24–9/6). Nucleic acids were extracted, and Salmonella was quantified by digital PCR using primers targeting the invA gene. WS data were returned to CDPH within 12-24 hours of sampling.

Following identification of the invA spike, additional investigation included assessment of non-WS data for GI signals, selective culture and isolate whole genome sequencing (WGS) to characterize Salmonella serotypes, assessment of DNC food preparation practices for possible contamination, and consultation with local wildlife experts.

Results

From 8/17 to 8/22, the population-normalized concentration of the Salmonella invA gene increased by >500% in 1 of the DNC-associated sewersheds, returning to pre-DNC levels on the day after the DNC ended (8/23). The spike was not evident in any other Chicago sewershed—including the other DNC-associated sewershed—nor did data from other surveillance sources corroborate the increase. During the DNC analysis period, ED visits for GI symptoms remained below pre-DNC levels, and no medical aid stations reported activity suggesting an enteric disease outbreak. Investigation of food preparation practices at the venue in question and local wildlife revealed nothing suggestive of possible Salmonella contamination.

All samples collected from the 2 DNC-associated sewersheds (n=25) were cultured; only the 5 samples associated with the observed invA spike grew Salmonella isolates, identified by MALDI-TOF mass spectrometry. WGS identified all 5 isolates as serotype Isangi. All isolates were closely related (1-3 SNP differences), and no closely related references were identified in NCBI Pathogen Detection.

Discussion
A large and sustained increase of Salmonella in wastewater was observed during the DNC near a primary event venue. Epidemiologic investigations were unable to confirm the source of the increase. No additional details were available in clinical data, food preparation investigations, or insight from wildlife colleagues. These isolates fall within a rare serotype, and we found no closely related specimens reported in NCBI Pathogen Detection, so potential sources for the isolates are unknown.

While WS detected a pathogen signal absent from other surveillance sources, follow-up epidemiologic investigation found no corroborating signal which deterred unnecessary public health intervention. WS can complement traditional data surveillance systems during large scale events.

Presenter: Samantha Smith, Chicago Department of Public Health, samantha.smith@cityofchicago.org

Poster 52
Robust Performance and Diverse Applications of the Clear Dx Fully Automated Genomics Solution
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The Clear Dx fully automated, cloud-integrated next generation sequencing (NGS) solution has revolutionize the way clinical and public health laboratories approach genomic and pathogen surveillance. Its ease of use with a single human touch point has enabled users to generate consistent and reliable results. The Clear Dx Microbial Surveillance application in particular has been pivotal in providing timely whole genome sequences of foodborne pathogens to PulseNet network for surveillance purposes. This application has also proven to be extremely versatile, enabling users to characterize healthcare-associated infections (HAI), antimicrobial resistance (AMR) profiles and help resolve disease transmission pathways. To date, more than 10,000 samples have been sequenced by the system with great success. Multiple additional use cases have also been added to its capabilities, such as sequencing of flu, mpox, norovirus and HIV amplicons. With the recent launch of Microbial ID tNGS application, the utility of the Clear Dx platform expanded multiple folds, continuously supporting and meeting the needs of public health laboratories in the United States.

Presenter: Justin Ng, Clear Labs, Inc., justin.ng@clearlabs.com

Poster 53
Utilizing a Common LIMS Framework for Both Environmental and Clinical Divisions Within Public Health Organizations
D. Crossett, Clinisys, L. Cummings, Clinisys, C. Stover, Clinisys, M. Cauthen, Clinisys, D. Robinson, Clinisys

Public health organizations handle a wide variety of testing needs, ranging from clinical assessments of individual health within a population to environmental testing that examines the impact of the environment on public health. In public health, "clinical" work involves directly interacting with individuals and populations to assess health needs, deliver interventions, and manage diseases. On the other hand, "environmental" work focuses on identifying and mitigating health risks related to factors such as air and water pollution, toxic exposures, and unsafe living conditions. Traditionally, due to these varying needs, public health laboratories conducting clinical and environmental testing have been forced to use separate information management systems due to differing terminologies and

regulatory concerns around housing patient data. This poster addresses the potential of utilizing a common underlying LIMS framework to meet the needs of both groups, thereby minimizing IT overhead and reducing reliance on disparate technologies.

Presenter: Donald Crossett, Clinisys, donald.crossett@clinisys.com

Poster 54
Enhancing NCBI Pathogen Detection Cluster Surveillance with NBCI Cluster Tracker
S. Baird, Colorado Department of Public Health and Environment

NCBI Pathogen Detection is a centralized system that clusters related bacterial and fungal pathogen genome sequences in the NCBI database to help identify possible links between cases in the context of outbreak investigations. The automated nature of NCBI Pathogen Detection makes it a useful tool for background cluster surveillance, but it can be challenging to keep track of a large number of isolates and their associated clusters over time. We developed a Python command-line tool, ncbi-cluster-tracker (github.com/CDPHE-bioinformatics/ncbi-cluster-tracker), to extend the functionality of the existing NCBI Pathogen Detection web interface by creating additional data aggregations and visualizations to enhance cluster surveillance.

Given a list of BioSample IDs for isolates of interest (referred to as “internal isolates”), ncbi-cluster-tracker downloads data about the isolates and their related clusters from Pathogen Detection’s BigQuery data warehouse and file server. In addition to BioSample IDs, users can optionally provide additional metadata for internal isolates like specific collection dates and alternate sample IDs that may not be published in the public NCBI records. ncbi-cluster-tracker then outputs a standalone, offline HTML report that can be securely shared through email or a shared drive. The HTML report is organized into three main tabs. The “Clusters” tab contains a filterable and exportable table of the clusters and their isolate counts, along with a Gantt chart showing the timelines of all of the clusters. The “Isolates” tab contains a table where users can find information about a particular isolate. The “Cluster details” tab allows users to search detailed information about a particular cluster, including a URL linking to the SNP cluster tree on the Pathogen Detection website, configured to highlight internal isolates by default. This tab also displays a pairwise SNP distance matrix and histogram of isolate counts over time, two new visualizations not currently available on the Pathogen Detection web interface.

While we developed ncbi-cluster-tracker for cluster tracking needs within the Colorado Department of Public Health and Environment, we designed the tool to be easily adoptable by any user, with the only requirements being access to an environment that can run Python and a Google Cloud Platform account (which is needed to access the public “pdbrowser” BigQuery dataset provided by NCBI). We hope that other public health professionals will find ncbi-cluster-tracker to be a useful addition to their bioinformatics and genomic epidemiology toolkit.

Presenter: Samuel Baird, Colorado Department of Public Health and Environment, sam.baird@state.co.us

Poster 55
Enhancing SARS-CoV-2 S-Gene Sequencing Coverage Using S-gene Specific Spike-in Primers
K. Castro-Vital, Colorado Department of Public Health and Environment, D. Mallal, Colorado Department of Public Health and Environment, S. Baird, Colorado Department of Public Health and Environment, A. Rossheim, Colorado Department of Public Health and Environment, S. Matzinger, Colorado Department of Public Health and Environment

The monitoring of SARS-CoV-2 Spike gene (S-gene) diversity is a vital part in monitoring vaccine effectiveness and therefore a major public health concern when it comes to surveillance for this virus. Popular tiled SARS-CoV-2 amplicon approaches such as ARTIC, are effective at sequencing a majority of the SARS-CoV-2 genome but continue to be plagued by primer dropouts caused by mutation accumulation in primer binding regions, as the virus evolves. This study describes the comparison of

three approaches in the hopes of capturing the majority of the SARS-CoV genome, while ensuring 100% S-gene coverage. This study consisted a comparison of the a CDC developed two amplicon approach to capture 100% of the S-gene region, the CDC two amplicon Spike gene approach combined with the ARTIC 5.3.2 tiled amplicon scheme to capture 100% of the S-gene and as much of the rest of the SARS-CoV-2 genome as possible, and the ARTIC 5.3.2 tiled amplicon scheme alone for comparison.

Three different primer combinations were used to amplify a subset of already sequenced SARS-CoV-2 samples consisting of lineages circulating in early 2025. Ct values were determined using the TaqPath COVID-19 Combo Kit run on the 7500 Fast Dx ABI. The samples were amplified using the Artic V5.3.2 panel and with custom CDC two amplicon S-gene primers spiked into the Artic 5.3.2 panel. After amplification both sets underwent bead cleanup and high-throughput sequencing using ONT DNA prep and the GridION platform.

Following sequencing, a comparative analysis was conducted. The results in this study will contribute to the ongoing efforts of SARS-CoV-2 surveillance through maximizing S-gene coverage to inform public health response, and specific SARS-CoV-2 vaccine reformulation.

Presenter: Kevin Castro-Vital, Colorado Department of Public Health and Environment, kevin.castrovital@state.co.us

Poster 56

Deconvolution of Influenza Viral Variants in Wastewater Samples Using the Viral Lineage Quantification (VQL) Pipeline and Freyja

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Background.
Wastewater-based next generation sequencing epidemiological surveillance has emerged as a powerful tool over the last 4 years. Although wastewater sequencing surveillance methods are relatively well established for SARS-CoV-2, work to expand wastewater sequencing surveillance to additional pathogens is ongoing. Here, we focus our efforts on exploring currently available methods and bioinformatic tools for performing deconvolution of Influenza viral variants in wastewater. Deconvolution is the process of identifying and estimating the relative abundance of viral variants in a mixed sample, which can be technically challenging. Because the genome of Influenza is segmented, which adds extra complexity, we focus our surveillance on the hemagglutinin (HA) gene segment. The HA gene is a highly variable region that encodes the receptor binding protein and is important for vaccine development and vaccine escape.

Methods.
We performed sequencing on both contrived mixed samples that were spiked with known concentrations of Influenza viral variant RNA and real-time wastewater samples that were positive for Influenza collected from sites within our sentinel wastewater surveillance network. We used a tiled amplicon based-sequencing approach using previously developed primer schemes that capture the HA gene segment for seasonal Influenza A H1N1 and H3N2 and Influenza B Victoria, respectively. We performed analysis using two available deconvolution methods: the VLQ pipeline as described in Baaijens et al. 2021 and Freyja as described in Karthikeyan et. al. 2022. For deconvolution, the VQL pipeline uses an algorithm (Kallisto) originally developed for quantifying RNA-seq data and a customized database of representative reference genomes, whereas Freyja uses a regression based method and a maintained barcode matrix of variant defining mutations.

Results.
For the VQL pipeline, we curated a custom database that included 88, 119, and 60 Influenza A H1N1 and H3N2, and Influenza B Victoria representative HA sequences which included all Influenza clades

present since 2018, respectively. For mixed contrived samples, all expected lineages were predicted in ratios that matched the spiked in concentrations. For Freyja we used the curated barcodes provided by the Freyja developers. Freyja's results for mixed contrived samples were similar to the VQL pipeline.

Discussion.
Both the VQL pipeline and Freyja are promising tools for the deconvolution of Influenza HA gene segment viral variants. Both pipelines required upfront work to apply them to the deconvolution of Influenza viral variants, but both yielded similar results. Deconvolution allows for the sequence characterization and abundance estimation of viral variants in wastewater at the community level which adds an additional tool to traditional sentinel surveillance based on sequence characterization of clinical samples.

Baaijens JA. et. al. Variant abundance estimation for SARS-CoV-2 in wastewater using RNA-Seq quantification. medRxiv [Preprint]. 2021 Sep 2.

Karthikeyan, S. et al. Wastewater sequencing reveals early cryptic SARS-CoV-2 variant transmission. Nature 609, 101–108 (2022).

Presenter: Molly Hetherington-Rauth, Colorado Department of Public Health and Environment, mchetheringtonrauth@gmail.com

Poster 57
Comparison of Mpox Subtyping Assays in Wastewater Samples by dPCR
G. Lequia, Colorado Department of Public Health and Environment, J. Romero, CO Dept of Public Health and Environment, N. Pysnack, CO Dept of Public Health and Environment, A. Rossheim, Colorado Department of Public Health and Environment, S. Matzinger, CO Dept of Public Health and Environment

Monkeypox virus (Mpox) is a zoonotic virus that causes smallpox-like illness in humans. Mpox exists in two genetic clades: Clade I and Clade II, which differ in geographic region and severity of illness. Increased global transmission of Mpox has occurred over the past few years, including an ongoing outbreak of Clade II that has been prevalent since 2022, and a more recent Clade 1b outbreak with a confirmed case in the United States as of January 2025. Clade I has been associated with more severe disease and as such it is important to be able to identify which clade an infected person has contracted. Since Mpox is shed via bodily fluids such as saliva, urine, and feces wastewater surveillance testing can serve as a cost effective and community level surveillance system to monitor the spread, providing early detection of infected people and helping identify potential clusters of infection before they escalate.
The Colorado Department of Public Health and Environment (CDPHE) Laboratory has been conducting routine testing of wastewater samples for MPXV Clade II and non-variola orthopox via digital PCR (dPCR) using a GT Molecular assay kit since early 2024. As Clade I is becoming more prevalent, detection of Clade I specifically has become public health impactful. CDPHE conducted a comparison study between two multiplex dPCR protocols for the detection of non-variola orthopox (NVO), Mpox Clade II, and Mpox Clade I. Test samples consisted of raw wastewater samples known to be negative for Mpox Clade I and II virus either spiked or left unspiked with positive Mpox Clade I or Clade II material and extracted on the Thermo Scientific KingFisher Flex using the MagMAX Wastewater Ultra Nucleic Acid Isolation Kit. Wastewater samples were then run on digital PCR (dPCR) on the Qiagen QiAcuity Eight. Analysis of this comparison provides results for the optimization of an in-house assay for Mpox subtyping in wastewater samples.

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Poster 58
Validation of Group A *Streptococcus* Emm Typing PCR Using Standard Biotools Biomark x9
D. Mallal, Colorado Department of Public Health and Environment, A. Rossheim, Colorado Department

of Public Health and Environment, S. Matzinger, Colorado Department of Public Health and Environment

Group A Streptococcus (GAS) is a bacteria which can cause severe invasive disease. The emm typing of GAS is highly related to virulence. CDC has developed a sequential quadriplex real-time PCR strategy to identify the 20 most common emm types among invasive GAS as a rapid and cost-effective method of determining emm typing. This assay has the standard limitations of traditional real-time PCR having limited fluorescent channels, requiring up to 5 quadriplex reactions to screen for all 20 emm types.
Standard Biotools Biomark X9 is a microfluidic thermocycler capable of performing real-time PCR on up to 96 targets and 96 samples simultaneously. Here, we validated the primers from the CDC emm typing assay on the X9 as a way to screen all 20 emm typing targets and a control S. pyogenes specific target (SPY) at once.
The X9 is set up to detect in the FAM channel of fluorescence so we redesigned the probes from the CDC emm typing assay to all have the FAM fluorophore. CDC provided us with a set of 32 isolates with emm types that have been confirmed by whole genome sequencing, as well as a plasmid which contains all 20 emm genes targeted in this assay in order to perform this validation. Isolates were extracted using the Qiagen DNEasy blood and tissue kit. We used Standard Biotools 48.48 IFC (up to 48 samples and 48 targets) and genotyping protocol to confirm the emm types of the 32 isolates, as well as perform a limit of detection for the plasmid. Inter and Intra run replicates were performed in order to assess reproducibility.
The emm types for all isolates were correctly detected in all replicates of this validation showing high reproducibility and accuracy. For isolates that were confirmed as an emm type that was not one of the 20 included in this assay, they correctly showed no amplification for any target other than the SPY gene demonstrating the high specificity of the assay.
The X9 is a viable platform for multiplexing emm typing PCR on a single run. This PCR was originally designed to be ran sequentially in order to detect 20 of the most common emm types, however the X9 is able to consolidate these 5 quadriplex reactions into a single plate, saving both time and reagents. Furthermore, with the X9 it would be possible to expand the number of targets beyond these 20, with the ability to multiplex up to 96 targets at once.
References:
Velusamy S, Jordak K, Kupor M, Chochua S, McGee L, Beall B. 2021. Sequential quadriplex real-time PCR for identifying 20 common emm types of group A Streptococcus. J Clin Microbiol 59:e01764-20. https://doi.org/10.1128/JCM.01764-20.

Presenter: Daniel Mallal, Colorado Department of Public Health and Environment, daniel.mallal@state.co.us

Poster 59
Olivar Tiled Amplicon Sequencing for Influenza Viruses' HA Gene Segment and Its Targeted Domains
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Background: Wastewater is a complex matrix that likely contributes to the fragmentation of DNA and RNA, making sequencing of fragile viral RNA genomes challenging. Sequencing of the whole genome of Influenza A viruses is achieved using an amplicon approach targeted at the universal ends of the viral gene segments and works well in clinical specimens. Given the fragmentation of viral RNA in wastewater a targeted tiled amplicon approach may be more effective at characterizing regions of the Influenza A virus in wastewater. We previously developed a tiled amplicon primer scheme for capturing the complete hemagglutinin (HA) gene segment which encodes the receptor binding protein. The receptor binding domain is a highly variable region known to accumulate mutations. Studies performing deep mutational scanning across the receptor binding domain (e.g. Dadonaite et al. 2024) are able to predict mutations in genomic "hot spots" that, if they were to occur, could result in increased host

receptor binding, immune evasion and potentially novel host ranges. Targeted sequencing approaches aimed at capturing these predicted regions, may provide greater sensitivity and prove more useful for wastewater surveillance of mutational “hot spots”, when compared to targeted whole genome approaches. We compared our previously developed HA tiled amplicon approach for capturing the complete HA gene segment to a targeted sequencing of individual amplicons covering “hot spot” regions in contrived and real-time wastewater samples to assess sensitivity.

Methods: Previous work described using the Olivar tool developed by the Treangen Lab at Rice University, tiled amplicon primers were designed for each of the main circulating strains of influenza: influenza virus A H1, influenza virus A H3, and influenza virus B Victoria. In this work, specific primers from these tiled amplicon schemes covering regions of concern in the Influenza A virus HA gene were tested in parallel with the previously described tiled amplicon approaches on contrived and real-time Influenza A virus positive wastewater samples.

Results: Comparison of sequencing depth and coverage of tiled amplicon HA gene and single target amplicon sequencing of HA regions of interest on contrived and wastewater samples demonstrates increased sensitivity when performing more targeted approaches.

Conclusions: This work describes the ability to sequence smaller regions of the HA genome with more consistency and efficiency when a more targeted approach is applied. The ability to increase the likelihood of detection of known regions of interest in the Influenza A HA gene can be applied to additional gene segments within the Influenza A virus genome and other pathogens, and help to utilize wastewater as an efficient population level surveillance tool to respond to specific public health concerns for these pathogens.

Dadonaite et al. 2024. Deep mutation scanning of H5 hemagglutinin to inform influenza virus surveillance. bioRxiv preprint.

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Poster 60

Leveraging Cloud to Automate Sequencing Instrument Data Ingestion and Preparation

D. Polanco, Colorado Department of Public Health and Environment, M. Hetherington-Rauth, Colorado Department of Public Health and Environment, L. Bankers, Colorado Department of Public Health and Environment, S. Matzinger, Colorado Department of Public Health and Environment

Background: Pathogen genomic surveillance demands efficient data processing. Traditional “on-prem” methods often struggle with large datasets and computationally intensive analyses leading to delays in public health response. Cloud-based solutions are capable of significant improvements in speed, scalability, and reliability. We use Google Cloud Platform (GCP) services to automate data ingestion and preparation, accelerating analysis and enhancing our Genomic Surveillance Program's effectiveness against public health threats.

Methodology: Illumina and Oxford Nanopore Technologies (ONT) sequencing platforms generate FASTQ files. Illumina data, initially stored in BaseSpace, are accessed via their CLI on a Cloud Run job, scheduled by Cloud Scheduler, and transferred to a Cloud Storage "instruments" bucket. ONT data is directly ingested from the instrument using a scheduled Docker container (cronjob) with the GCP CLI, landing in a Cloud Storage "inbox" bucket. A Cloud Run service, triggered by Pub/Sub messages, prepares these FASTQ files for analysis. Cloud Monitoring and Cloud Logging track service performance and errors. Basic file size and data freshness validations are implemented prior to downstream processing.

Results: Our automated data ingestion pipeline, currently undergoing testing, is designed to significantly enhance our genomic surveillance capacity. Leveraging Cloud Run and Cloud Scheduler, the system is projected to increase our max daily data ingestion by at least five-fold, handling

anticipated future growth. Automated validation routines, implemented to ensure data integrity, are demonstrating a reduction in processing errors compared to our current manual methods. Full system deployment is anticipated by the end of the year, enabling rapid response to future public health emergencies.

Conclusions: This project demonstrates the feasibility and benefits of leveraging cloud-based solutions for automated pathogen genomic surveillance data ingestion. The automated pipeline significantly improves data standardization, reduces human error, and mitigates the risk of data loss compared to manual methods. While initial development costs (time, design, training) were incurred, the long-term cost savings and improved efficiency will outweigh these investments. Future enhancements will focus on integrating more robust error handling, exploring multi-threaded processing languages for further optimization, and expanding the system to encompass additional data sources and analytical tools. The robust security and data isolation features of the cloud environment further enhance data protection and compliance.

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Poster 61
Implementing CDC Sendout ETOR Orders and Results in Horizon LIMS
C. Stark, Colorado Department of Public Health and Environment, J. Nucci, Colorado Dept of Public Health and Environment

The Colorado State Public Health Laboratory sends samples to CDC laboratories for several types of samples, including organisms of public health concern. Previously, in order for the results to be delivered to the ordering body, Lab staff would submit paperwork and retrieve the results manually, as well as manually transmit them to our clients. Implementing HL7 ordering and results allows the Lab to bypass these steps and streamline the process, saving time on all ends and reducing the possibility of human error.

HL7 messages were developed in two phases: results and orders. Because many CDC units are already set up for electronic result delivery, a single prototype process could be developed with additional testing and results added and validated over time. Test and analyte codes are mapped into OBR/OBX fields in HL7 messages and, when received, are placed into the appropriate samples directly. The results are then directly reported to the client. This allows those CDC units which have implemented electronic results delivery to report quickly and accurately, while preserving the current process for those units which are not yet set up in this fashion. Ordering follows a similar process to our current Horizon LIMS CDC Sendout process, with the addition of a single line with a dropdown menu entitled “Test Ordered”. Mapping the currently available tests for ordering allows Lab staff to bypass entering the same information into ELIMS while preserving the ability to see status and results in that system.

Presenter: Corina Stark, Colorado Department of Public Health and Environment, corina.stark@state.co.us

Poster 62
Applying One Health Principles at a Local Public Health Laboratory
J. Breher, County of San Diego Health and Human Services Agency Public Health Laboratory, J. Corrigan, County of San Diego, E. Trumbull, County of San Diego

Per the Centers for Disease Control and Prevention (CDC), One Health is a collaborative, multisectoral, and transdisciplinary approach — working at the local, regional, state, national, and global levels — with the goal of achieving optimal health outcomes recognizing the interconnection between people, animals, plants, and their shared environment. A One Health approach is needed now more than ever due to our changing interactions with animals, plants, and our environment (e.g., population growth and expansion into new geographic areas, climate change and land use, and

increased cross-border movement of people, animals, and animal products). The County of San Diego (COSD) identified this need for a local One Health approach, saw an opportunity to improve the health of humans, animals, and the environment, and in May of 2023, launched the COSD One Health Epidemiology Program (OHEP).

OHEP consists of a cross-sectoral group of subject matter experts that includes epidemiologists, physicians, veterinarians, nurses, laboratorians, and other support personnel. A network of connectivity with federal, state, academic, commercial, and local partners has been created with relationship-building and communication key features of the program.

The four primary goals of OHEP are to: 1) enhance disease outbreak management (e.g., with robust animal specimen testing capability), 2) to develop a centralized data hub consisting of local human, animal, and environmental disease information, 3) conduct surveillance for spillover risk management, and 4) provide education and outreach to our local partners and beyond.

One successful example of OHEP's efforts is the COSD Public Health Laboratory's influenza A virus (IAV) testing program. Through collaboration with a commercial veterinary diagnostic laboratory, specimens from mammals submitted for rabies testing, local wildlife, as well as other sources are tested for IAV via PCR with a turn-around-time of < 7 days. The results are communicated to the COSD Epidemiology Unit, and California Department of Public Health (CDPH) and CDC guidelines are followed for monitoring exposed humans for clinical signs and testing. Non-negative specimens get forwarded to the USDA's National Veterinary Services Laboratory for confirmation, subtyping, sequencing, and uploading into the national database. Results are also shared with state partners such as California Department of Food and Agriculture (CDFA), California Department of Fish and Wildlife (CDFW) and CDPH.

The COSD OHEP is a recently developed initiative that employs a One Health approach to create connectivity and communication across sectors. The COSD PHL specifically engages in zoonotic disease testing in animals and database management to support this program. Scrutinizing the human-animal interface and rapidly identifying potential spillover events will create an opportunity for enhanced surveillance of humans who come in contact with zoonotic disease carrying animals.

Presenter: Julie Breher, County of San Diego Health and Human Services Agency Public Health Laboratory, julie.breher@sdcounty.ca.gov

Poster 63

Bioinformatic Approaches for Streamlined Analysis of Viral NGS Data in a Public Health Laboratory Setting

F. Kabir, Dallas County Health and Human Service, E. Plaisance, Dallas County Health and Human Services, J. Stringer, Dallas County Health and Human Services, L. Short, Dallas County Public Health Laboratory, F. Kabir, Dallas County Health and Human Services

Introduction: The field of viral genome sequencing has been experiencing unprecedented surge with emerging, and recurrent virus transmission and continuous evolution in the post pandemic era. Newly identified strains of known viruses are constantly being subjected to sequencing and analysis by rapidly developed next generation sequencing (NGS) techniques and Bioinformatic frameworks. Extracting meaningful sequencing information from pathogen genomes continues to pose challenges and conventional methods for data comparison and verification may require adaptations or modifications through suitable analytical workflows or established pipelines. We aim to consolidate our Bioinformatic approaches for viral pathogens by comparing sequencing data using multiple analysis platforms.
Methods: Illumina whole viral genome sequencing based on targeted protocols such as amplicon-based and hybrid capture, were routinely carried out to generate the raw sequencing data. Three different viral Fastq data obtained from SARS-CoV-2, MPXV and HIV pathogens were exported from MiSeq to perform quality control (FastQC), and assembly steps on in-house and commercial platforms including, TerraBio and CLC WB.
Results: We sequenced >100 samples for each viral pathogen and performed data analysis using relevant amplicon-based or hybrid capture workflows from individual analysis platforms. We cross-compared the quality metrics resulted from each analysis and determined the consistency of the result derived from several critical features including cleaned reads, depth of coverage, percent reference genome coverage, assembly length, assembly consensus fasta, percent host DNA contamination,

detected variants or mutations in target organisms. We also correlated these sequencing read-outs with specimen status attributed with Ct values, viral load or viral absolute quantitation in digital PCR assay.
Conclusions: Our Bioinformatic analysis of viral sequencing data establishes robust workflows streamlined with comparative approaches to ensure data QC and validate results in a limited PHL setting. The results and insights derived from the sequencing data will be instrumental for our public health outbreak investigators and clinicians.

Presenter: Farruk Kabir, Dallas County Health and Human Service, farruk.kabir@dallascounty.org

Poster 64
Empowering Public Health: Transforming Dallas County Labs Through AI Innovation
K. Cirrincione, Dallas County Health and Human Services, A. Portman, Dallas County, D. Silva, Dallas County Public Health Laboratory, L. Short, Dallas County Public Health Laboratory

Artificial Intelligence (AI) is revolutionizing public health laboratories by streamlining workflows and enhancing efficiency. At Dallas County Health and Human Services, generative AI tools are accelerating the creation of regulatory-compliant documents, improving training programs, and uncovering advanced data insights. By automating routine processes, AI enables staff to dedicate more time to complex, high-value work, improving both productivity and accuracy.
Current applications focus on leveraging generative AI for content creation, data analysis, and operational support. Future potential includes advanced lab automation and predictive analytics to further optimize workflows and preempt disruptions. While these tools transform traditional practices, they require thoughtful oversight to address ethical considerations, maintain data security, and mitigate limitations such as reliance on precise prompts and potential inaccuracies.
Through strategic implementation, AI complements human expertise, empowering public health laboratories to adapt to evolving demands while preserving operational integrity and advancing public health goals.

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Poster 65
Development of a Novel Multiplex PCR Assay for Rapid Diagnosis of Mpox, Syphilis and Herpes Infections
D. da Silva, Dallas County Health and Human Services, E. Plaisance, Dallas County Health and Human Services, F. Kabir, Dallas County Health and Human Services, J. Stringer, Dallas County Health and Human Services, L. Short, Dallas County Public Health Laboratory

Introduction: Mpox is a significant emerging public health concern, with an increasing number of community transmission cases reported in the US. The virus spreads through close contact with infected individuals such as exposure during intercourse, often leading to the development of genital lesions. This community spread coincides with a rise in sexually transmitted infections (STIs), further complicating diagnostics. In Dallas County Health and Human Services (DCHHS), the syphilis positivity rate stands at 29%, based on samples collected from 2022-2023, with an incidence of primary and secondary syphilis rising by 52% from 2016 to 2020 in the US. Meanwhile, herpes remains one of the most common STIs in the US, with an estimated 12% of the population aged 14-59 infected. Given the overlap in lesion presentations between Mpox and other STIs like syphilis and herpes, a multiplex PCR (MPCR) assay capable of diagnosing these infections simultaneously could streamline testing protocols, enabling a quicker, more efficient response to public health needs while providing accurate, timely results.
Methods: The protocol was evaluated using the TaqPath™ BactoPure™ Microbial Detection Master Mix (ThermoFisher A52702) for its high specificity and sensitivity for microbial DNA detection, the CDC primers/probe sets for the Mpox and extraction control detection, and the primers/probe sets for HSV1/2, and Treponema pallidum detection previously reported by the DCHHS. DNA was extracted from samples using the QIAamp DSP DNA Mini Kit (QIAGEN 61304) from UTM and dry swabs. Over

50 samples were tested, including at least 30 dry swabs and 20 UTM. The protocol was tested on an ABI 7500 fast (ThermoFisher).
Results: The protocol demonstrated good efficiency (> 90%) for all fluorescent markers. Both Mpox and herpes samples showed 100% agreement with previous results performed by the laboratory. The MPXV data was further verified by NGS assay and confirmed for the detection of MPXV clade I and II. Treponema pallidum was also detected on samples that tested negative for rapid plasma reagin test, demonstrating that the assay developed can detect the presence of the bacteria on the lesions prior to a host humoral immune response.
Conclusion: The development of a multiplex assay for the detection of Mpox, herpes, and syphilis can improve testing efficiency, particularly for cases with atypical presentations. It enables the simultaneous identification of potential pathogens in cases involving genital lesions associated with Mpox. This assay offers significant practical benefits for public health laboratories by reducing testing time and resources required for diagnosis. As there are no commercially available NAT for syphilis to compare against the results from the developed multiplex assay, this protocol offers a novel approach for detecting active Treponema pallidum bacteria at the infection site, providing a more rapid diagnosis.

Presenter: Danilo da Silva, Dallas County Health and Human Services, drsilva90@outlook.com

Poster 66
Next Generation Sequencing and Drug-resistant (DR) Mutation Analysis of HIV Specimens with Moderate Viral Load (VL)
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Introduction: The persistent global public health challenges posed by HIV/AIDS warrants substantial efforts and strategies to prevent and limit the ongoing HIV epidemic. Due to its extremely high genetic variability and rapid evolution, the HIV genome can evade conventional laboratory detection, which impedes disease surveillance and targeted antiviral treatment and result in exacerbating outbreak risk. The consequent prevalence of DR HIV strains needs to be monitored as Pre-exposure Prophylaxis (PrEP) is scaled up with changing HIV transmission dynamics. We aim to develop a streamlined HIV next-generation sequencing (NGS) workflow with DR mutation analysis on the most efficient and scalable protocol. This method could be valuable for genomic surveillance, clinical assessment, and also extended to other sexually transmitted pathogens.
Methods: HIV RNA NAAT (Aptima HIV Quant Dx) positive and Antigen-Antibody (Bioplex 2200 Geenius HIV Ag/Ab assay) reactive serum samples were collected from DCHHS patients. Viral RNA was extracted using an automated magnetic bead-based protocol (MagMax Viral Pathogen Kit, Kingfisher DuoPrime). A custom digital PCR (dPCR) assay targeting gag/pol regions was performed to screen sample's viral load (VL) using QIAcuity One 5plex. HIV library preparation was performed using targeted enrichment hybrid-capture and an amplicon-based protocols and with final sequencing step on Illumina MiSeq. The Bioinformatic analyses were performed by assembling fastq data on Illumina DRAGEN apps, CLC Genomics Workbench 23.0.5, and Stanford HIV Drug Resistance Database (HIVDB) tool.
Results: Nearly 150 HIV patient samples, the majority Ag/Ab reactive, were analyzed for assessing viral copies and sequencing depth or reference coverage. Using dPCR assay we determined >100 copies/uL as optimal viral titer for successful analysis of over 100 patient samples. We also sequenced reference materials (SeraCare/LGC) as QC controls to validate HIV-1 subtypes A, B, C, D, and HIV-2 A/B, including a HIV-1 B recombinant construct harboring 49 DR mutations. Sequencing QC was assessed based on individual run metrics and parameters: >85% Q30, >90% PF and optimal cluster density (~1000) for both protocols. Patient-derived samples were verified for their read counts of >1 million, >85% genome mapped with respective HIV genotyping and subtyping. Both sequencing protocols indicated a standard sequencing depth (>20) in samples with viral titer of ≥100 copies/uL. We identified DR mutations in patient samples. These DR mutations were analyzed by HIVDB analysis tool

and were found across HIV gag and pol genes in the HIV genome conferring resistance to PI, NRTI, NNRTI and INSTI related groups of antiviral drugs.
Conclusion: Our overall findings provide a robust lab-validated NGS protocol and a scope for HIV genome sequencing and DR analysis for specimens with moderate viral load. This sequencing approach may strengthen public health surveillance of HIV infection, reveal novel variants, and identify outbreaks, potentially leading to the identification of index cases. The next phase of our future study involves the analysis of DR mutations in HIV samples from individuals on PrEP, as well as providing supportive information in the management and treatment of HIV patients.

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Poster 67
Laboratory Response Network First Responder Training in Preparation for the 2026 FIFA World Cup in Dallas, Texas
A. Marfo, Dallas County Health and Human Services, A. Portman, Dallas County, D. Kuret, Dallas County Health and Human Services, M. Williams, Dallas County, K. Cirrincione, Dallas County, J. Stringer, Dallas County Health and Human Services

Dallas, Texas, population 1.3 million, will host matches for the 2026 FIFA World Cup. In anticipation of thousands of attendees, the Dallas LRN has partnered with Biowatch and key first responders—including the FBI, Hazmat teams, and Postal Inspectors—to bolster emergency preparedness. To address potential risks posed by large-scale events, this year's annual First Responder Training highlights Phase 1 sample collection following a Biowatch Actionable Result (BAR) declaration. The training curriculum covers aseptic sample collection, personal protective equipment usage, accurate labeling, robust chain-of-custody documentation, and specialized exercises on donning, doffing, and glow germ contamination assessment. We expect to train approximately 100 first responders—from the FBI, Hazmat teams, and Postal Inspectors—in correct sample collection, packaging, and shipping protocols for the upcoming 2026 FIFA World Cup in Dallas. This inaugural LRN First Responder Training for a global event sets a precedent for future emergency preparedness initiatives, ensuring swift, coordinated sample collection and rapid testing

Presenter: Agnes Marfo, Dallas County Health and Human Services, agnes.marfo@dallascounty.org

Poster 68
Evaluation of a dPCR Assay Using Nanoplates with 8.5k and 26k Partitions for HIV Samples with Moderate Viral Load
E. Plaisance, Dallas County Health and Human Services - Public Health Laboratory, J. Stringer, Dallas County Health and Human Services, L. Short, Dallas County Public Health Laboratory, F. Kabir, Dallas County Health and Human Services

Introduction: Due to high genetic variability and rapid evolution, the HIV genome can evade conventional laboratory detection, making it difficult to screen for Next-Generation Sequencing (NGS) workflows. We aim to evaluate digital PCR (dPCR) assays that may allow minimal template input for screening absolute quantification of genome copies for NGS downstream applications. This assay could confirm initial screenings of samples tested by qualitative Ag-Ab reactive assays and NAT that could be correlated with sequencing data.

Methods: HIV samples were originally screened by Panther RNA NAT Aptima HIV Quant Dx and Bioplex 2200 and Geenius HIV Antigen/Antibody assays collected from Dallas County Health and Human Services (DCHHS) patients. Viral RNA was extracted using the MagMax Viral Pathogen Kit on the Kingfisher DuoPrime into a 50ul elution volume. Aliquots from same eluates were assessed on the QIAcuity One, 5plex with 3uL and 10uL template volume to compare the 8.5k nanoplate (12uL total reaction volume) and 26k nanoplate (40uL total reaction volume) respectively. Current dPCR assay targets the pol/gag regions of HIV to confirm presence of the viral genome and eligibility for library preparation and sequencing steps. Samples with concentration of >30cp/uL proceeded to sequencing and were correlated to sequencing data output, for determining a threshold required for optimal data

analysis.

Results: Nearly 100 HIV patient samples were compared with both 8.5k and 26k partitioned nanoplates to determine optimal reaction and template volume for conserving reagents and extracted materials. We obtained comparable dPCR data for the respective sample volumes used in both partition formats. Our sequencing data showed >100 copies/uL from the dPCR assay as the required viral titer to successfully analyze patient samples with higher reads, consensus sequences, and >85% genomic positions mapped.

Conclusion: Our overall findings provide a transition to ideal dPCR assay conditions that will allow conservation of template material and produce quality assessment of concentration for NGS workflows. This dPCR assay may be modified and more reliable with added regions of the HIV genome (ENV/LTR) to improve detection limit of where mutations may be occurring relative to our sequencing output. Our updated dPCR approach will allow more concrete screening of HIV specimens for NGS workflows to better inform clinicians and surveillance teams in managing HIV transmission dynamics.

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Poster 69

Streamlining Laboratory Processes: The Impact of LIS Modernization on Turnaround Times at DCHHS-PHL

M. Shabazz, Dallas County Health and Human Services, Public Health Laboratory, V. Amadi, Public Health Laboratory Dallas County Health and Human Services, D. Silva, Dallas County Public Health Laboratory, F. Kabir, Dallas County Health and Human Services, J. Stringer, Dallas County Health and Human Services, L. Short, Dallas County Public Health Laboratory

Introduction:
Prior to June 2023, the DCHHS-PHL struggled with inefficiencies in processing over 200,000 samples annually to serve a population of 2.6 million. Delays in manual workflows, including 1–2 hours of data entry during the pre-analytical phase, a 24-hour administrative lag between the analytical and post-analytical phases, and 2–4 hours daily for faxing and mailing test reports, led to an average turnaround time (TAT) of five days for positive specimens and three days for negatives. The National public health standards recommend a TAT of 48 hours for positive specimens, highlighting the significant gap in DCHHS-PHL's processing capabilities. To ensure reliability, the laboratory reported a conservative seven-day TAT, which posed challenges to timely patient care and public health responses. These inefficiencies underscored the need for laboratory information system (LIS) modernization to streamline operations, reduce delays, and enhance result reporting.

Methods:
To address inefficiencies and improve TATs, DCHHS-PHL implemented a comprehensive LIS modernization initiative, deploying over 100 test codes and algorithms across five laboratory units. The initiative aimed at automating workflows, reducing redundancies, and enhancing data accuracy and speed through the following strategies: Pre-Analytical Phase: A client-side web portal was used to allow for the direct entry of patient demographic information. Analytical Phase: HL7 integration was implemented to establish communication between laboratory instruments and the LIS. Post-Analytical Phase: Batch review capabilities were utilized to enhance technical reviews, enabling simultaneous validation of related results.

Results:
The client-side web portal accelerated data entry to under 30 seconds per sample, streamlining pre-analytical workflows. HL7 integration automated data transfer for 60% of test volume, reducing redundancies and ensuring seamless operations. In the analytical phase, HL7 integration between laboratory instruments and the LIS automated test result uploads. It eliminated two hours of manual data entry per batch, doubling efficiency and minimizing

transcription errors. Post-analytical enhancements, including batch reviews and electronic result delivery, reduced processing time from 31 hours to less than one hour, representing a 98% improvement. These optimizations improved average TATs to two days for positive specimens and less than one day for negatives, enabling faster diagnoses and timely public health responses.

Conclusion:
This is the first report on optimizing operations through improved TATs at DCHHS-PHL. It demonstrates the transformative potential of LIS modernization in public health diagnostics. Workflow enhancements reduced processing times from 31 hours to less than one hour, improving average TATs to two days for positives and less than one day for negatives. These advancements directly support timely diagnoses and enhance public health responses. The project offers a scalable model for laboratories seeking to improve efficiency, data accuracy, and patient outcomes through LIS modernization.

Presenter: Makayla Shabazz, Dallas County Health and Human Services, Public Health Laboratory, makayla.shabazz@dallascounty.org

Poster 70
Implementing and Monitoring Targeted Interventions to Reduce Unsatisfactory Specimen Rejection Rates in a Public Health Laboratory
S. Borna, Dallas County Public Health Laboratory, D. Silva, Dallas County Public Health Laboratory, S. Borna, Dallas County Public Health Laboratory, E. Kirkscey, Dallas County Public Health Laboratory, L. Brown, Dallas County Public Health Laboratory, R. Watkins, Dallas County Public Health Laboratory, L. Short, Dallas County Public Health Laboratory

Background:High rejection rates of unsatisfactory specimens hinder patient care, delay diagnoses, and strain laboratory resources. In public health laboratories, where timely and accurate results are essential for disease surveillance, data-driven prioritization is critical for addressing challenges in the pre-analytical process. A review of our 1.76% rejection rate identified two primary areas for intervention: sample quality and specimen stability. Lipemia accounted for 51% of rejected specimens, suggesting either a higher prevalence among our patients or unclear criteria at both bench and clinician levels. Client batching practices and accessioning bottlenecks further delayed specimen processing, compromising stability and testing timelines.

Objective: To reduce specimen rejection rates below 1% by tackling primary quality and stability concerns, ensuring operational efficiency, and enabling accurate, timely testing for public health surveillance.

Methods:

Our Laboratory Information Management System (LIMS) enabled real-time monitoring of rejection rates, providing immediate feedback to staff and clients. Data collected through the LIMS informed continuous process adjustments and ensured timely resolution of emerging issues. Three key interventions were implemented to reduce unsatisfactory specimen rejection rates:

1. Client Communication Campaign: A multi-pronged campaign clarified specimen collection and submission guidelines, identified recurring issues, and conducted targeted site visits to improve client compliance.

2. Workflow Optimization for HIV Testing: Internal workflows were revised to prioritize blood specimen processing, minimizing delays during accessioning and sample handling. Specific adjustments included implementing continuous loading practices and reducing batching.

3. Lipemia Criteria Standardization: Manufacturer-defined quantitative thresholds were converted to a qualitative benchmark to standardize lipemia acceptability, reducing unnecessary rejections.

Results:

The unsatisfactory rate for Whole Blood and Serum specimens decreased from 1.76% in Q4 2023 to 0.10% in Q4 2024. Updated lipemia criteria significantly reduced rejection rates, while enhanced client communication improved compliance with collection and submission standards. However, positivity rates for HIV Antibody & Antigen and Syphilis Total Antibody tests declined after lipemia criteria modifications. The HIV positivity rate dropped from 1.87% (N = 252/13,252) to 1.47% (N = 150/10,070) ($\chi^2 = 5.543$, $p = 0.0186$), and the Syphilis positivity rate declined from 21.6% (N = 2855/13,214) to 18.7% (N = 1846/9859) ($\chi^2 = 33.325$, $p = 0.001$).

Conclusions:

This initiative demonstrated that targeted interventions, including client education, workflow optimization, and standardized acceptance criteria, can significantly reduce unsatisfactory specimen rejection rates and enhance laboratory efficiency. The observed decline in test positivity rates highlights the need for ongoing monitoring and evaluation to ensure interventions remain appropriate. Additionally, fluctuations in STI incidence in the Dallas County population may confound the results, underscoring the importance of further analysis to align laboratory processes with public health objectives.

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Poster 71
Success and Challenges in the World of Radiochemistry as a Fellow
Z. Watkins, DCLS

The world of radiochemistry is incredibly fascinating and constantly evolving. Being a part of this community of talented radiochemists and scientists has been an eye-opening experience, to say the least. Despite the interesting research that lies ahead of the field, there are many challenges and successes that seem to cause trouble along the way. In order to have a method up and running, there are numerous steps and actions that must be considered, which were observed when starting a FERN food method looking for Americum-241. It started with doing background research on the method of interest, to consider safety while handling the needed chemicals, and making sure each instrument needed is smoothly running without issues. The method under examination called for hydrofluoric acid to be used in the digestion and extraction, and this required extensive training from the safety team before usage. The discussion of safety when using the chemical, how to properly clean up a spill, and how to drills to run through if there were to be a serious accident. A microwave digestor had to be used with the food method above, and with that came numerous technical and mechanical issues that needed to be resolved. This challenge came with contacting the manufacturer to have issues fixed and asking around to other labs to see what daily practice was for others. Before loading a sample, there must be an understanding of how the alpha spectrometry mechanically works, how maintenance should be performed, and what standard guidelines should be followed. Even establishing standard policies and guidelines can be a challenge, because it may depend on the qualifications of the method or how the manufacturer manuals explain proper maintenance. With challenges like these, many teach valuable lessons and create spaces for scientists to collaborate, however, also shed light on the hardships these same scientists face. Due to the community being so small, there are only so many people that one can reach out to or only so many people may understand what is being discussed. This creates significant gaps in making speedy progress in method optimization, validation, and verification. Expansion of the community is vital when battling the toughest challenges and sharing ground-breaking successes.

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Poster 72
Application of Language Model Learning for Genomic Sequence Classification in Pathogen

Surveillance: A Case Study of Fine-tuning genSLM
J. Rowell, Deloitte Consulting LLP, J. Cosme, Deloitte Consulting LLP, R. Campbell, Deloitte Consulting LLP, S. Lehman, Deloitte Consulting LLP, J. Jones, Deloitte Consulting LLP, K. O'Connell, Deloitte Consulting LLP

Biosurveillance, the real-time detection and monitoring of pathogenic organisms, plays a critical role in monitoring and preventing the spread of infectious diseases. Genomic data can be instrumental in identifying and characterizing pathogens for effective surveillance. However, a preliminary step to using genomic data for pathogen surveillance is the removal of host reads, a task that currently relies on kmer-based classification tools such as Kraken2. While these methods typically classify reads with high accuracy, they can be relatively slow with large datasets and often struggle with species-level classification. These limitations underscore the need for faster and more accurate methods of sequence classification. The emergence of Large Language Models (LLMs) offers a promising solution. LLMs have been successfully applied in bioinformatics and proteomics tasks such as protein structure prediction and gene feature identification. By treating biological sequences as a language, these models can potentially revolutionize sequence classification tasks, including host read removal. In this study, we fine-tuned an existing LLM – genSLM, for the classification of human and SARS-CoV-2 sequences. Our model was trained on Genome in a Bottle (GIAB) human references and >1,000 representative SARS-CoV-2 genomes. We benchmarked our model against Kraken2 and STAT for host DNA identification, evaluating model speed, accuracy (using known read IDs), and financial cost to run the model. We evaluate if our model outperforms traditional methods in speed, accuracy, and cost-efficiency for host DNA identification. This innovation underscores the potential of LLMs in enhancing sequence classification tasks for effective host read removal, marking a significant step towards the application of Generative AI models to public health genomics. The use of LLMs can substantially advance the field of bioinformatics, providing more accurate, faster, and cost-effective tools for public health genomics.

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Poster 73
Implementation of a Bioinformatics SOP for Improved Functionality of Bioinformatics in a Public Health Laboratory
L. Fink, Division of Consolidated Laboratory Services

The rapid integration of bioinformaticians into the public health workforce has addressed many gaps related to data management, genomic epidemiology, and sequencing, but it has also created new challenges in the fields of regulation, onboarding, and standardization of best practices. After evaluating the lack of written protocol to guide bioinformatic workplace function at The Department of General Services Division of Consolidated Laboratory Services (DGS-DCLS), the Sequencing and Bioinformatics Group (SBG) created, refined and implemented a new standard operating procedure (SOP) outlining the general rules and protocols that it was decided that the group bioinformaticians should abide by. It has been useful in establishing trust amongst the team, it has provided a way to codify the release of products and software, it has given the team timelines for updating software and competencies, and establishes guidance for how to validate, verify, course correct, code review, and respond to certain problems and situations that are unique in the public health lab to bioinformatics. The bioinformatics SOP could theoretically be adopted by any public health laboratory, and it provides a generalizable framework that allows enough flexibility to be modified and implemented in any institution.

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Poster 74
Antimicrobial Activity of Non-regulatory Polyherbal Formulation on Clinical Isolates — *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*: An In Vitro Study
K. Madaki, East Milton Keynes Primary Care Network, I. Agyigra, National Ear Care Centre, P. Malau, National Ear Care Centre, M. Sani, National Ear Care Centre, C. Uwan, National Ear Care Centre

Introduction: Traditional Medicine (TM) in Africa is divided into three categories, namely spiritualism, divination and herbalism. This combination makes African traditionalists belief that there is always an explanation to pain and suffering. The WHO defined Herbal Medicine (HM) to include herbs or herbal materials, herbal preparations and finished herbal products that contain plant parts or other plant materials or a combination as active ingredients.
Objectives: The purpose of this study is to determine the efficacy of some popular herbal medicines on common bacteria. It aims to test the sensitivity pattern of three herbal medicines: Answer STD, Madam F. Kayes and Goko cleanser and establish minimum bactericidal concentration (MBC) and minimum inhibitory concentration (MIC).
Methods: Three bacterial samples, Staphylococcus aureus, Pseudomonas aeruginosa and Escherichia coli were harvested in the laboratory of the National Ear Care Centre. The method used is sensitivity testing using nutrient agar. Identified bacteria were inoculated unto nutrient agar, and wells were cut at equidistant, and the herbal mixtures were introduced. Antibiotic cefuroxime was used in one well as a positive control and distilled water in another as negative control. Nutrient agar was then stored at 37oc for 24hours.
Results: The antibiotic recorded zones of inhibition between 20-30mm across all three plates, distilled water had a zone of inhibition of 0mm as this was negative control. The three Herbal Mixtures used all showed a zone of inhibition of 0mm. Implying all three bacteria were resistant to the herbal mixtures used.
Conclusion: Prior studies have shown that high doses of these herbal mixtures had negative effect on the liver and kidneys, this study aimed to find the MBC through diluting the HM and introducing it to the bacteria at different concentrations of 100%, 75%, 50% and 25% to find a lower dosage with the best MBC which should exert the least damage to the liver and kidneys, at absolute concentration, all three bacteria were resistant to the HMs.
Keywords: Herbal medicine, susceptibility testing, antimicrobial resistance, bacteria, antibiotics, antibiotic resistance.

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Poster 75
Tracking Trouble: Using Wastewater Monitoring to Track Pathogens During Hurricane Season, FL 2024
B. McGruder Rawson, Florida Bureau of Public Health Laboratories- Jacksonville, G. Crafts, Florida Department of Health Bureau of Public Health Laboratories-Jacksonville, D. Kelly, Florida Department of Health Bureau of Public Health Laboratories-Jacksonville, A. Sakhichand, Florida Department of Health Bureau of Public Health Laboratories-Jacksonville, K. Stockdale, Florida Department of Health Bureau of Epidemiology

With the support of the National Wastewater Surveillance System many states now have an infrastructure available to explore additional applications of wastewater surveillance, including surveillance of natural disasters. Preparedness and response activities usually supersede disease surveillance during a natural disaster- ensuring the physical safety and care of people. Wastewater testing might be able to fill the gap in disease surveillance during a disaster. Compared to tornados or earthquakes, which occur with limited notice, the state of Florida copes with annual hurricane season. Hurricane season runs from June through November and while the exact path is unknown the storms are identified well before landfall. For the 2024 hurricane season the Florida Wastewater Surveillance Program, partnering with QIAGEN, developed and tested a pilot panel of targets that focused on waterborne diseases commonly found post hurricane. The panel included Aeromonas hydrophila, Shigella sonnei, Leptospira interrogans, and Vibrio vulnificus. As a part of the pilot there was limited testing done to determine if there was any cross-reactivity between the panel targets and other species that are tested for by our laboratory. The pilot utilized samples that were already collected as a part of the regular surveillance effort by BPHL-Jacksonville. The goal was to determine 1. if any of the targets were already present in the wastewater, 2. if targets increased post-hurricane, and 3. if the increase in wastewater correlated with confirmed epidemiological cases. This study highlights the need to appropriately screen targets prior to use in a surveillance panel and that environmental pathogens have

the potential to obscure human cases.

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Poster 76
Evaluating the Similarities and Differences of Molecular Process Within the Field of Virology
D. Gibson Jr, Florida Department of Health - Tampa, E. Kopp, Florida Department of Health - Tampa, P. Dumoulin, Florida Department of Health - Tampa, P. Merriman, Florida Department of Health - Tampa

The aim of this qualitative study is to compare and contrast molecular workflows of public health laboratories. Three laboratories were visited and evaluated, located in Florida, New York, and New Hampshire. Evaluated processes included sample collection, shipping, accessioning, extraction, testing, waste disposal, sample storage, result entry and approval. Observations of testing processes were recorded verbally and as written notes. Consolidated information from all three locations will be sorted to identify commonalities and differences among workflows. Differences in standards set by accreditation bodies such as CLIA, CLEP, and CAP will be noted when they impact workflows. These comparative results may help identify areas where an exchange of ideas or methodologies between laboratories can result in improvements in safety, efficiency and testing accuracy.

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Poster 77
Scaling Up Genomic Surveillance Capacity for Better Epidemic Preparedness and Response in the WHO African Region
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Genomic surveillance has proven critical in tracking emerging pathogen variants in previous pandemics, including SARS-CoV-2, and is becoming an increasingly fundamental global public health tool for laboratory detection, characterization, monitoring, and response to various infectious disease epidemics and pandemics. The 6th IHR Emergency Committee for COVID-19 recommended increasing global genomic sequencing capacities and encouraged rapid sharing of genomic data; WHO to provide active support to member states in strengthening systematic genomic surveillance by leveraging the Global Influenza Surveillance and Response System (GISRS) and other relevant networks [1]. Furthermore, there is a crucial need for enhanced capacity in laboratory services and bioinformatics to improve the quality and timeliness of molecular epidemiological output in support of epidemic or pandemic control. The WHO Regional Office for Africa (WHO/AFRO) aims to ensure that all its 47 Member States have in-country laboratory capacity to conduct genomic surveillance for detection, characterization, monitoring the evolution, and response to pathogens of interest. While the existing capacities in the region have helped to facilitate the detection of SARS-CoV-2 variants of concern, thereby informing appropriate response activities, the capacities for laboratory-based genomic sequencing have been limited. Thus, a crucial need to strengthen laboratory capacities to scale up pathogen genomic surveillance. Therefore, WHO-AFRO through the financial commitment of the European Union’s DG-HERA is implementing this project to scale up the genomic surveillance capacity in 6 African countries. The implemented activities up to date have been focused on country assessment, consultation with regional reference laboratories, and development of an operational plan for building genomic sequencing and bioinformatics capacities through the development of human resources expertise and acquisition of laboratory reagents and equipment. Overall, the country assessment activities demonstrated that none of the six countries have yet established a national genomic surveillance strategy nor have developed a priority pathogens list for genomic surveillance. However, all 6 countries have the Oxford Nanopore Minion sequencing platform available, but for Comoros, the sequencing platform is housed at the National Research Institute for Agriculture, Fisheries and the Environment (INRAPE) under the leadership of the Ministry of Agriculture, Fisheries,

Environment, Tourism, and Craftsmanship. While most countries have human resources capable of performing the sequencing, very few have the capacity for bioinformatics analysis (CAR and Togo). Besides Togo and CAR, none of the countries has performed sequencing activities after the recession of the COVID-19 pandemic mainly because of reagents shortage or expiration. The project's next step is to foster on-site training in Genomic sequencing and bioinformatics, supply countries with reagents, and foster collaboration with regional reference laboratories. In conclusion, the target countries have the potential and are willing to implement genomic surveillance

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Poster 78

Development and Validation of a Short-read Amplicon-based Whole Genome Sequencing Assay for Respiratory Syncytial Virus in a Public Health Laboratory

K. Smith, Georgia Public Health Laboratory, J. Harrison, Georgia Public Health Laboratory, H. Yu, Georgia Public Health Laboratory, B. Bertrand, Georgia Public Health Laboratory, T. Parrott, Georgia Public Health Laboratory, A. Ramaiah, Georgia Public Health Laboratory

Background: Respiratory syncytial virus (RSV) is a negative-sense RNA virus with a 15.2 Kbp genome size that causes acute respiratory and lung infections primarily in young children and older adults. The two RSV subtypes (RSV-A and RSV-B) pose a major public health burden globally due to the high evolution rate and emergence of new lineages, necessitating genomic surveillance to monitor circulating types in the community. The Georgia Public Health Laboratory (GPHL) conducts surveillance of respiratory viruses using the Thermo Fisher TaqMan™ Gene Expression Capillary assay, which detects and subtypes RSV from nasopharyngeal swabs collected in Viral Transport Medium. To expand RSV surveillance in Georgia, GPHL developed and evaluated an amplicon-based whole genome sequencing (WGS) assay for routine RSV genomic surveillance to characterize circulating strains.

Methods: For the initial validation studies of the RSV WGS assay, we used 88 deidentified remnant clinical specimens (44 RSV-A and 44 RSV-B) collected from September 2023 to November 2024 with cycle threshold values below ≤25. Commercially available RSV transcripts were also extracted and processed as positive controls. The specimens were amplified using two sets of primers for each RSV subtype, a previously published primer set from the Minnesota Department of Health (MDH) and a custom primer set designed by GPHL. Library preparation and sequencing were performed on the specimens amplified by the MDH primers using an adapted and customized protocol from the Illumina COVIDSeq Test with CovidSeq kit reagents. Sequencing was performed on the NextSeq 2000 instrument using a P2 flow cell to ensure high-quality data generation. The FASTQ raw reads were processed by a custom-built Nextflow pipeline, GPHL-RSV-PIPE within AWS HealthOmics.

Results: By multiplexing the RSV-A and RSV-B primers, we amplified viral genetic material from the clinical samples with an amplicon size of 400 bases. A total of 57 of 88 were successfully sequenced, while the remaining specimens were dropped due to insufficient viral load or technical issues. The depth of RSV-A sequences was between 10,433.9x and 100,896.0x (median, 66,078.1x; mean, 57,435.8x), and the coverage was between 91.4% and 99.1% (median, 97.6%; mean, 96.8%). The depth of RSV-B sequences was between 7,148.1x and 105,228.0x (median, 54,041.6x; mean, 53,096.8x), and the coverage was between 90.2% and 99.7% (median, 99.1%; mean, 97.9%). Using Nextclade Web v3.10.0, the dominant lineages were identified as A.D.3.1 and B.D.E.1 for RSV-A and RSV-B, respectively. Although the sequences had sufficient depth and coverage, the RSV-A sequences experienced amplicon dropouts in the variable G gene region, which is prone to mutation.

Conclusions: The preliminary results of the WGS assay using the pre-existing primer set demonstrate a proof of concept to identify RSV lineages in clinical specimens. We will repeat this protocol using the GPHL custom-designed primer set on the same clinical specimens to identify their robustness and efficiency in terms of their sensitivity and specificity. We plan to use the more efficient primer set in the final implementation of this assay at GPHL for near real-time genomic surveillance of RSV that will

ultimately improve public health responses to virus outbreaks and genomic surveillance in Georgia.

Presenter: Keri Smith, Georgia Public Health Laboratory, keri.smith@dph.ga.gov

Poster 79
Wastewater Monitoring Provides a Rapid, Cost-effective Approach to Assess Community-wide H5 Avian Influenza and Longitudinal Testing
L. Robison, GT Molecular, A. Jones, GT Molecular, M. Zvyagin, GT Molecular, C. McKee, GT Molecular

Wastewater-based epidemiology (WBE) continues to grow in span and provide a non-invasive, lower-cost compliment to traditional, clinical-based epidemiology. Herein, we report influenza WBE surveillance studies, subtyping (including influenza A subtypes), and microbial source tracking (MST). Initial studies were performed on all wastewater samples to determine what type and quantity of influenza was present in wastewater and if enough influenza A viral material was present for H5 subtype detection. Multiplexed digital PCR (dPCR) assays were developed to detect pan-influenza A (FluA or infA), pan-influenza B (FluB or infB), SARS-CoV-2 (SC2), respiratory syncytial virus (RSV), bovine coronavirus (BCoV, an internal process control), and H5* (avian influenza H5 subtypes, including H5N1 from avian, cattle, and human origin designed using an advanced bioinformatic pipeline). Samples containing detectable FluA levels were then subtyped for H1N1, H3N2, and H5* discrimination, resulting in only H5* detection. Additionally, the presence of fecal matter was studied to determine human or animal origin. As expected from human wastewater, human marker, HF183, was robustly recovered, and nucleic acid dilutions were required to obtain results within the linear dynamic range. Avian markers CL, Gull2, and Gull4 were negative in all replicates tested. In contrast, the cowM2 marker was recovered at ultra-low levels, meaning cattle are a possible source of the positive data observed. From these studies, we can simultaneously detect pan-influenza A and H5* in a wastewater matrix, with confidence no other common influenza A subtypes are being amplified. Sites testing for H5N1 can use highly specific assays developed by GTM to longitudinally monitor H5* from both human and animal sources.

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Poster 80
Investigating the Gasdermin- B Gene to Address Early-onset Asthma in Adolescent African Americans
M. Tongue, Howard University, D. Davis, APHL-CDC

The 17q12-21 asthma locus, a widely recognized biomarker for early-onset asthma and susceptibility to respiratory viruses, provides critical insights into airway responses to environmental pathogens. However, its scope is limited in accounting for the cumulative effects of multiple genetic variants and environmental exposures, particularly in African Americans, who experience a higher prevalence and severity of asthma compared to European populations. Despite this disparity, asthma research predominantly centers on Europeans, leaving an urgent need to explore susceptibility factors in African Americans. Recent findings have highlighted the gasdermin-B gene and its associated single nucleotide polymorphisms (SNPs) as key contributors to asthma risk in African Americans. This study aims to investigate the role of the gasdermin-B gene through gene-environment interaction (GxE) analyses to identify precise biomarkers for early-onset asthma in African American children. By elucidating the molecular and cellular mechanisms of gasdermin-B, this research will inform the development of targeted strategies to reduce its expression. These findings promise to advance practical interventions and preventative measures, ultimately reducing asthma prevalence in this high-risk population. This work aligns with precision medicine and public health goals by addressing critical gaps in asthma research and providing actionable insights to mitigate health disparities.

Presenter: Morgan Tongue, Howard University, morganktongue@gmail.com

Poster 81
Performance of Multiple NGS Methods for Genomic Characterization of Co-circulating Respiratory Viruses
R. Stinnett, Illumina Medical & Scientific Affairs, T. Mazurski, Illumina, J. Koble, Illumina, P. Prashar, Illumina, S. Kuersten, Illumina, R. Stinnett, Illumina, R. Schlaberg, Illumina

The seasonal co-circulation of rapidly evolving respiratory viruses can create pressure on healthcare systems, as seen with so-called tripledemics in recent years. Genomic surveillance can uniquely augment response to these “predictable emergencies” but can be costly in time and expense. Enrichment of viral nucleic acid by molecular strategies, e.g. PCR or probe hybridization-based enrichment, is one strategy to enable cost-effective, rapid sequencing directly from samples. The benchtop MiSeq™ i100 Series (Illumina) provides numerous technological advancements over legacy short read platforms, including reduced sequencing time, high output, onboard DRAGEN™ analysis, room temperature reagents, and longer read formats while maintaining high base quality (M-GL-02244; www.illumina.com).

In this study, the performance of multiple sequencing assays on the Illumina MiSeq i100 system were evaluated using the same panel of remnant nasopharyngeal swab (NP) samples. All NP samples previously tested positive by diagnostic RT-PCR assays for the following: influenza A (H1N1) or (H3N2), respiratory syncytial virus (RSV-A and RSV-B) or SARS-CoV-2 at a specialized commercial laboratory. De-identified samples were provided with permission for inclusion in this study. A range of predicate Ct values were represented. Library preparation methods evaluated were Illumina Microbial Amplicon Prep (IMAP) with custom primer designs and 2 enrichment panels designed for the parallel detection of multiple viruses (Viral Surveillance Panel v2 and Respiratory Viral Enrichment Kit). Specifically, IMAP-Flu primers, IMAP-RSV primers and COVIDseq with ARTIC v5.4.2 primers were used to prepare libraries from influenza-, RSV-, and SARS-CoV-2 positive samples, respectively. Read data (2x151) were analyzed using Illumina’s Dragen Microbial Amplicon and Dragen Microbial Enrichment Plus bioinformatics solutions.

Enrichment assays detected each expected viral target in all samples with Ct 30 in this study, an amplicon assay was more likely to detect and characterize the expected virus than enrichment assays, e.g. COVIDSeq for SARS-CoV-2. In general, the completeness of genome assembly correlated with predicate Ct value. Type/subtype were correctly assigned for all detected viruses, in some cases with < 70% callable bases. Bioinformatic analysis was possible in < 2 hours, with analysis time dependent on run size and sample complexity. Surveillance goals should inform the relative prioritization of various capabilities in genomic surveillance assay development, notably analytical sensitivity, potential for full genome assembly, and other workflow considerations including adaptability of a “universal” assay for broad parallel vs focused characterization of viral surveillance targets. However, each workflow presented here is suitable for rapid, reliable detection and typing of RSV and influenza and SARS-CoV-2 viruses directly from respiratory samples.

Presenter: Rita Stinnett, Illumina Medical & Scientific Affairs, rstinnett@illumina.com

Poster 82
Enhancing Public Health Response through Interagency Collaboration: The Role of the Kentucky Public Health Laboratory in Influenza and Rabies Cross-Testing
M. Sauceda, Kentucky Department For Public Health Division of Laboratory Services, T. Fields, Kentucky Department for Public Health Division of Laboratory Services, S. Preston, Kentucky Department for Public Health Division of Laboratory Services, R. Zinner, Kentucky Division of Laboratory Services, M. Johnson, Kentucky Department for Public Health Division of Laboratory Services, V. Arora, Kentucky Department for Public Health Division of Laboratory Services

Zoonotic disease surveillance of rabies and Highly Pathogenic Avian Influenza (HPAI) A(H5N1) is enhanced though collaborative efforts between local, state, and federal partners. This poster explores the pivotal role of the Kentucky Department for Public Health’s (KDPH) Division of Laboratory Services

(DLS) in a collaborative diagnostic project involving concurrent HPAI and rabies testing in cats. The project brings together KDPH DLS, KDPH Division of Epidemiology and Health Planning, University of Kentucky Veterinary Diagnostic lab (UKVDL), Murray State University Breathitt Veterinary Center (BVC), and National Animal Health Laboratory Network (NAHLN)/U.S. Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS). This dual testing approach streamlines the surveillance process, enabling faster identification of both diseases and more effective public health responses. In partnership with the UKVDL, DLS coordinates the collection and transfer of brain samples from cats for rabies and subsequent HPAI testing. This partnership ensures that rabies negative samples from local health departments and veterinary clinics are also tested for HPAI, a critical consideration in areas with recent avian outbreaks. We discuss the workflows, interagency communication, and logistical strategies that make this dual testing approach efficient, as well as the implications for improved disease detection and prevention in human and animal populations. This collaborative model exemplifies the importance of multi-agency coordination in managing emerging zoonotic risks and enhancing public health preparedness.

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Poster 83
Cultivating Engagement: Inspiring Students and Connecting Communities
R. Zinner, Kentucky Division of Laboratory Services, E. Lee, Texas Department of State Health Services, A. Galusha, Wadsworth Center, NYS DOH, A. Keese, Texas Department of State Health Services, H. Reiss, Association of Public Health Laboratories (APHL)

Public health laboratories are crucial in protecting and improving the health of our communities, yet face significant challenges exacerbated by workforce flux, public trust, and visibility of their work. Outreach initiatives play a vital role in addressing these challenges by fostering collaboration, establishing credibility, and promoting health literacy. The Association of Public Health Laboratories (APHL) Public Health Laboratory Ambassadors program aims to recruit the next generation of laboratorians by promoting careers through targeted outreach efforts. Academic and community outreach can be utilized as a recruitment tool to engage and excite students, while building positive ties to the local, national, and global communities. The Ambassadors program focuses on inspiring students to explore career paths in public health laboratories by using their own unique stories to increase accessibility of the sciences to a broader segment of the population. The program began as an initiative by Cohort 16 of the Emerging Leader Program and has expanded to a network reaching communities throughout the country. The program currently has 112 Ambassadors across 32 states and U.S. territories engaging in local outreach events and raising the visibility of public health laboratory careers. In 2024, Ambassadors organized more than 40 opportunities to connect with students of all ages. Outreach events and long-term programming with local universities provide platforms for students to experience firsthand the role of public health laboratories in both daily operations and emergent situations such as outbreaks. Moreover, these events present an avenue for laboratory staff to share their personal pathways into public health, allowing students to explore laboratory careers through meaningful connections. Continuation for such channels into public health are pivotal in cultivating and fostering future laboratorians.

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Poster 84
Development of a Real-time PCR Assay for the Detection of TR34/L98H Azole Resistance Markers in *Aspergillus fumigatus* Isolates
S. Bailey, Maryland Department of Health Laboratories Administration, T. Maruca, Maryland Department of Health Laboratories Administration, J. Stegman, Maryland Department of Health Laboratories Administration, D. Torpey, III, Maryland Department of Health Laboratories Administration, R. Myers, Maryland Department of Health Laboratories Administration

The emergence of azole resistance in Aspergillus fumigatus presents a significant public health

concern in multiple countries. This mold can lead to allergic diseases and severe invasive infections, such as aspergillosis, in immunocompromised individuals. The mortality rate associated with invasive infections is greater than 50%. The primary drivers of azole resistance in A. fumigatus are the use of azole antifungal agents in human therapies and as agricultural fungicides. A primary mechanism of azole resistance in A. fumigatus is polymorphisms in the lanosterol 14 α-demethylase gene (cyp51A), which encodes the CYP51A protein. In some cases, this resistance is linked to a combination of the cyp51A polymorphism and tandem repeats within the promoter region of the cyp51A gene. Of significant relevance is the A. fumigatus cyp51A TR34/L98H genotype, which has been associated with the use of azole fungicides and resistance to itraconazole, voriconazole, and posaconazole. As azole resistance in A. fumigatus continues to rise, it is essential for laboratories to have the capability to affordably detect resistant isolates and enhance surveillance efforts. This is a key objective of the Antimicrobial Resistance Laboratory Network (AR Lab Network), where MDH Laboratories Administration serves as a regional reference laboratory for azole resistance screening. In this study, we developed a multiplex real-time PCR assay for the detection of TR34/L98H mutations in A. fumigatus isolates based upon whole genome sequencing of the cyp51A region. Broth microdilution assays were performed to phenotypically evaluate azole resistance in the A. fumigatus isolates. A. fumigatus DNA was extracted using a mechanical bead beating method followed by a spin-column-based method which was shown to be the optimal technique for DNA extraction. Primers and probes were designed to specifically target mutations TR34 in the cyp51A promoter region and the L98H polymorphism in A. fumigatus isolates. Additional primers and probes were created to target the ITS1 and ITS2 regions to confirm the presence of fungal DNA. A standard curve was established to assess amplification efficiency. The qPCR assay was optimized to determine the optimal primer and probe concentrations, cycling conditions, and the quality and concentration of the template DNA. The functionality of the qPCR assay was optimized by testing it against A. fumigatus isolates with known wildtype and mutant cyp51A regions. The cyp51A genotypes were confirmed through whole genome sequencing conducted by the Mycotic Diseases Branch at the Centers for Disease Control and Prevention. The development of this multiplex qPCR assay will allow laboratories to reliably detect the TR34/L98H mutations that contribute to azole resistance in A. fumigatus, allowing for increased surveillance of this emerging threat without the need to conduct whole genome sequencing.

Acknowledgments
Mycotic Diseases Branch, Centers for Disease Control and Prevention (CDC)
Antimicrobial Resistance Laboratory Network (AR Lab Network)

Presenter: Sydney Bailey, Maryland Department of Health Laboratories Administration, sydney.bailey@maryland.gov

Poster 85

Cronobacter Surveillance in Infant Foods: Enhancing Preparedness for the Next Outbreak

J. Bedolla, Maryland Department of Health Laboratories Administration, K. Lozinak, Maryland Department of Health - Laboratories Administration, S. Urban, Maryland Department of Health - Laboratories Administration

Cronobacter Surveillance in Infant Foods: Enhancing Preparedness for the Next Outbreak

Jesus Bedolla, Kristen Lozinak, and Sinisa Urban
Maryland Department of Health - Laboratories Administration, 1770 Ashland Avenue, Baltimore, Maryland, 21205

Cronobacter spp. are bacteria found naturally in the environment that can persist in dry foods like powdered infant formula and cereals. Although Cronobacter infections in infants can be deadly, they are rare and became a nationally reportable condition only in 2024. As a result, key tools like genomic sequence databases needed to investigate Cronobacter infections remain incomplete. To help close this gap, this APHL Fellowship project is aimed at characterizing the prevalence of Cronobacter contamination of infant foods, sequencing the isolates, and submitting their genome sequences to the National Center for Biotechnology Information (NCBI) database. To date, 0 out of 75 infant formulae, but 17 out of 186 infant cereals, were found to be contaminated with Cronobacter, and 70 whole

genome sequences were generated and uploaded to NCBI. This ongoing project promises to help solve future outbreaks, with the hope of eventually preventing them from occurring in the first place.

Presenter: Jesus Bedolla, Maryland Department of Health - Laboratories Administration, Division of Environmental Sciences, jesus.bedolla@maryland.gov Topic: Food Safety

Presenter: Jesus Bedolla, Maryland Department of Health Laboratories Administration, jesus.bedolla@maryland.gov

Poster 86
Carbapenem Resistance and Public Health Concerns: A Review of CRAB Surveillance in Maryland (2021–2024)
Presenter: Victoria Campodonico, Maryland Department of Health Laboratories Administration, victoria.campodonico@maryland.gov

Poster 87
Identifying New PFAS Compounds in Maryland's Drinking Water Using Non-targeted Analysis
S. Fleegal, Maryland Department of Health Laboratories Administration, Z. Cao, Maryland Department of Health Laboratories Administration, S. Urban, Maryland Department of Health - Laboratories Administration

Per- and polyfluoroalkyl substances (PFAS) are a group of synthetic chemicals that are widespread and persist in the environment. Human exposure to PFAS is linked to negative health effects, including reduced responses to vaccines, developmental defects or delays in children, and increased risk of cancer. While the EPA recognizes over 14,000 chemicals as PFAS, its current drinking water testing methods encompass only 29 of these compounds. As part of an APHL Fellow project, we sought to gain a better understanding of the total PFAS contamination in Maryland by recollecting drinking water from all 63 water systems that we previously found to be contaminated with PFAS above EPA's maximum contaminant levels. The samples were first extracted and quantified using EPA Method 533 before being subjected to non-targeted analysis using high-resolution mass spectrometry. We identified an additional 28 putative PFAS compounds, suggesting that Marylanders are being exposed to triple the number of PFAS chemicals in their drinking water than previously thought. Some of these newly identified PFAS were present in every contaminated water system, while others were less common, and some were specific to a single water system. This study may be the first complete fingerprint of PFAS contamination in any state's drinking water.

Presenter: Sarah Fleegal, Maryland Department of Health Laboratories Administration, saraha.fleegal@maryland.gov

Poster 88
The link Between Social Vulnerability Indices (SVI) and Antimicrobial Resistance: An Analysis of Maryland's NARMS Retail Meat Data
O. Ijabadeniyi, Maryland Department of Health Laboratories Administration, Baltimore, S. Wozny, Maryland Department of Health - Emerging Infections Program, M. Boyle, 2Maryland Department of Health - Emerging Infections Program, P. Ryan, Maryland Department of Health - Emerging Infections Program, K. Lozinak, Maryland Department of Health - Laboratories Administration, S. Urban, Maryland Department of Health - Laboratories Administration

Introduction: Antimicrobial resistance (AMR) is a global health threat associated with 4.95 million deaths annually, 1.27 million directly linked to drug-resistant pathogens. AMR hinders Sustainable Development Goals such as newborn survival, healthy aging, and poverty reduction. In the United States, AMR bacteria are prevalent in beef, turkey, pork, and chicken, with livestock antibiotic usage as a key contributor. This study examines Social Vulnerability Indices (SVIs) associated with resistant and non-resistant, as well as multidrug-resistant (MDR) and non-multidrug-resistant (NonMDR) bacterial isolates.

Method: Maryland's 2008-2021 NARMS Retail Meat datasets were geocoded and matched to SVI values according to isolate collection locations. The isolates were sorted into susceptible versus resistant groups, and their average SVIs were compared across 27 antibiotics, categorized by all isolates, species-specific isolates, commodity-specific isolates, and species-commodity combinations. Statistical significance between the SVIs as well as between MDR and non-MDR for 27 antibiotics were calculated using GraphPad Prism.

Results: Significant differences between SVI (resistant) and SVI (susceptible) were detected. Streptomycin resistance displayed significance ($P \leq 0.05$) across all isolate groupings. Comparisons between MDR and non-MDR isolates revealed significant differences ($P \leq 0.05$), particularly in Enterococcus (species-specific), Turkey (commodity-specific), and species-commodity combinations such as Turkey/Salmonella, Chicken/Enterococcus, Turkey/Enterococcus, and Turkey/Campylobacter.

Discussion: While notable differences between resistant and susceptible isolates were expectedly sparse, streptomycin's distinct profile highlights its relevance in AMR dynamics. MDR isolates consistently exhibited significant SVI disparities, suggesting key social variables influencing resistance patterns.

Conclusion: This study identifies significant correlations between SVIs and resistance patterns in specific antibiotics and isolate groupings. These findings offer valuable insights into social factors driving AMR, underscoring the need for targeted interventions and enhanced surveillance to combat this global challenge.

Presenter: Oluwatosin Ijabadeniyi, Maryland Department of Health Laboratories Administration, Baltimore, tosynolu@yahoo.com

Poster 89

Automation of PulseNet's Rapid Quarter Volume DNA Library Preparation Method

B. Sabina, Massachusetts Department of Public Health, E. Dart, Massachusetts Department of Public Health, K. Cleary, Massachusetts Department of Public Health, H. Tan, Massachusetts Department of Public Health, E. Fortes, Massachusetts Department of Public Health, S. Bhattacharyya, MA State Public Health Laboratory, N. Epie, Massachusetts State Public Health Laboratory

With an emphasis on ensuring high quality data, Massachusetts State Public Health Laboratory (MA SPHL) continues to evaluate cost-saving measures to enhance offering whole genome sequencing (WGS) to complement infectious disease surveillance. In 2023, MA SPHL transitioned from Illumina's Nextera XT DNA library preparation kit to DNA Prep library preparation kit. This resulted in improved genome coverage and evenness for genomes with both low and high GC content, as well as in longer average read lengths, fewer contigs, and fewer ambiguous bases in assemblies. Here we report findings from the feasibility study with modified DNA library prep for CDC's PulseNet protocol using both manual and automated preparation method on the Eppendorf epMotion 5075t liquid handler to reduce hands-on time and reagent volume.

Briefly, twenty (20) paired genomic libraries were prepared at reduced volume, manually and on the epMotion 5075t liquid handler. Libraries obtained from both methods were sequenced on the Illumina MiSeq using a v2 500-cycle reagent kit and compared to the full reagent volume paired samples. Manually prepared libraries performed comparably at quarter and full volume. Automated libraries prepared at quarter reagent volume produced identical high-quality genome assemblies that passed all required quality metrics required for submission to CDC's PulseNet group. The cost to prepare and sequence a library decreased approximately 20% and the hand-on time was reduced from approximately 6 hours to 3 hours.

Moving forward, we plan to use Carbapenem-resistance organisms (CROs), invasive Group A streptococcus pyogenes (iGAS), and Mycobacterium tuberculosis (MTB) isolates to determine if

high-quality genome assemblies can be generated from automated prepared quarter volume DNA libraries and reduce costs further.

Presenter: Brandon Sabina, Massachusetts Department of Public Health, brandon.sabina@mass.gov

Poster 90
Applying a Sample Size Calculation Framework to Evaluate and Improve SARS-CoV-2 Variant Surveillance in Massachusetts
Z. Thompson, Massachusetts Department of Public Health, C. Casiello, Massachusetts Department of Public Health, E. Decker, Massachusetts Department of Public Health, L. Jones, Massachusetts Department of Public Health, M. Burns, Massachusetts Department of Public Health, E. Fortes, Massachusetts Department of Public Health, S. Wohl, Massachusetts Department of Public Health

BACKGROUND: The Massachusetts Department of Public Health (DPH) monitors sequenced SARS-CoV-2 (SC2) clinical specimens and presents variant prevalence estimates in surveillance reports. With the reductions in reported SC2 cases, DPH, as part of the work with the New England Pathogen Genomics Center of Excellence, applied a sample size calculation framework to evaluate the impact of potential biases on the number of sequences needed to detect emerging variants. The goals were to determine sample size targets for SC2 variant surveillance and convey confidence in variant detection and prevalence to improve surveillance reports.

METHODS: The phylosamp R package combines binomial power analyses with bias corrections accounting for variability in testing rate and sensitivity, asymptomatic rate, and genomic characterization success rate across SC2 variants. This correction term (“C ratio”) enables more accurate sample size calculations when these common sources of bias affecting variant detection are present. Using sequencing data from the Massachusetts surveillance and case management system, we examined two scenarios ─ being half as likely and just as likely to detect an emerging variant (C ratios 0.5-1) ─ to calculate variant detection probabilities. To determine sample size targets, we set a variant detection threshold of 3% with 80% confidence. For variant prevalence estimates, we computed 95% confidence intervals (CIs).

RESULTS: We determined that a weekly sample size target of 53-105 successfully sequenced specimens should allow detection of an emerging variant at 3% prevalence with 80% probability given our range of bias scenarios, provided that underlying assumptions for sampling homogeneity and representativeness are met. Meeting this sample size target also increases the precision of variant prevalence estimates. Sample size calculation code and outputs were integrated into SC2 surveillance reporting to plot variant detection probabilities alongside weekly sample sizes and display 95% CIs for variant prevalence estimates alongside variant proportions graphs.

CONCLUSIONS: Applying a sample size calculation framework helped establish weekly targets for SC2 sequences and informed the number of specimens the Massachusetts State Public Health Laboratory requests from clinical partners during respiratory virus season. Evaluating confidence in variant detection and prevalence enhances SC2 reporting and allows more complete assessment of variant data and surveillance gaps. Limitations include that underlying assumptions of sampling representativeness may not be met due to small numbers of samples and submitting laboratories. Our methods use a flexible framework that can be adapted to a jurisdiction’s variant surveillance goals and account for biases due to changes in virus biology over time.

Presenter: Zachary Thompson, Massachusetts Department of Public Health, zachary.thompson@mass.gov

Poster 91
Medical Misogyny: An Epidemiological Review of Societal Stress on Women's Mental Health
M. Quinn, MCPHS

Contrasting modern day neuroscience research and employed public health survey methods against contemporary literature explores the relationship of societal effects and expression on women's mental health. Contemporary literature viewed from the lens of narrative medicine communicates the ever-present effects of societal pressures on the brain as evidenced by neuro-scientific literature on diverse women populations and strengthened by local survey conducted to evidence symptomatic expressions to identify risk reduction practices in medical settings.
Discussion centers around how women's mental health decline is debilitated by societal pressures and enabled by misogyny in medicine. These novels follow women through their lives as they are driven into madness and uncharacteristic behaviors, while their medical "hysteria" goes unperturbed. It is necessary to use literature in this study as it gives nuance and introspection to real life themes of mental health stigma and enhances the power of narrative in medicine.
Neuroscience proves how the brain reacts to stress caused by society in neural anatomy in diverse women populations to depict the representation found in literature. Stigma, stress, and discrimination causes changes in cortisol levels and neural damage which depicts mental illness ignorance, which is perpetrated by patriarchal society. The Public Health Survey targets adult women who experience mental health challenges. The survey will analyze the symptoms expressed in the population and identify the self-reported contributing factors. These methods used harmoniously will help identify key themes in society and women's experiences highlighted in literature that affect adverse neural health outcomes and hypothesize risk reduction implementations.

Presenter: Madelyn Quinn, MCPHS, maddyquinn02@gmail.com

Poster 92
Medical Misogyny: Epidemiologic Examination of Kang's Vegetarian and Murakami's Sleep
M. Quinn, MCPHS

Contrasting modern day neuroscience research and employed public health survey methods against contemporary literature explores the relationship of societal effects and expression on women's mental health. Contemporary literature viewed from the lens of narrative medicine communicates the ever-present effects of societal pressures on the brain as evidenced by neuro-scientific literature on diverse women populations and strengthened by local survey conducted to evidence symptomatic expressions to identify risk reduction practices in medical settings.
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Neuroscience proves how the brain reacts to stress caused by society in neural anatomy in diverse women populations to depict the representation found in literature. Stigma, stress, and discrimination causes changes in cortisol levels and neural damage which depicts mental illness ignorance, which is perpetrated by patriarchal society.

Presenter: Madelyn Quinn, MCPHS, madelynquinnpremed@gmail.com

Poster 93
Ergonomic, Green and ADA Considerations for Renovation and Construction
D. Fayram, Merrick & Company, R. Glaser, Wadsworth Center, NYS Dept of Health, C. Fortune, Montana Laboratory Services Bureau, M. Marsico, APHL, S. Escott, Biosafety, Biosecurity and Bioterrorism Preparedness Training

Following the SARS-CoV-2 pandemic, many institutions supporting public health received funding for building or upgrading their current laboratory facilities. High-containment laboratories are notoriously expensive to construct and maintain due to their many required safety features. In addition, special accommodation for employees who need them are difficult to anticipate and may be tricky to incorporate due to limited space. This poster will review some of the relevant considerations for integrating "green" principles into new construction, as well as designing or upgrading various types

of laboratory facilities to include ergonomic and accessibility provisions, such as those required by the ADA (Americans with Disabilities Act), Disability Discrimination Act 1995 (DDA) in the U.K., or the Accessible Canada Act (ACA), to name a few examples. References will be available as a handout during the poster session.

Presenter: Drew Fayram, Merrick & Company, drew.fayram@merrick.com

Poster 94

Measurement of BHA, t-BHQ and Phase II Metabolites (t-BHQ- glucuronide and -sulfate) in Rat Serum by Liquid Chromatography Tandem Mass Spectrometry

A. Deeds, Michigan State University Diagnostic Laboratory, J. Buchweitz, MSU VDL, J. Zyskowski, MSU VDL

Background: BHA (butylated hydroxyanisole) and its metabolite t-BHQ (tert-butylhydroquinone) are well-known synthetic antioxidants used to preserve oils and fats by preventing lipid peroxidation. They are commonly found in various consumables, including vegetable oils, animal feeds, and personal care products such as cosmetics. It has been shown that low concentrations of t-BHQ and its phase II metabolites can induce inflammatory and other immune effects in some rodent models. Consequently, excessive exposure to these antioxidants through food may pose a serious threat to food safety and human health. Since these compounds are nearly ubiquitous across diets it is important to understand what concentrations of these antioxidants are circulating in blood. Of the analytical techniques available, liquid chromatography-tandem mass spectrometry (LC-MS/MS) offers the greatest sensitivity, specificity, and accuracy. The objective of this study was to develop and validate an LC-MS/MS method capable of simultaneously quantifying BHA, t-BHQ and its phase II metabolites in animal and human serum samples, with the ultimate goal of assessing pharmacokinetics.

Method: A multiple reaction monitoring (MRM) method was developed and optimized to quantify t-BHQ and BHA. Chromatographic separation was achieved on a Shimadzu LC 30 AD ultra high-performance liquid chromatography (UHPLC) system (Shimadzu, Kyoto Japan) equipped with a Waters (Milford, MA) ACQUITY UPLC BEH 1.7um C18 130 Å (2.1 mm x 50 mm) column. 10 µL of sample was injected onto the column with a flow rate of 0.20 mL/min. Tandem mass spectrometry identification and quantitation was accomplished using an ABSciex 6500+ triple quadrupole mass spectrometer (Framingham, MA).

Results: Our initial studies indicate that t-BHQ can be reproducibly detected and quantified in spiked-serum extracts at concentrations as low as 5 ng/mL. The liquid-liquid extraction procedure for t-BHQ resulted in a 95% recovery rate. Standard curves were generated by spiking charcoal-stripped serum with concentrations of t-BHQ ranging from 25 ng/mL to 500 ng/mL, yielding a linear curve with an R-value of 0.994. Additionally, serially diluted standard curves were generated using an analytical stock mixture of t-BHQ, with concentrations ranging from 0.1ng/mL to 25ng/mL. This included the limit of detection (LOD) of 0.39 ng/mL, with a coefficient of variation (%CV) of 18.1.

Conclusion and Future Direction: The current method is more sensitive and precise than alternative non-LC-MS methods. The next phase of this research aims to refine the extraction process to achieve lower detection limits for all t-BHQ -related analytes and to establish baseline values for these in serum across multiple species.

Presenter: Alyssa Deeds, Michigan State University Diagnostic Laboratory, alyssa_deeds@aol.com

Poster 95

Simultaneous Extraction of Fat-soluble Vitamins A, E and D Metabolites (25OHD2 and 25OHD3) in Animal Tissue Followed by Quantitation with Liquid Chromatography Tandem Mass Spectrometry

Z. Rice, Michigan State University Veterinary Diagnostic laboratory, J. Buchweitz, MSU VDL, J. Zyskowski, MSU VDL

Fat-soluble vitamins (FSV), A, E, D, and K are organic compounds essential for life and normal growth. All FSV are acquired from the diet, with a few exceptions. The physiological requirements vary widely across species, and imbalances have been linked to many diseases. Thus, vitamins are considered a prophylactic measurement, and their accurate determination in animal tissue and feeds is crucial. Herein, we describe a liquid chromatography tandem-mass spectrometry (LC-MS/MS) method for detecting and quantifying vitamin A (retinol), vitamin E (alpha tocopherol), and two vitamin D metabolites, 25(OH) D2 and 25(OH) D3, in animal tissue. Additional analytes, including retinyl esters and alpha tocopherol quinone, are monitored to assess relative hydrolysis efficiency for vitamin A and oxidative stability of vitamin E.
The method consists of sample homogenization and alkaline saponification prior to supported liquid extraction (SLE) with n-hexane. Extracts were evaporated, reconstituted in acetonitrile, and split into two aliquots. One aliquot was chromatographically separated for analysis of vitamins A, E, alpha tocopherol quinone, and retinyl esters. In the second aliquot, vitamin D metabolites were derivatized with 2-nitrosopyridine. It was similarly separated chromatographically. Multiple reaction monitoring (MRM) settings were developed and optimized for each target analyte. Chromatographic separation was achieved on a Shimadzu Nexera LC40XR ultra high-performance liquid chromatography (UHPLC) system (Shimadzu, Kyoto, Japan) equipped with a Waters (Milford, MA) AQUITY BEH 1.7um C18 130 Å (2.1 mm x 50 mm) column. Tandem mass spectrometry detection and quantification was accomplished using an ABSciex 6500+ triple quadrupole mass spectrometer (Framingham, MA).
Fetal bovine liver spiked at four concentrations ranging from 50 ug/g to 400 ug/g for vitamins A and E, and 100 ng/g to 1000 ng/g for vitamin D metabolites yielded the following recoveries: 102-120% for vitamin A, 100-131% for vitamin E, 96-125% for 25(OH)D2, and 94-118% for 25(OH)D3. Intraday precision of neat standard curves averaged 100% for vitamin A, 95% for vitamin E, 99% for 25(OH)D2, and 100% for 25(OH)D3, respectively. Linearity ranged from 5 ng/g to 2500 ng/g for vitamin D metabolites and 1 ug/g to 500 ug/g for vitamins A and E. Hydrolysis successfully increased retinol concentrations by reducing retinyl esters to ≤15% with minimal effects on vitamin E, 25(OH)D2, and 25(OH)D3 concentrations. Stability of vitamin E was maintained as the relative oxidation post-extraction averaged <10% in clinical samples.
Although the current method has been optimized with animal tissue for diagnostic use, our overall goal is to extend it to a variety of commercial feed products. Hepatic tissue, one of the more difficult animal tissue matrices, is a major component of commercial meat products, and marks the beginning of this objective.

Presenter: Zoe Rice, Michigan State University Veterinary Diagnostic laboratory, zrice20@gmail.com

Poster 96
Simplification and Automation of a Biological Sequencing Workflow
K. Yeh, MRIGlobal, A. Jorns, MRIGlobal, J. Russell, MRIGlobal, K. Yeh, MRIGlobal, J. Bogan, MRIGlobal

Traditional nucleic acid amplification tests and immunoassays require a priori knowledge of the threat being targeted, whereas nucleic
acid sequencing can be done in a non-targeted fashion that allows detection of any and all biological threats, both known and unknown.
For this reason, incorporation of biological sequencing technology into the DoD's threat detection infrastructure is critical to address the
rise in novel, emerging, and engineered biothreats. Recent advances in field portable sample preparation and sequencing devices have
created an opportunity to revolutionize the DoD's capability to detect, identify, and characterize biological threats via sequencing at the
point of need, thereby accelerating the timeline from sample collection to actionable intelligence. Despite these advancements,
instrument size, method complexity, turnaround time, reagents that require cold-storage, and data analysis requirements are still hurdles

limiting successful deployment of sequencing capabilities to field environments.
To address this gap, we performed feasibility testing of a simplified workflow that includes highly-portable sequencing devices,
automated sample and library preparation modules, room-temperature stable reagents, and push-button data analysis for in-field
detection, identification, and characterization of known, emerging, and engineered biological threat agents with minimal training. Results
from this testing have proven the feasibility of a rapid, simplified, automated workflow for whole genome sequencing (WGS) in austere
field settings. The workflow developed provided high-confidence, species-level detection of a simulant bacteria (Bacillus thuringiensis
subsp. kurstaki) in < 1 hour from sample to answer and is fully automatable. We also performed studies to assess the feasibility of this
workflow with additional sample/pathogen types, yielding valuable information on the current potential of the developed workflow and
underlying technologies, as well as the remaining gaps and challenges that must be addressed.
After performing feasibility testing with the air-gapped workflow, we initiated development of a fully integrated, sample-to-answer
sequencing device for agnostic detection, identification, and characterization of known, emerging, and engineered biological threat
agents. This revolutionary low size, weight, and power (SWaP) device will integrate and automate nucleic acid extraction, sequencing
library preparation, sequencing, data analysis, curation, and reporting into a portable, ruggedized, and battery-operated device. The
device in development would be approximately 8” L x 6” W x 5”H, and weigh approximately 8 lbs. Furthermore, it would require no user
intervention after loading a sample, making it an ideal technology for users that are not professional molecular biologists and/or have
other duties to perform while the sample is being prepared for sequencing. Complete sample-to-answer automation will also facilitate
future integration into autonomous environmental monitoring systems for aerosols, water, and other matrices, enabling early warning
systems to detect novel threats.

Presenter: Kenny Yeh, MRIGlobal, kyeh@mriglobal.org

Poster 97
Use of Censored and Contaminated Data to Generate Reference Intervals Through Indirect Approaches
M. Marsh, MSU Veterinary Diagnostic Laboratory, J. Buchweitz, MSU VDL, B. Puschner, MSU Veterinary Diagnostic Laboratory

Introduction:
Reference intervals (RIs) are fundamental tools used by veterinary and laboratory professionals for interpreting clinical test results, yet their accurate determination can be challenging in veterinary diagnostics. The most common approach is to base RIs on the central 95% of a normal distribution of test values observed for a reference population that is free of diseases that influence that laboratory test result. But identifying suitable reference populations in veterinary medicine is challenging due to asymptomatic disease conditions, sample size requirements, and other factors. In contrast, the indirect approach establishes RIs based on real-world data consisting of mixed healthy and unhealthy populations. Additionally, left-censored data arises when measurements fall below the method's quantitation limits. This study aimed to evaluate indirect methods for establishing RIs for trace nutrients in bovine liver using such complex, real-world datasets.

Methods:
Data were sourced from 14,744 bovine liver samples submitted to the MSU Veterinary Diagnostic

Laboratory. Measurements for seven trace nutrients (cobalt, copper, iron, manganese, molybdenum, selenium, and zinc) were included. Animals under 30 days old were excluded, as well as observations with measurements that fell in the range of toxicity. The remaining values were filtered using the Latent Abnormal Values Exclusion (LAVE) method, which iteratively removes values likely to represent disease states, determined by the quantity of trace nutrients that fall outside of the central 95% range of the remaining data. This leaves only values that appear to be nonpathological. Statistical handling of censored data involved the Robust Regression on Order Statistics (ROS) method, chosen for its ability to provide robust estimates in large datasets with low levels of censoring (< 30%). This method estimates statistics with censored data by imputing censored values based on an assumed normal or lognormal distribution.

Results:
After filtering, 10,779 samples were retained for analysis. RIs estimated using the Robust ROS method closely matched those currently used by the laboratory. The central 25th–75th interquartile range (IQR) represented healthy populations, while marginal values extended from the IQR to the 2.5th and 97.5th range. These results support the effectiveness of the method in estimating RIs from censored and contaminated data.

Conclusions:
This study demonstrates the validity of applying indirect approaches to generate RIs from complex datasets. The Robust ROS method effectively handles censoring and contamination, producing results consistent with established standards. Improving the accuracy of RIs requires not only the collection of sufficient data and the use of correct statistical methods, but also proper stratification of heterogeneous subpopulations. Proper implementation of these methods can drastically reduce cost and time associated with generating RIs.

Presenter: Madelyn Marsh, MSU Veterinary Diagnostic Laboratory, madelynfmarsh@gmail.com

Poster 98
Use of Rolling Medians to Evaluate Data Suitability for Indirect Reference Interval Generation: A Case Example of Canine Liver Copper
M. Marsh, MSU Veterinary Diagnostic Laboratory, J. Buchweitz, MSU VDL, B. Puschner, MSU Veterinary Diagnostic Laboratory

Introduction:
A reference interval (RI) defines the expected value range for a healthy population. This is typically achieved either through direct testing of healthy individuals or indirectly using real-world data mining of large clinical databases and rigorous statistical techniques. In dogs, copper-associated hepatopathy (CAH) is defined by excessive liver copper, leading to chronic hepatitis. Hepatic copper concentrations are critical for diagnosing CAH, as clinical pathology often cannot differentiate it from other inflammatory liver diseases. With increasing literature reports of CAH in canines, this study evaluates the use of rolling medians as a statistical tool to assess the temporal stability of large datasets for generating canine liver copper RIs indirectly.

Methods:
Data included 1,821 observations for canine liver copper submitted to the MSU Veterinary Diagnostic Laboratory between April 2015 to December 2023. A composite, static, indirect RI was initially generated for the dataset. The indirect method involved filtering the data based on age and literature-based thresholds for toxicosis. The remaining values were filtered further using a Latent Abnormal Values Exclusion (LAVE) method, which iteratively removes cases deemed abnormal with respect to measured quantities of other trace nutrients (cobalt, copper, iron, manganese, molybdenum, selenium, and zinc) observed outside of their central 95% range. To address inconsistencies in case number submissions and to transform the data into a time series format, median values were calculated by month. Rolling medians (k=7) were calculated from this data using a computational method coded in the software R to smooth short-term fluctuations and highlight trends temporally. Statistical analysis included a t-test of the linear regression for rolling medians.

Results:
The application of an indirect method for reference interval generation effectively identified a plausible lower quartile limit (90 µg/g) but yielded an upper quartile limit (985 µg/g) significantly greater than historic intervals (137 - 400 µg/g). This suggested marked inconsistencies in the data. Both a monthly median regression ($p < 0.004$) and a rolling median analysis of monthly data ($p < 2.0e-11$) revealed a positive temporal trend in liver copper concentrations over time

Conclusions:
The rolling medians approach detected a shift, or trend, in the data that may have otherwise been missed when looking at static data in its totality. This variability in monthly data implied an increased temporal contamination of the dataset with CAH cases. The dataset's inconsistency, influenced by unequal monthly sample sizes and increasing copper concentrations, rendered it unsuitable for indirect RI generation with the given exclusion criteria. However, the observed trends highlight a growing prevalence of CAH, underscoring the need for direct or more refined indirect approaches to liver copper RI establishment.

Presenter: Madelyn Marsh, MSU Veterinary Diagnostic Laboratory, madelynfmarsh@gmail.com

Poster 99
Beryllium Exposure and Workplace Medical Surveillance
L. Barker, National Jewish Health

Beryllium is a lightweight, thermostable, metal that can be found in both high tech metal and ceramics industries. It is most often found as an alloy where it confers its properties to other, less expensive metals. US exposure estimates of 64,000 - 134,000 currently exposed workers in both private industry and in some federal facilities. Since the OSHA beryllium rule was published in 2017, employers are required to offer medical surveillance to workers exposed or likely to be exposed at or above the action level of 0.1 ug/m3 for 30 days or more per year. Individuals with genetic susceptibility to beryllium are at risk of beryllium sensitization (BeS) with exposures around the current PEL of 0.2 ug/m3, and regular medical surveillance is an important tool in risk reduction for all workers but particularly those who are susceptible. An abnormal BeLPT in an exposed worker can be important for the individual worker, and also those who work in the same area, as an abnormal response could indicate a work area where exposure levels are elevated.
OSHA compliant medical surveillance includes metrics related to exposure levels and periodicity of exams to provide on-going confidence in the safety of the workplace and includes: medical and work history, pulmonary function tests (PFT), beryllium lymphocyte proliferation test (BeLPT), and low dose chest CT when recommended by the health care provider. Exam results are shared with the employee including any need for work restriction or follow-up with a medical provider. Employers are provided with the date of exam and an explanation of work restrictions (if any) needed as a result of findings on workers exam.
The BeLPT is an immunologic test that detects a response to an antigen (beryllium) in the individual's blood. This cell-mediated immune response defines beryllium sensitization (BeS) in an exposed worker. There are no symptoms associated with BeS. Approximately 2-20% of exposed workers will develop BeS, depending on exposure level in a facility. A worker needs to proceed to clinical evaluation which includes: comprehensive occupational/medical/social history, exercise testing, blood work, enhanced PFTs, bronchoalveolar lavage (BAL) with transbronchial lung biopsies to identify non-caseating granulomas in the lung. This finding, along with abnormal BeLPT, is the hallmark of progression to chronic beryllium disease (CBD). BeS progresses to CBD at 6-8% per year. CBD is often mistaken for other respiratory diseases. This is one of the reasons it is so important to identify beryllium exposed workers with health effects and provide long-term follow up to maintain the highest quality of life for the longest duration.
Beryllium is an important metal, used in numerous applications. Public health professionals should be aware of its use and potential health effects in their state and local industries. It is also important to understand that beryllium exposure confers a lifelong risk of BeS and CBD. These workers may develop non-specific respiratory disease as older individuals and understanding past beryllium

exposure can assist with an appropriate diagnosis and appropriate treatment for these workers.

Presenter: Lisa Barker, National Jewish Health, barkerl@njhealth.org

Poster 100
Rapid Detection and Response to a Local Brucellosis Cluster in North Carolina
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Brucellosis is a zoonotic infectious disease that can be caused by one of four clinically relevant Brucella spp. Consumption of unpasteurized dairy products from infected animals is the most common route of transmission. The species associated with brucellosis are categorized as BSL-3 agents due to the reported low infectious dose and ease of aerosolization. Clinical laboratorians that unknowingly manipulate cultures derived from cases of brucellosis, in the absence of BSL-2 facilities and/or BSL-3 work practices, are at elevated risk for infection. Since these types of facilities and practices are not universal in clinical labs and laboratorians typically aren't informed of suspected brucellosis in order to take necessary precautions, it is commonly reported as a laboratory-acquired infection.

The North Carolina State Laboratory of Public Health (NCSLPH) works collaboratively with epidemiologists at the State and Local level and sentinel laboratory staff to investigate cases where bacterial isolates cannot be ruled out when using the ASM Sentinel Level Clinical Laboratory Guidelines for Brucella spp. When these cases occur, members from each team come together on a conference call to discuss the case and the lab work practices used to develop a risk assessment for potential lab exposures.

Between October and December 2024, the NCSLPH facilitated the timely identification of a local brucellosis cluster and conducted laboratory risk assessments at 2 sentinel laboratories that processed and tested isolates from the 3 hospitalized patients. The isolates could not be ruled out as Brucella spp. and were quickly transported to NCSLPH for rapid molecular detection, followed by traditional biochemical characterization using established LRN assays. All isolates were confirmed to be B. melitensis and were sent to the CDC for further molecular characterization. Based on sequence analysis at CDC, the clinical isolates were determined to be highly genetically related suggesting a single source of transmission.

The epidemiological investigation revealed that all cases consumed unpasteurized imported goat cheese. Remnant cheese from the affected households was not available; but a sample of locally sold unpasteurized imported cheese was submitted to CDC for testing. This sample was negative for Brucella spp.

Lab exposure risks were identified in the 2 sentinel labs that processed and tested isolates from the 3 hospitalized patients. The initial processing steps used in both labs were conducted within a biosafety cabinet. However, downstream processes, such as, Gram staining, oxidase testing, and VITEK® 2 slide preparation were conducted on an open bench, leading to 6 high-risk laboratory exposures.

Due to the 6 high-risk laboratory exposures observed and the potential for additional cases in the community, the NC DHHS's Division of Public Health distributed a memo to NC healthcare providers, to advise of the potential risk of additional cases of brucellosis. Furthermore, NCSLPH Outreach Consultants provided refresher training to sentinel labs to reinforce safe work practices that will limit lab exposures when handling Gram negative isolates derived from blood cultures prior to the completion of rule-out testing.

Presenter: Leticia Barton, NC State Laboratory of Public Health, Leticia.Barton@dhhs.nc.gov

Poster 101

Response of the Nebraska Public Health Laboratory to a Sensitive Public Records Request

P. Iwen, Nebraska Public Health Laboratory, J. Khoury, University of Nebraska Medical Center, J. Klintoe, University of Nebraska, S. Koepsel, University of Nebraska Medical Center, E. McCutchen, Nebraska Public Health Laboratory

IIntroduction: According to state law, the public has an opportunity to inspect or obtain copies of public records that "are of or belonging to" any public body within the State of Nebraska. As a result, the University of Nebraska, is subject to the Nebraska public records statutes. There are, however, instances whereby records may be withheld from the public. This report describes a process taken by the Nebraska Public Health Laboratory (NPHL) to respond to a sensitive public health record request. Scenario: On Day 0, the NPHL received a request from a "news organization" for CLIA validation/verification documents ensuring compliance to 42 CFR, 493.1253 for the following tests: Ebolavirus, Burkholderia pseudomallei, smallpox, lead filter paper blood spot, lead capillary whole blood, and lead venous whole blood. The Associate General Counsel (AGC) for the University of Nebraska (NPHL is under administrative support of the university) was informed of this request. On Day 4, the AGC submitted to the requester a copy of the NPHL's CLIA Certificate of Accreditation and CAP Certificate of Accreditation confirming compliance to CLIA. On Day 9, the requester replied, "your response may have some oversight" and again requested the validation/verification documents for the specific tests that were issued or modified after 24Apr2003. On Day 15, NPHL contacted LRN-B Operations to inquire if other LRN-B laboratories had received a similar request, indicating a more widespread inquiry. LRN-B Operations responded on Day 16 that they were unaware of any other requests and would continue to monitor this situation. On Day 22, NPHL contacted advisors at CAP and CLIA/Centers for Medicare and Medicaid Services (CMS) to inquire if validation/verification documents were public domain information. On Day 24, the CMS inspector responded that "federal FOIA rules do not apply here since they are not requesting federal records" and the CAP response was that "CAP does not provide validation data unless requested by a federal request" (suggesting that responding to the request was up to the laboratory). On Day 43, the AGC responded to the requester "the requested documents are not subject to disclosure as they are quality assurance materials protected by Nebraska statutory peer review privileges (Neb Rev Stat 71-7903)". On Day 70, the requester challenged the decision to withhold these documents to the NE Attorney General (AG). The AG agreed on Day 84 with the claim of "peer review privileges" to not release the documents. There was no further response from the requester (as of 31Dec2024). Conclusion. Ultimately, the AG agreed with the reply that release of these sensitive documents "could potentially result in substantial or debilitating impact to our state and nation's ability to effectively respond to bio- and chemical terrorism events should reverse engineering or exploitation of limitations of the test system be explored". The scenario describe provides guidance to others who might encounter a similar request for laboratory documents.

Presenter: Peter Iwen, Nebraska Public Health Laboratory, piwen@unmc.edu

Poster 102

Detection of the Lassa Fever Virus by the Nebraska Public Health Laboratory

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Introduction. Historically, the detection of the Lassa fever virus (LFV) has only been available through federal laboratories such as the Centers for Disease Control and Prevention (CDC). Recently, the BioFire® FilmArray® Global Fever Special Pathogens panel (GFSPP) (bioMerieux, Inc, Cambridge, MA),) was FDA-cleared to test for 14 targets to include LFV. This report describes the use of the GFSPP at the Nebraska Public Health Laboratory (NPHL) to identify a rare case of LFV infection in the US. Pre-analytical. NPHL was notified by the Iowa State Epidemiologist on 27Oct2024 at 1645, that a person under investigation (PUI) at the University of Iowa Health Care in

Iowa City required diagnostic testing to rule-out/refer LFV. Seven blood specimens were collected in EDTA vacutainer tubes in 1921 in Iowa City, IA packaged for category A shipment and transported by car using a government courier to the NPHL in Omaha, NE (approximately 235 miles). The shipment was received at 2308 and via a chain-of-custody process, was accessioned for testing at the NPHL. Analytical. One EDTA blood specimen was processed and tested by the GFSPP following manufacturer's instructions in a biosafety level 3 laboratory. The results of specimen testing were available at 0111 on 28Oct2024 and reported as "presumptive-positive for LFV RNA by RT-PCR". Post-analytical. The Federal Select Agent Program (FSAP) was notified of a presumptive identification of LFV via e-mail on 28Oct2024. NPHL, in consultation with the CDC Special Pathogens Lab (SPL), packaged seven blood specimens (which had been initially sent to NPHL) in a category A container with dry ice and transported these via FedEx overnight to the CDC in Atlanta. Specimens were received at the CDC at 0722 on 29Oct2024. On 30Oct2024 at 2155, the CDC reported "test results are positive; this is consistent with an acute infection caused by Lassa virus". An additional e-mail from the SPL was received on 08Nov2024 at 1114 that stated, "(the CDC SPL) isolated infectious Lassa virus from the blood specimen". Sections C and D of Form 4 were completed following this confirmed identification of LFV by culture for submission to FSAP. Subsequent PUI testing completed at NPHL included three additional tests with an average of 2 hours from specimen receipt to result reported. Conclusion. Prior verification of the GFSPP at the NPHL allowed for rapid identification of a LFV infection within approximately 2 hours after specimen receipt in the laboratory. This situation highlighted the need to expand the laboratory network to have strategically available CLIA-approved testing for high consequence pathogens (HCPs). Dialogue among the laboratories that support the LRN-B programs and the laboratories that support the 13 Regional Emerging Special Pathogen Treatment Centers (RESPTC labs) should be considered to enhance this network. Additionally, the ability to transport specimens that might contain a HCP continues to be problematic and requires clearer guidance from overseeing regulatory agencies of not only when a specimen becomes a select agent but also about how these specimens can be transported by commercial couriers to optimize patient management.

Presenter: Peter Iwen, Nebraska Public Health Laboratory, piwen@unmc.edu

Poster 103

Rare Case of Septicemia Caused by *Salmonella enterica* serotype Gaminara Linked to Exposure to a Pet Bearded Dragon

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Introduction: Salmonellosis is a major cause of illness in the United States with most of the cases linked to ingestion of contaminated food. However, Salmonella is endemic among reptiles, which can be shed in their feces, creating a path for transmission to pet owners. We present a case of septicemia caused by exposure to a bearded dragon that was shedding Salmonella enterica serotype Gaminara (Salmonella Gaminara).
Case Report: An adolescent presented to a hospital with septicemia, bloody diarrhea, nausea, vomiting, and fever. Blood and stool samples were collected, and both were culture positive for Salmonella species, not serogroup A, B, C, D, E, G, and Vi. The isolate was susceptible to ampicillin, ceftriaxone, and trimethoprim/sulfamethoxazole. The patient was not hospitalized and was prescribed oral azithromycin with full recovery. Epidemiological investigation revealed no other household contacts were ill. Within the timeframe of exposure, the patient attended a childcare center but had no recent travel history or exposure to recreational water. The patient owned the bearded dragon for 17 days before getting sick There was exposure to a pet bearded dragon that the patient received as a gift 17 days before the illness began. The patient and the patient's father were the main caretakers and had multiple exposures to the reptile and the animal's environment.
Bacteriology: A blood culture from the patient and stool sample from the bearded dragon both were culture positive for Salmonella. Whole genome sequencing (WGS) was conducted on the samples

and analyzed with BioNumerics pipeline (as part of the federal PulseNet program). Both samples were identified as Salmonella Gaminara (serogroup I), however during sequencing a QC error was noted with the bearded dragon isolate indicating the potential for multiple strains of Salmonella present. The stool sample was replated and allowed to incubate longer. Subsequently, multiple Salmonella isolates were identified. Following WGS of two isolates, Salmonella Gaminara and Salmonella, serogroup F, unsubtypeable were identified. Upon bioinformatics analysis, the Salmonella Gaminara, was determined to be indistinguishable from the patient isolate.
Conclusion: This case represents the pairing of an epidemiological investigation with genomics to identify the root cause of a rare case of septicemia caused by Salmonella Gaminara. The mixed isolates from the breaded dragon sample demonstrated the complications that can occur with sequencing that can make a challenge to link an environmental source (bearded dragon) with the clinical case.

Presenter: Michael Wiley, Nebraska Public Health Laboratory, mike.wiley@unmc.edu

Poster 104
Genomic Diversity, Antibiotic Resistance and Stress Adaptation of *Salmonella* Isolates: Insights from a Four-year Surveillance Study in New Hampshire
A. Diamond, New Hampshire Public Health Labs, C. Benton, New Hampshire Public Health Labs

Background
Salmonella is a leading cause of foodborne illness globally, presenting significant public health challenges due to its ability to acquire antibiotic resistance and adapt to environmental stressors. These adaptations increase the complexity of treatment and the risk of outbreaks. Genomic surveillance is essential for monitoring Salmonella's genetic diversity, detecting emerging strains, and informing public health strategies. This study examined the genomic and phenotypic features of Salmonella isolates collected over four years (2020–2023) from clinical samples in New Hampshire.

Objectives
This research aimed to characterize the genomic diversity, antibiotic resistance, and stress adaptation mechanisms of Salmonella isolates over time. By identifying novel or atypical strains, the study sought to improve understanding of Salmonella’s evolution and inform public health interventions.

Methods
DNA was extracted from clinical Salmonella samples and sequenced to generate paired-end FASTQ files. The Terra platform, developed by the Broad Institute and MIT, was used for analysis. Sequence data were retrieved from the NCBI Sequence Read Archive using the SRA_Fetch workflow. Genomic analyses included the TheiaProk_Illumina_PE_PHB workflow for de novo assembly, quality assessment, sequence typing, and antimicrobial resistance detection, and the Snippy_Variants_PHB workflow for reference-based SNP identification. Quality metrics (assembly length, N50, GC content, coverage) were evaluated to ensure sample suitability. Phylogenetic clustering was conducted using kSNP3_PHB and Snippy_Tree_PHB, generating core- and pan-genome phylogenetic trees to identify distinct isolates and their relationships.

Results
More than 500 clinical Salmonella isolates were analyzed, revealing extensive genomic diversity across sequence types (STs) and serotypes. Several isolates lacked predicted sequence types, highlighting the need for enhanced sequence typing methods. Adaptive variations were observed in stress resistance genes (e.g., gold, mercury, arsenic resistance) and virulence factors (e.g., iroC/B, sinH, iucA). Notably, some isolates exhibited significant genomic divergence, suggesting the emergence of novel strains. Antibiotic resistance patterns remained consistent, with frequent resistance to aminoglycosides, quinolones, and tetracyclines.

Significance
This study highlights the critical role of genomic surveillance in identifying emerging Salmonella

strains, monitoring resistance trends, and informing outbreak response strategies. The Terra platform's robust workflows enabled reliable phylogenetic insights and revealed gaps in current reference databases. Strengthened collaboration with clinicians for timely sampling and reporting is essential to improve outbreak detection, reduce Salmonella-related illnesses, and guide public health interventions.

Presenter: Aron Diamond, New Hampshire Public Health Labs, aron_diamond@yahoo.com

Poster 105
Development of Modified EPA Method 1668 for the Detection of 209 PCB Congeners in Fish Tissues
K. Balaji, New Jersey Department of Health, S. Du, New Jersey Department of Health, C. Riker, New Jersey Department of Health, Z. Fan, New Jersey Department of Health

A growing body of scientific literature has associated Polychlorinated biphenyls (PCBs) with a multitude of health concerns, and the detection of these pollutants is a pertinent undertaking. The NJDOH-ECLS is prompted to develop the inquired methods from Bureau of Freshwater and Biological Monitoring (BFBM) of NJDEP to perform whole tissue analyses of PCBs collected from their fish tissue monitoring program. We aim to develop and validate effective method by integration with modern automated sample preparation instruments to reduce solvent and labor cost in comparison to the original EPA method 1668B. Specifically, we have developed and validated the selective accelerated solvent extraction (ASE) in reference matrix by combining a serial of adsorbents in extraction cells, and developed the effective Gel Perpetration Chromatograph (GPC) for the subsequent clean-up of fish tissue extracts, followed by the automated Solid Phase Extraction (SPE) modules for lipid removal. In addition, we have developed instrumental analysis method for the successful separation, detection, and quantification of all the listed PCBs in EPA method using Isotope Dilution techniques. This developed method significantly reduced the sample preparation from over a week to 3 days. The reproducibility of 28 spiked reference material was below 20% for all the analytes. Mean recoveries (n=7) of the spiked samples ranged from 40% to 74% and 61% to 108% for labeled and native compounds respectively. The method development results demonstrated that this method was precise, accurate and robust with high-throughput potential that suitable for monitoring studies.

Presenter: Keerthi Balaji, New Jersey Department of Health, keerthibalaji2024@gmail.com

Poster 106
Short-term and Long-term Frameworks for Public Health Laboratories Responding to the Public Health Mercury Crisis
E. Bind, New Jersey Department of Health, Z. Fan, New Jersey Department of Health

Mercury is a toxic metal to which people are exposed routinely through diet (e.g., fish and seafood), preservatives (e.g., thimerosal), consumer product (e.g., lightbulbs, thermometers), and cultural practices (e.g., religion, skin lightening product [SLPs]). Acute and chronic exposure to mercury can cause many adverse health outcomes in adults, including neurological, cardiovascular, and reproductive disorders. These effects are even more pronounced in children, especially developing fetuses where adverse birth outcomes that persist into adulthood can occur. Data from NJ Biomonitoring (NJB) indicates a mercury public health concern that is echoed by other public health jurisdictions nationally and globally. NJB data indicate >11% of the state’s population have blood mercury levels above our 5 µg/L health limit and that 60% of babies tested through the prenatal screening work are exposed to mercury at levels that could potentially cause adverse health outcomes. Mercury is a public health crisis that has remained inconspicuous until lately; therefore, few public health laboratories (PHLs) have the knowledge of the issue, expertise, and resources allocated to support residents exposed to mercury in their jurisdictions. This poster will provide a) a background of the mercury issue, b) NJB exposure data, c) opportunities to leverage resources already existing in PHLs to provide immediate, short-term solutions to individuals and communities,

and d) a framework for long-term solutions.
An ideal mercury public health response has a three-pronged approach aimed at a) reducing public demand for products containing mercury, b) reducing the supply of mercury in the market, and c) screening to identifying exposed individuals and contaminated goods. PHLs can leverage their current outreach capabilities to raise awareness to reduce demand (a). PHLs with instrumentation (e.g., XRF, mercury analyzers) can test products and provide data to their jurisdiction's existing enforcement branch in support of solutions b and c. Further, PHLs with existing LRN-C level 1 or 2 capabilities already have the capability to screen blood and urine for mercury. Jurisdictions can rely on their existing tracking expertise (e.g., child lead, occupational exposure) to begin their mercury response efforts.

In the long-term, NJB has identified mercury-related public health gaps including the lack of reference values, screening policies, medical treatment protocols, tracking programs, environmental response protocols, and more. The mercury public response can build upon historically successful responses (e.g., lead) as PHLs design their mercury tracking programs. These programs need to include provisions for outreach/communication, case management, field operations, enforcement and compliance, data management, and laboratory analysis. Examples of expertise needed by a comprehensive program includes toxicology, epidemiology, health education, investigation, instrumentation, hazardous materials remediation, and data analysis.

Due to the short half-life of mercury in blood (1-2 months vs. years for some PFAS), individual exposure levels can be reduced dramatically in a short amount of time. These short- and long-term solutions can help PHLs prepare and implement their response to the mercury crisis, and ultimately, improve health outcomes.

Presenter: Eric Bind, New Jersey Department of Health, Eric.Bind@doh.nj.gov

Poster 107
How the NJDOH-ECLS Uses the CDC-APHL Fellowship Program to Increase Capacity and Improve Public Health Service Provision
E. Bind, New Jersey Department of Health, C. Yu, New Jersey Department of Health, A. Steffens, NJDOH, E. Cook, New Jersey Department of Health, L. Zhong, NJDOH, S. Du, New Jersey Department of Health, C. Xu, NJDOH, D. Wene, NJDOH, Z. Fan, New Jersey Department of Health

The NJ Department of Health (NJDOH) – Environmental & Chemical Laboratory Services (ECLS) expanded its range of services significantly to better address emerging public health issues in the past 10 years. Beyond water testing to support regulatory efforts, ECLS developed biomonitoring, food, and cannabis testing programs. Expansion requires analytical method development, outreach, and data management and analysis. The expansion of the activities requires significant manpower and skill to conduct. ECLS leveraged the great opportunity created by the CDC-APHL Public Health Laboratory Fellowship Program (PHLFP) to successfully onboard 18 fellows since 2021. While the fellows provide their talents and manpower to ECLS, the extensive experiences and mentoring expertise of the ECLS also provide great training opportunities to the fellows, matching the core goals of the PHLFP, i.e., training the future of the public health workforce. The recent creation of Environmental Health (EH) and Informatics fellowships have proven invaluable to ECLS.

ECLS onboarded the 18 fellows to conduct clinical metals analysis, toxic metals in food analysis, biomonitoring implementation and data management, method development for a variety of chemicals/matrices (PFAS in water and fish, drugs of abuse, and LRN-C testing), and upgrading the LIMS. Of the 18 fellows, 4 are still in their first year, 9 extended for the optional second year, 4 were hired full-time by NJDOH, 2 entered medical school, 1 entered a master's program, 3 entered the public health workforce elsewhere, and 1 entered the US Fulbright Program. During this time, ECLS also brought in a CDC PHAP associate to conduct biomonitoring work and interns to support every program. ECLS mentors found the streamlined PHLFP application process and periodic reporting requirements to be more straightforward and easier to navigate than other programs. The project funds provided by APHL were used for supplies required for project implementation and to increase

NJDOH's capabilities and capacity, providing another benefit from the program.

Overall, the fellows are professional, talented, productive, and innovative. They have collectively contributed significantly through the development of a half dozen new analytical methods, the improvement in efficiency of existing methods, the analysis of >10,000 specimens and samples, the recruitment and consenting of nearly all the statewide biomonitoring study participants, the extraction, management, and analysis of thousands of data points, outreach and education at community events, and much more. Beyond the APHL-provided training and support, ECLS provides important technical and professional experience through individual training and group mentoring to prepare the fellows for their careers. Select mentors offer addition leadership and soft skills training. Notably, ECLS mentors find the PHLFP to be the best and most mutually beneficial fellowship program with whom it has partnered.

This poster will detail how ECLS has worked with the APHL fellowship program to enhance its workforce allowing for the completion of projects that would have otherwise not been feasible and how ECLS mentors helped prepare the fellows for their professional careers. ECLS will continue to find innovative ways to maximize the mutual benefits of the PHLFP.

Presenter: Eric Bind, New Jersey Department of Health, Eric.Bind@doh.nj.gov

Poster 108
Conducting Subject Matter Expert Calls with Study Participants with Elevated Results from the Ongoing NJHANES Biomonitoring Study
E. Bind, New Jersey Department of Health, A. Steffens, NJDOH, L. Zhong, NJDOH, C. Yu, New Jersey Department of Health, E. Cook, New Jersey Department of Health, Z. Fan, New Jersey Department of Health

Presenter: Eric Bind, New Jersey Department of Health, Eric.Bind@doh.nj.gov

Poster 109
Data Modernization in Practice: Streamlining Large-scale Environmental Testing for Statewide Childcare Drinking Water Through Integration of REDCap and LIMS
C. Donohoe, New Jersey Department of Health, C. Yu, New Jersey Department of Health, D. Haltmeier, New Jersey Department of Health, R. Litvak, New Jersey Department of Health, E. Berg, New Jersey Department of Health, Z. Fan, New Jersey Department of Health

The New Jersey Department of Health (NJDOH)-Environmental and Chemical Laboratory Services (ECLS), in collaboration with the Department of Children and Families' (DCF) Childcare Drinking Water Project, is providing free lead and copper testing for 4,000+ childcare centers in New Jersey. This testing is a requirement for each childcare centers' annual licensure renewal and a critical initiative directly impacting the health and safety of young children particularly vulnerable to the effects of lead and copper exposure through drinking water. This new initiative challenged ECLS with managing a large-scale project in a designated timeframe with limited resources, as the size and complexity of the project overwhelmed current ECLS capacity and strained the aged Laboratory Information Management System (LIMS). To address the time-consuming and error-prone manual processes of contacting, registering, and reporting thousands of samples, ECLS developed an integrated electronic solution.

The DCF Project streamlined data collection using an online survey platform (REDCap), significantly reducing manual entry errors, email correspondence, and centralizing data management. By developing R-based middleware to integrate participant survey information from REDCap with our LIMS, we have established a semi-automated workflow that can be adapted across multiple data systems. This integrated solution dramatically improved data quality by virtually eliminating transcription errors in data management and reporting, while simultaneously reducing staff time dedicated to these tasks by 80%. This project demonstrated how existing infrastructure and staff expertise can be leveraged to address immediate data challenges while building toward broader

modernization goals.

The Centers for Disease Control and Prevention (CDC) established the Data Modernization Initiative (DMI) to transform the nation's public health data infrastructure and management. The DCF Project exemplifies how ECLS is advancing practical implementation of DMI through targeted workflow enhancements, strengthening our ability to protect public health through efficient, data-driven decision making, and adapting to evolving public health challenges.

Presenter: Colleen Donohoe, New Jersey Department of Health, Colleen.Donohoe@doh.nj.gov

Poster 110

Strategic Modernization of Public Health and Environmental Laboratory's LIMS: A Case Study of NJDOH-ECLS's Transition from ELEMENT® to Clinisys®

C. Yu, New Jersey Department of Health, D. Yoder, New Jersey Department of Health, C. Donohoe, New Jersey Department of Health, Z. Fan, New Jersey Department of Health

The Laboratory Information Management System (LIMS) is essential for automating routine laboratory processes, including sample login, batching, analysis, and result reporting. The Environmental Chemistry Laboratory Services (ECLS) within the New Jersey Department of Health (NJDOH) has utilized Promium's ELEMENT® system since 2009. ELEMENT efficiently manages environmental samples while maintaining compliance with the Environmental Protection Agency (EPA) and New Jersey Department of Environmental Protection (NJDEP) regulations. In 2014, ECLS expanded its analytical capabilities to include clinical specimens and diverse food matrices, supporting Centers for Disease Control and Prevention (CDC) state biomonitoring grants (2014-2024) and Food and Drug Administration (FDA) food safety initiatives (2020-2025). This expansion exposed several critical limitations in the current system's architecture and functionality.
This LIMS modernization was driven by key challenges: inability to handle Health Level 7 (HL7) messaging, local-only data management preventing direct sharing data with public or clients, and lack of integration with external systems such as Electronic Test Orders and Results (ETOR) and Electronic Laboratory Reporting (ELR) capabilities required by the medical/clinical field. After comprehensive vendor evaluation, ECLS selected Clinisys® over STARLIMS® based on technical capabilities, support services, ease of migration from ELEMENT®, and the State procurement requirements. Primary lessons learned during the selection process included the importance of thorough requirement analysis, compatibility with existing infrastructure, operational efficiency, and cross-divisional coordination. The remaining challenges include critical considerations in data migration strategy, system validation protocols, regulatory compliance documentation, and comprehensive staff training requirements.
The ECLS LIMS upgrade, targeted for 2027, has provided valuable insights for other state laboratories. Key success factors included early involvement of State OIT and NJDOH IT specialists, establishment of clear communication channels with parties involved, and development of comprehensive validation protocols. The transition to a serverless environment presents unique challenges in data security and system architecture that can be addressed through iterative planning and implementation. The upgraded LIMS will deliver optimized workflows, enhanced interoperability, and robust data exchange capabilities (e.g., real-time data sharing, automated reporting) for the ECLS. These improvements, combined with lessons learned from the modernization process, provide a valuable framework for other state laboratories undertaking similar LIMS upgrades.

Presenter: Chang Ho Yu, New Jersey Department of Health, Chang.Yu@doh.nj.gov

Poster 111

Survey Weighting Methods and Initial Health Outcome Findings from the New Jersey Health and Nutrition Examination Survey (NJHANES)

C. Yu, New Jersey Department of Health, C. Donohoe, New Jersey Department of Health, M. Sirimis, New Jersey Department of Health, K. Lam, New Jersey Department of Health, E. Cook, New Jersey Department of Health, E. Bind, New Jersey Department of Health, Z. Fan, New Jersey Department of Health

The New Jersey Health and Nutrition Examination Survey (NJHANES) is the first statewide biomonitoring initiative to assess environmental exposures and health outcomes among residents of New Jersey (NJ). The study aims to recruit 500 NJ residents aged six years and older for the assessment. Employing a multistage, probability-based sampling design, participants were recruited by mail from randomly selected households, stratified and clustered from census tracts throughout NJ. The survey includes demographics and health questionnaires, physical examinations, and laboratory analysis of blood and urine samples, providing a comprehensive dataset of environmental exposures and public health indicators.
As of today, 503 sample persons (SPs) out of 734 respondents have been successfully enrolled, with field visits, specimen collection, physical measurements, and survey collection completed for 430 SPs. Remaining collections and subsequent analysis/results reporting are currently underway. Given the complex survey design, challenges such as recruitment bias and data discrepancies may arise. To address these, the NJHANES team developed and implemented a systematic approach that included 1) appropriate sample weighting procedures, 2) variance estimation methods, and 3) reliability measures for population estimates.
The effectiveness of these statistical methodologies was demonstrated through initial health outcome analyses, which revealed meaningful relationships between environmental exposures and health conditions. The multinomial logistic regression analysis, while controlling co-variates (i.e., diet, physical health, gender, race/ethnicity, and income), explored associations between toxic blood metals (cadmium, lead, and mercury) and serum perfluoroalkyl and polyfluoroalkyl substances (PFAS), and health outcomes such as obesity and hypertension. Initial results suggest significant associations: cadmium and mercury were associated with obesity risk, while lead and specific PFAS compounds (perfluorooctanoic acid [PFOA] and perfluorohexane sulfonic acid [PFHxS]) were associated with hypertension risk.
These findings demonstrate the feasibility of conducting state-level population surveillance with statistical rigor comparable to national efforts such as the Centers for Disease Control and Prevention's (CDC's) National Health and Nutrition Examination Survey (NHANES). Early results highlight state-specific patterns of environmental exposures and associated health risks for metals and PFAS exposures. NJHANES provides meaningful, state-specific data for health assessment and environmental exposure monitoring, as well as valuable insights into localized public health challenges.

Presenter: Chang Ho Yu, New Jersey Department of Health, Chang.Yu@doh.nj.gov

Poster 112
Building a Public Health Laboratory STEM Program for High School Students in New Jersey
T. Mizglewski, New Jersey Department of Health - Public Health and Environmental Laboratories, S. Patel, New Jersey Public Health and Environmental Laboratories

A 2024 National Science Foundation (NSF) report shows the number of STEM graduates exceeding STEM jobs with some vacancies seen in specialties and in remote locations. This implies adequately prepared numbers and types of job candidates. Concurrently, New Jersey 8th Grade Student Learning Assessments (NJSLA) for 2022-2023 indicate only 37.6% proficiency in Math and only 24.9% proficiency in science with an expanding computer science K-12 curriculum. On balance, the need for a more STEM-savvy student body is borne out by the ever-growing need for public health laboratory workplace skills in statistics, computer sciences and AI.

Our focus in building a STEM program in New Jersey has been five-fold: 1) Build a sustainable internal infrastructure with newly hired scientists; 2) build relationships with local Community Based Organizations (CBOs) with a shared focus; design student experiences which reflect current workforce STEM skills, and highlight public service; serve a local underserved community; 5) provide a leadership experience for interns (lead Committee meetings)

To those ends we formed a committee composed of new and experienced scientists and educators, led by interns, wrote a STEM Committee Charter, defined goals and objectives for the year, built

relationships with the Trenton Area STEM Counsel (TASC) members and reached out to additional partners at the NJ Department of Education STEAM Program and the NJ Department of Agriculture. The program, to be offered July 2025, will include the following five hands on experiences for up to 100 TASC students: 1) DNA/RNA Extraction and Sequencing - Students perform mock DNA extraction, compare sequences to references, and identify variants; 2) Vector-Borne Parasitic Infections - Students screen mock blood samples, observe infected vs. uninfected cells under a microscope, and identify parasite stages related to human infections; 3) Biomonitoring/Chemical Terrorism - Students modify R code, analyze pre-collected data, and create reports with graphs, tables, and figures; 4) Metals Lab - Students test water samples using ICP-MS, measure lead concentrations, and fit concentration curves; 5) Newborn Screening - Students use mock blood samples, load gels, simulate running them, and interpret results based on varied outcomes.

Presenter: Tyler Mizglewski, New Jersey Department of Health - Public Health and Environmental Laboratories, Tyler.Mizglewski@doh.nj.gov

Poster 113

Advancing Public Health Laboratory Innovation Through Strategic Data Science Internships

S. Patel, New Jersey Department of Health - Public Health and Environmental Laboratories, S. Mikorski, New Jersey Public Health and Environmental Laboratories

Public health laboratories face increasing demands to leverage innovative technologies to address emerging health challenges. One means the New Jersey Public Health and Environmental Laboratories (NJ PHEL) is using to cultivate a skilled workforce and create data-driven solutions is to engage interns in projects which illustrate these solutions across the organization. This year our internship program focused on recruiting talented computer and data science students from leading New Jersey universities, including Rutgers University, NJIT, Rowan University, and The College of New Jersey who come prepared to meet these challenges.
Over the past three semesters (Spring, Summer, and Fall 2024), the program received close to 200 applications and hosted 20 interns across diverse projects. Key initiatives included:
• Developing AI-powered tools: Utilizing computer vision for parasite detection in blood smears and machine learning for improved malaria diagnostics.
• Enhancing disease surveillance: Implementing data-driven approaches to monitor H5N1 influenza in livestock and visualize COVID-19 genomic data for actionable insights.
• Modernizing laboratory operations: Automating sample submission workflows, optimizing equipment maintenance and inventory tracking, and streamlining data management for environmental and chemical analyses.
• Addressing current workforce needs: Conducting a training needs assessment to develop learning pathways for public health professionals in data science and analytics.

These projects provide interns with invaluable hands-on experience, fostering a deep understanding of the public health data lifecycle and equipping them with the skills to contribute meaningfully to future challenges. In turn, our staff and management learn more about the skills new graduates can bring to the table to meet our needs.

The NJ PHEL internship program demonstrates the power of strategic partnerships between academia and public health. By integrating cutting-edge data science into core laboratory functions, this program not only strengthens the public health workforce but also drives innovation and improves public health outcomes for the entire state.

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Poster 114

Assessing Data Modernization Skill Level in New Jersey Public Health Laboratory Personnel

S. Patel, New Jersey Department of Health - Public Health and Environmental Laboratories, J. Studebaker, New Jersey Public Health and Environmental Laboratories

A Data Modernization survey was conducted for the purpose of assessing the level of knowledge and experience among New Jersey Public Health and Environmental Laboratory (PHEL) technical and scientific staff members in the areas of programming languages, data analysis/management/visualization, statistical analysis tools, coding, and artificial intelligence. The goal of the survey, combined with a study of use cases, is being used to ultimately determine training needs and develop a Data Modernization training plan.
The survey was developed in summer of 2024 with the assistance of inhouse Subject Matter Experts (SMEs) and a summer MPH intern from Boston University and funded by the Centers for Disease Control and Prevention (CDC), Epidemiology and Laboratory Capacity (ELC) Cooperative Agreement #6 NU5OCK000525-01-04 for the purpose of laboratory workforce development. One hundred and forty-five (145) personnel were surveyed using MS Forms within the following service areas: Public Health Laboratory Services (PHLS), Clinical Laboratory Improvement Services (CLIS) and Environmental Chemical Laboratory Services (ECLS). The participants included the following Civil Service title series: Microbiologists, Chemists, Research Scientists, Clinical Laboratory Evaluators, Lab Technicians, Medical Technologists, Quality Assurance Officers and Laboratory Technicians. The survey measured the participants' self-perceived abilities and level of experience in programming languages, data analysis/management/visualization, AI, and statistical analysis tools and how often they use them within their organizational unit.; Participants were given the option to remain anonymous. A desired response rate of 80% was set and the survey administered three times.
One hundred three responses were received. (103/145) 71%; of the responses received, 98 were included in the analysis. Apart from the Informatics team within the PHLS, and the Data Management and Analysis team within the ECLS, all staff reported limited experience with data analysis/management/visualization and statistical analysis tools. Most staff reported a good working knowledge of Excel.
Based on these results, a PHEL Data Modernization Task Force was formed comprised of representatives from these program areas: Virology, Microbiology, Newborn Screening, Inorganics, Organics, Biomonitoring and Blood Bank Improvement Services. These program representatives are charged to work alongside Advanced Informatics Subject Matter Expert (SMEs identify use cases for data modernization applications in each of the Service Areas and make recommendations regarding a training plan. Use cases to be identified should improve data quality, analysis, visualization and interoperability.
With these coding and data management skills, microbiologists and chemists can efficiently process, analyze and interpret the vast amounts of data generated in PHLs, streamline data transfer and centralize data management, contributing to improved disease surveillance, outbreak investigation and overall public health efforts.

Presenter: Satyam Patel, New Jersey Department of Health - Public Health and Environmental Laboratories, Satyam.Patel@doh.nj.gov

Poster 115
VectorSurv Helper: Automating the Transfer of Data from PCR Instruments to an Online Database
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We have developed VectorSurv Helper, an automated system to streamline processing and database entry of PCR test results for vector-borne disease detection in New Jersey. Laboratory staff members test samples, such as ticks and mosquitoes, for arboviral diseases using 96-well plates in PCR instruments and upload the results to the online VectorSurv (VS) database.

The process has three main components:

Windows Scheduler Task: Runs every five minutes to trigger a batch file.
Batch File: Monitors a designated network folder for newly exported files from PCR instruments.
Python Program: Processes these files, creating a review file and a VS import file.

The system can handle plates with multiple diseases per well and some empty wells. Although a manual step is necessary to initiate the bulk import into VS, the automated process significantly reduces the time, effort, and the probability of making manual transcription errors, thus enhancing accuracy efficiency in arboviral surveillance workflows and reporting.

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Poster 116

NYC PHL Quickie Labs for Routine Sexual Health Testing Services Showcase an Efficient Tool for Emergency Preparedness Efforts

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The PHL Quickie Labs offer rapid molecular testing for Chlamydia trachomatis (CT) and Neisseria gonorrhea (NG) for walk-in clients without prior appointment. The acceptance of self-collected specimens (urine, anorectal and/or oropharyngeal swabs) eliminates the requirement to see a physician, and the automatic reporting of results to a personal patient portal helps to reduce turnaround time (TAT).
Modeled after the Dean Street Express in London (UK) the first NYC PHL Quickie opened at Chelsea's NYC Health Department (DOH) Sexual Health Clinic (SHC) in June 2019. The PHL Quickies are equipped with Cepheid GeneXpert® Infinity48s systems, which ensure quick, accurate results and are easy to switch to different assays when needed due to a single cartridge system specific to the intended target pathogen(s).
During the COVID-19 pandemic response, the Quickie model demonstrated that investment in lab services makes it possible to pivot quickly from routine testing to detection and surveillance of an emerging pathogen of public health significance. Sexual health testing for CT/NG had been suspended during the peak of the COVID-19 pandemic, and the Chelsea Quickie was transformed into a "COVID Express Lab" along with 7 new developed sites during the summer 2020. Co-located with the NYC DOH's SHCs throughout the 5 boroughs in areas with existing health inequities, COVID Express labs provided underserved communities with enhanced access to critical testing services with the same day results. It was a rapid and effective emergency response during a time when COVID-19 test reporting through conventional health systems was not well established.
CT/NG testing at the Chelsea PHL Quickie resumed in July 2021 while continuing testing for SARS-CoV-2, Flu A and B and RSV until June 2022.
With decreasing demand of respiratory testing and in collaboration with DOH's SHCs, the PHL COVID Express Lab at the Fort Greene SHC (Brooklyn) was converted into a PHL Quickie in June 2022, offering CT/NG testing similar to the Chelsea's SHC.
After resuming STI testing at Chelsea's SHC in 2021, approximately 8,000 specimens were tested with 98.9% of results reported the same day of specimen collection. In 2022, the number of specimens increased to approximately 29,000 (including both Quickies) which resulted in a decrease of same-day reporting to 77.0%. In 2023, the number of specimens increased further to 46,000, however, optimization of specimen delivery from collection site to the lab in certain intervals throughout the day allowed for an improved same-day reporting rate of 84.6%. Furthermore, in August 2024 NYC PHL implemented a new Laboratory Information System (LIS) with auto verification of test results, and the number of same day reports increased from 88.5% (January - July 2024) to 98.8% (August - November 2024).
By combining existing patient-centered clinical services with on-site laboratory capacity supported by the PHL an efficient form of service was created. The main advantages of the PHL Quickie labs are: 1. self-collected specimens with real-time transport to the lab; 2. the short turnaround time for result

reporting and, consequently, 3. decreased median time to treatment.
The experience gained from this collaboration could be extended to other locations or for testing other infectious diseases of public health concern. The quick and nimble switch to Covid Express testing in the pandemic illustrated the benefits of the PHL Quickies as a framework for emergency preparedness efforts.

Presenter: Madina Shakirzyanova, New York City Public Health Laboratory, mshakirzyanova@health.nyc.gov

Poster 117
Detecting and Measuring Siloxane Isomer Contaminants in Various Water Sources
M. Chowdhury, New York State Department of Health, B. Duffy, New York State Department of Health, J. Wineriter, New York State Department of Health

Siloxanes, used in a wide range of consumer and industrial products, are contaminants of emerging concern. Cyclic D3, D4, and D5 isomers (hexamethylcyclotrisiloxane (D3), octamethylcyclotetrasiloxane (D4), decamethylcyclopentasiloxane (D5)) and linear L2, L3, L4, and L5 isomers are among the most commonly used. When these isomers are not effectively removed, they can enter different water sources and pose significant risks of environmental contamination. These short-chain methyl siloxane solvents have chemical and physical properties such as environmental persistence, lipophilic characteristics, and high vapor pressure, which further enhance their potential for water contamination and bioaccumulation. In New York State, inadequate solvent recovery practices have historically led to groundwater contamination from fluids used in dry cleaning and parts degreasing. Similar to these solvents, the selected siloxane isomers present similar level of environmental risks due to their common usage patterns. This work aims to construct an aqueous solid-phase extraction (SPE) method and quantitative instrumental analysis using gas chromatography-mass spectrometry (GC-MS). Preliminary full-scan GC-MS results have been analyzed for selective ion monitoring for each isomer. The developed method will then be used to examine more than twenty-five ground, surface, wastewater, and drinking water samples across New York State. Given the potential for siloxanes to bioaccumulate and contaminate water sources, this study aims to identify regions with high concentrations of siloxane contamination, thereby providing critical information to help mitigate environmental exposure.

Presenter: Maanal Chowdhury, New York State Department of Health, maanal2001@gmail.com

Poster 118
***Pseudomonas Aeruginosa* Clinical Isolates Dependence on Autoinducers for Quorum Sensing Gene Regulation**
E. Key, New York State Department of Health, E. Knorr, Carnegie Mellon University, J. Paczkowski, Wadsworth Center

Cystic fibrosis (CF) is an uncurable genetic disease characterized by a thick mucus lining in the lungs. This hyper mucoid lining creates an ideal environment for recurrent bacterial infections. Because of this, individuals with CF are at high risk for chronic bacterial infections, despite the use of antibiotics. Pseudomonas aeruginosa is a multidrug resistant bacterium that is often isolated from the lungs of CF patients. It is responsible for a large portion of chronic and acute hospital-borne infections. A key aspect of these chronic infections is the ability of P. aeruginosa to sense their environment via a mechanism of bacterial communication called quorum sensing (QS). QS is a cell density dependent process that allows bacteria to regulate virulence factor gene expression. QS is regulated by production and detection of homoserine lactone, an autoinducer produced by a LuxI-type synthase that binds to a LuxR-type receptor transcription factor. In P. aeruginosa, this pathway is driven by synthase/transcription factor receptor pairs lasI/R and rhlI/R and binding of autoinducer, 3OC12HSL and C4HSL, respectively. The production and binding of these autoinducers creates a positive feedback loop that coordinates bacterial behaviors important for establishing chronic infections. In addition to these systems, the Pqs system follows a similar mechanism in which the receptor PqsR bound to the quinolone molecule called PQS upregulates the pqsABCDE operon.

Relevant to our research, previous research showed that PqsE is required for virulence factors and pathogenesis through its interaction with QS transcription factor RhlR. The RhlR-PqsE complex allows for higher binding affinity and more stable binding of DNA, leading to higher levels of virulence gene expression. The primary objective of this study was to determine the important regulators of QS in P. aeruginosa CF clinical isolates. Using phenotypic assays, our data confirm that disrupting the RhlR-PqsE complex and the binding of autoinducer C4HSL cause a disruption of QS pathways. Without presence of C4HSL binding and/or PqsE dimerization there was a significant decrease in virulence factor production. However, RNAseq data indicate PqsE does not influence RhlR-dependent gene expression relative to the laboratory strains of P. aeruginosa. Thus, RhlR-dependent QS gene expression is predominantly C4HSL-dependent in these isolates. Overall, these findings will provide insight to the regulation of important gene expression for virulence and pathogenesis in CF infections.

Presenter: Elizabeth Key, New York State Department of Health, Elizabeth.key@health.ny.gov

Poster 119

Enhancement of a Carbapenemase-producing Organism Colonization Screening Workflow: Decreasing Time, Increasing Data and Maximizing Resource Capabilities

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Carbapenemase-producing organisms (CPOs) contribute to over 54K infections and more than 4K deaths per year in the US. Due to this public health burden, the Antimicrobial Resistance Laboratory Network (AR Lab Network) provides CPO colonization screenings (CS) to assess the spread of CP Enterobacterales, Acinetobacter and Pseudomonas species. The CDC recommends rectal swab CS with an initial dual swab specimen. A culture-first method is often utilized for carbapenem-resistant Acinetobacter baumannii (CRAB), but results require 3 workdays or more.
Wadsworth Center (WC), as the Northeast Regional AR Lab Network laboratory, wields a molecular first algorithm, applying 1 swab for initial PCR and preserving the remaining swab for culture of positive specimens. In this approach, screening of the “Big 5” most common carbapenemase genes blaKPC, blaNDM, blaVIM, blaOXA-48-like and blaIMP is performed with the Cepheid Xpert® Carba-R assay (Carba-R). The remaining Cepheid lysate, from the initial swab, undergoes DNA extraction by EMAG® (BioMerieux) followed by multiplex real-time PCR for blaOXA-23-like, blaOXA-24/40-like and blaOXA-58-like genes, specific for CRAB screenings.
WC’s algorithm has consistently produced all CPO CS gene screening reporting turnaround times of 1 workday or less for the past 5 years while preserving specimen material for culture and additional testing, even when all carbapenemase gene screening targets were assessed. Although the Carba-R assay is employed in AR Lab Network Regional laboratories, WC also provides reportable screening results for emerging blaOXA carbapenemases in under 24 hours from rectal swab accessioning, which is not widely utilized. An analysis of testing at WC over the past 5 years has also revealed that CPO CS using molecular methods, including Carba-R and CRAB, are more cost effective annually than screening all specimens using a culture-first approach.
Utilization of the WC testing algorithm improves efficiency, laboratory reporting and allows for more rapid healthcare protocol initiation and epidemiological response.

Presenter: Majie Foster, New York State Department of Health, Wadsworth Center, majie.foster@health.ny.gov

Poster 120

Feasibility of Wastewater Surveillance for Enteric Pathogen Detection: A Pilot Study in New York State

R. Robinson, New York State Department of Health: Wadsworth Center, J. Connors, Wadsworth

Center, A. Gray, Wadsworth Center, K. Law, Wadsworth Center, D. Wroblewski, Wadsworth Center, L. Mingle, Wadsworth Center, K. Musser, Wadsworth Center

OBJECTIVE
Bacterial infections present a significant threat to public health. Early detection through innovative surveillance methods is crucial to prevent outbreaks. While clinical testing requires patients to be symptomatic and seek healthcare, wastewater surveillance can detect pathogens from the 80% of American households served by municipal wastewater systems. This pilot study evaluates the feasibility of wastewater surveillance as a tool for monitoring common food- and water-borne bacterial pathogens.
METHODS
For testing, 500mL of wastewater influent was centrifuged for 10 minutes and the pellet was resuspended in PBS with 20% glycerol. The concentrated samples were inoculated into 5mL of Tryptic Soy Broth and grown overnight at 37°C. Samples were screened for enteric pathogens using the BIOFIRE® FILMARRAY® Gastrointestinal Panel. Enriched samples positive for Yersinia enterocolitica, Vibrio species, and/or Salmonella species were plated on the following selective agar plates: Cefsulodin-Irgasan-Novobiocin, Thiosulfate Citrate Bile Salts Sucrose Agar/CHROMagar Vibrio, and Xylose-Lysine-Tergitol 4/Salmonella CHROMagar Plus respectively. All enriched samples were cultured for Listeria using CHROMagar.
Suspected colonies of interest were confirmed via MALDI-TOF mass spectrometry and submitted for whole genome sequencing (WGS) through the GenomeTrakr network.
RESULTS
A total of 110 24-hour composite wastewater samples from 45 wastewater treatment plants across New York State were screened using the BIOFIRE® FILMARRAY® Gastrointestinal Panel. 100% of the samples were positive for an enteric pathogen. Of the samples tested, 99% were positive for Yersinia enterocolitica, 76% for E. coli O157, 73% for Shiga toxin-producing E. coli, 48% for Salmonella enterica, 12% for Campylobacter species and 3% for Vibrio species. 241 pathogens of interest were isolated (126 Yersinia enterocolitica, 85 Salmonella enterica, 29 Listeria monocytogenes, 1 Vibrio cholerae). 122 samples have been successfully whole genome sequenced and submitted to NCBI’s Pathogen Browser. 45 sequenced isolates clustered with a clinical sample from New York State.
CONCLUSIONS
Our findings highlight both the potential of wastewater surveillance to provide valuable insights into community health and the scope of bacterial outbreaks, as well as some of the challenges to data collection and interpretation. These results underscore the promise of this approach for informing public health interventions.

Presenter: Reed Robinson, New York State Department of Health: Wadsworth Center, reed.robinson@health.ny.gov

Poster 121

Enhancement of Newborn Screening Reporting Interpretations for Severe Combined Immunodeficiency (SCID) in North Carolina

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Objective:
The North Carolina Newborn Screening (NBS) Program began screening for Severe Combined Immunodeficiency (SCID) in 2017. Newborns at risk for SCID are detected by the measurement of T-cell receptor excision circles (TRECS) in dried blood spot (DBS) specimens via a lab-developed Real-Time PCR method. The SCID method is critical for detecting mutations that impair the immune system and can lead to devastating consequences if left untreated. Since the inception of NBS for SCID in NC, the NBS Lab has reported interpretations that include Normal, Borderline, Abnormal,

Inconclusive, and Unsatisfactory. Population data was analyzed to support the addition of a sixth interpretation, Abnormal-Urgent as well as more specified follow-up actions for certain gestational age (GA) groups. The updates aim to enhance the information available to follow-up care providers and prioritize individuals at the highest risk for SCID and SCID-Like disorders. This study examined the data that informed the development of this additional interpretation and the corresponding updates to newborn screening reporting for SCID.

Method:
We conducted a thorough review of current SCID reporting practices in collaboration with a multidisciplinary team of clinical Lab staff, follow-up specialists, and immunologists. The original screening algorithm included follow-up actions specific to GA groups < 35w and ≥35w. The updated screening algorithm includes follow-up actions specific to GA groups < 28w, 28-35w, and ≥35w, and the Abnormal-Urgent (Cq ≥40 or 'No Cq') interpretation. Population data from 2023 was reviewed to determine how this change affects the number of NBS notifications to follow-up for SCID. Reported interpretations from 2022 to 2024 were analyzed for cases with SCID and SCID-like outcomes.

Results:
The analysis of 2023 population data revealed that 255 initial screens were reported to NBS Follow-up with the original screening algorithm, including 65 Abnormal < 35w, 38 Abnormal ≥35w, 98 Borderline ≥35w, and 54 Inconclusive. With the updated algorithm applied to this data set, the number of initial screens that would be reported to Follow-up include 25 Abnormal-Urgent, while reducing the overall number of reports to 199. Outcome data from 2022-2024 revealed that 100% of True SCID confirmed cases were identified by the Abnormal-Urgent interpretation, and 45% of SCID-like cases were identified by the Abnormal-Urgent interpretation. Confirmed cases of ADA SCID did not fall into the classification of Abnormal-Urgent. The updated interpretation simply provides more concise information to Follow-up and Specialists for both preterm births and those most at-risk.

Conclusion:
To support of early detection, timely treatment, and improved outcomes, laboratories play a critical role by identifying patients at increased risk of potentially life-threatening conditions. Regularly reviewing and updating reporting practices ensures these goals are met efficiently and effectively. Because SCID reporting is based on the measurement of TRECs, which are typically low in preterm babies, specified reporting is essential. The refined reporting comments developed in NC provide more precise recommendations for follow-up testing and specialist consultation, improving the utility of test results and supporting more timely clinical decisions.

Presenter: Jared Clausen, North Carolina State Laboratory of Public Health, jared.clausen@dhhs.nc.gov

Poster 122
Implementation of Illumina Clarity LIMS: Experiences and Perspectives from the NC Public Health Lab
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The increase in next-generation sequencing (NGS) has created a large quantity of data, including sample metadata, library prep metrics, and bioinformatics pipeline results, which do not always fit neatly into existing public health laboratory information management systems (LIMS). As part of an ongoing data modernization process and expansion of sequencing capabilities at the North Carolina State Lab of Public Health (NC SLPH), we have implemented Illumina Clarity LIMS into our sequencing and bioinformatics response unit as the primary method of running sequencing workflows. Through cross-collaborative efforts involving bench scientists, Illumina support, and our

internal IT department, we have performed over 30 successful sequencing runs with nearly 700 samples sequenced across four workflows using Clarity LIMS as of January 2025. Clarity LIMS allows samples to be assigned to a workflow that associates reagent lots and expiration dates, master mix calculations, library pooling and quality control data to the run in addition to connecting runs to Illumina Connected Analysis (ICA) for downstream analyses using various bioinformatics pipelines. This usage has allowed us to achieve multiple funded ELC (Epidemiology and Laboratory Capacity) milestones, by increasing NC's sequencing capacity with an additional four trained staff focused on sequencing and bioinformatics development and increasing our computational infrastructure. Though not without challenges, utilizing Clarity LIMS has allowed us to streamline sample sheet generation, centralize wet lab lot number monitoring, and integrate these processes with BaseSpace for usage with bioinformatics pipelines. The utilization of Clarity LIMS for sample sheet generation has allowed our unit to reduce the usage of paper documentation and Excel based workbooks as the primary tracking method, reducing accidental document modification, saving paper and physical storage space, and enabling easier electronic review, both for internal users and documentation for external review. The integration of Clarity LIMS with BaseSpace software and MiSeq sequencers has allowed for a streamlined workflow that supports the seamless flow of data through BaseSpace to bioinformatics pipelines and population of sequencing run metrics into Clarity LIMS. These ongoing efforts will support our plans to integrate additional sequencing platforms including the Illumina NextSeq and i100 platforms, increase the number of pathogens with validated NGS testing, and incorporate Clarity LIMS into other units at the NC SLPH. Overall, the NC SLPH has innovated onboarding a sequencing specific LIMS that has increased bioinformatics capabilities, improved organization of sequencing data, and allowed for the future expansion and modernization of public health sequencing in North Carolina. Our findings support that Clarity LIMS is a useful tool for public health labs with rapidly expanding sequencing capacity seeking a LIMS to accurately track lab metrics and integrate bioinformatics pipelines.

Presenter: Jordan Ferguson, North Carolina State Laboratory of Public Health, jordan.ferguson@dhhs.nc.gov

Poster 123

Evaluation and Verification of the Diasorin Simplexa *C. auris* Direct Assay for Colonization Screenings: The North Carolina State Laboratory Experience

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Candida auris (C. auris) continues to pose challenges to public health because of its ability to cause severe illness, asymptomatically colonize patients, survive on surfaces, spread within healthcare facilities, and be resistant to antifungal drugs. Screening of patients in healthcare facilities aids in the identification of colonized patients to support transmission prevention and infection control efforts. In July 2024, the Diasorin Simplexa C. auris Direct Assay became the first FDA authorized molecular assay for the detection of C. auris in patient specimens submitted for suspicion of colonization. The Simplexa C. auris Direct Assay is intended to be performed on a sample obtained from the bilateral axilla/groin regions using the ESwabs Liquid Based Collection and Transport System. These samples are tested via the Diasorin LIAISON MDX platform. The ARLN Laboratory at the North Carolina State Laboratory of Public Health (NC SLPH) trained on and subsequently performed a verification of the Diasorin Simplexa C. auris Direct Assay to evaluate the performance characteristics of the assay whilst in our hands. A panel of 30 samples comprised of 12 contrived axilla/groin composite ESwabs in Liquid Amies spiked with C. auris, 6 contrived axilla/groin composite ESwabs in Liquid Amies spiked with near-neighbors or other yeasts, 5 Diasorin contrived samples containing synthetic C. auris DNA, and 7 residual patient samples from previous colonization screens (6 positive and 1 negative) were utilized to evaluate and determine agreement. Reproducibility was evaluated and verified utilizing a panel of 4 samples comprised of ESwabs in Liquid Amies spiked with 3 C. auris clades and 1 other yeast tested in triplicate on three separate days by two separate operators. The Diasorin Simplexa C. auris Direct Assay at the NC SLPH was determined to be 100% for Positive and Negative Agreement and Reproducibility. Our results were

comparable to Diasorin's performance characteristics which were 94.8% and 98.7% (Positive and Negative) Agreement and 100% for Reproducibility. Of note, Diasorin reported a reproducibility of 98.9% for Clade I at the low concentration tested. Implementation of the Diasorin Simplexa C. auris Direct Assay is a suitable option for public health laboratories looking to quickly standup C. auris colonization screenings in their jurisdiction. Up to 6 specimens can be set up per run along with positive and negative controls. Specimens do not require extraction or liquid handlers with results in under 2 hours. Future plans will consist of evaluating the 96 well disc format of the assay which can be tested on the instrument as a lab developed test.

Presenter: William Glover, II, North Carolina State Laboratory of Public Health, William.Glover@dhhs.nc.gov

Poster 124

Mycoplasma genitalium Prevalence Among Individuals Seeking Care in New York City Sexual Health Clinics During June 2022 – July 2023

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Background:
Mycoplasma genitalium (M. genitalium) is a sexually transmitted bacterium that infects both men and women. The estimated prevalence of M. genitalium is 1%–3% in the United States and it is currently on CDC's watch list as one of the top antimicrobial resistance threats. M. genitalium is usually detected in the urogenital tract and infections have been associated with nongonococcal urethritis in men, and in women, they can lead to pelvic inflammatory disease, infertility, and preterm birth. M. genitalium is commonly found as a coinfection with Chlamydia trachomatis or Neisseria gonorrhea. An infection with M. genitalium is not a reportable disease or as well-known as chlamydia and gonorrhea, and due to its asymptomatic nature, the infection is frequently overlooked. This results in patients only being treated for chlamydia and/or gonorrhea. Due to the location of M. genitalium within the host cell, standard treatments for chlamydia and gonorrhea do not effectively eliminate M. genitalium and may contribute to increased antibiotic resistance of the bacterium. The New York City Department of Health and Mental Hygiene (NYC DOHMH) is evaluating the potential implementation of routine testing for M. genitalium. However, there is a lack of local prevalence data that would guide medical providers for optimal screening practices. With this study, we aimed to better understand the prevalence of M. genitalium in specimens collected at NYC DOHMH sexual health clinics and to develop guidance for routine testing of M. genitalium at NYC DOHMH sexual health clinics.

Methods:
Rectal swabs, endocervical swabs, urethral swabs, and urine specimens from both symptomatic and asymptomatic patients were routinely collected at the NYC DOHMH sexual health clinics for the detection of C. trachomatis and N. gonorrhoeae. Remnant specimens were subsequently tested for M. genitalium using the Hologic® Aptima® Mycoplasma genitalium assay on the Hologic® Panther® System (Hologic, Incorporated, Marlborough, Massachusetts).

Results:
Between June 2022 and July 2023, a total of 6,310 specimens were tested using the Hologic® Aptima® Mycoplasma genitalium assay and we observed a prevalence of 11.2% for M. genitalium. The highest positivity rate was observed in urethral swabs at 19.1%, followed by 13% in rectal swabs, 10.8% in endocervical swabs, and the lowest rate of 8.8% in urine specimens.

Conclusions:
The estimated prevalence of M. genitalium in the United States is relatively low. However, the prevalence of M. genitalium is much higher among individuals who visit the NYC DOHMH sexual health clinics. This increased prevalence is likely due to the presence of symptoms, which drives individuals to seek care. The high prevalence of M. genitalium among our clinic patients warrants further investigation. Future analysis will examine symptom status at the clinic visits, demographic factors, and the correlation of M. genitalium with other sexually transmitted infections, such as chlamydia, gonorrhea, syphilis, and HIV. The results obtained from this surveillance study will help inform decisions about whether M. genitalium should be included as routine testing at all NYC DOHMH sexual health clinics.

Presenter: Joangela Nouel, NYC Public Health Laboratory, jnouel@health.nyc.gov

Poster 125
Automated Detection of Rabies Virus Using the LN34 qRT-PCR Assay with the Panther Fusion Open Access System
B. Nguyen, Orange County Public Health Laboratory, M. Zhowandai, Orange County Public Health Laboratory, M. Crumpler, Orange County Public Health Laboratory, M. Kim, Orange County Public Health Laboratory

The Orange County Public Health Laboratory (OCPHL) routinely performs diagnostic rabies virus testing on animal brain material using the CDC Protocol for Postmortem Diagnosis of Rabies in Animals by Direct Fluorescent Antibody (DFA). The objective of this study was to expand testing to validate the CDC LN34 qRT-PCR assay, which detects rabies virus RNA, on the Hologic Panther Fusion Open Access System. Rabies PCR can be utilized as a confirmatory test for DFA and should not be used as the primary diagnostic test for rabies. An inactivation study was performed to test the viability of rabies virus in Hologic Specimen Transport Media using cell cultures of serial ten-fold dilutions. The inactivation study was performed by Wadsworth Center's Griffin Laboratory at New York State Department of Health. The Panther Fusion Open Access software allows for the optimization of custom parameters for the development of lab-developed tests. With this software, the optimal primer probe reconstitution (PPR) mix, limit of detection (LOD), thermocycler settings, and PCR analysis was determined for the LN34 assay for the detection of Rabies virus.

Presenter: Bryan Nguyen, Orange County Public Health Laboratory, bryan.nguyen3630@gmail.com

Poster 126
Inorganic Arsenic Speciation by High Performance Liquid Chromatography Inductively Coupled Mass Spectrometry in Environmental Matrices
A. Hernandez, Oregon Department of Environmental Quality

Arsenic is a highly toxic naturally occurring element. Arsenic comes in multiple species, with inorganic arsenic being the most toxic, and posing the greatest environmental and public health concern. To give the most accurate toxicological profile of arsenic, it is of the upmost importance for public health labs to perform arsenic speciation. While there are multiple methods for arsenic speciation, high performance liquid chromatography inductively coupled mass spectrometry (HPLC-ICP-MS) is quickly becoming the most robust technology. At Oregon Department of Environmental Quality, we have adapted a method from the United States Geological Survey to perform arsenic speciation using HPLC-ICP-MS in environmental matrices. In this presentation, we will discuss methodology, validation, and challenges we faced as we worked to get this method up and running. In doing so, we are working to maintain the health of all Oregonians and hope to serve as a resource as other public health labs conduct this analysis.

Presenter: Avery Hernandez, Oregon Department of Environmental Quality, avery.hernandez@deq.oregon.gov

Poster 127
Using Microbial Source Tracking Methods (qPCR) to Identify Potential Contributors to Fecal Pollution in Three Oregonian Watersheds
K. Maloney, Oregon Department of Environmental Quality

The Oregon Health Authority partners with Oregon Department of Environmental Quality to monitor bacteria levels at public beaches through the Oregon Beach Monitoring Program, OBMP. Elevated fecal bacteria can come from humans, pets, livestock or wildlife, and cause illness among beachgoers. Understanding contamination types can help resource managers target contamination sources to mitigate or stop contamination from entering the water.

To help identify contamination sources, OBMP shipped samples, collected in 2022 from 10 monitoring locations adjacent to Cannon and Tolovana beaches, to EPA's Manchester Lab. The samples, collected between June and November, were analyzed using microbial source tracking methods, and compared to genetic markers for humans, ruminants, and dogs using qPCR. The analysis detected the presence of the three genetic markers in each watershed, with ruminant and human markers being detected at almost every site. This study by DEQ indicates that multiple species are contributing to fecal contamination in the three watersheds, but further investigation is needed to confirm the direct sources of contamination.

Presenter: Kat Maloney, Oregon Department of Environmental Quality, kat.maloney@deq.oregon.gov

Poster 128
Occurrence of Norovirus and Other Viral Gastroenteritis Pathogens in Sporadic Medically-attended Acute Gastroenteritis Kaiser Permanente Northwest, 2023–2024.
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Background: The objective of this project was to describe the occurrence of norovirus, and other viral gastroenteritis (GE) pathogens, among individuals with sporadic medically-attended acute gastroenteritis (MA-AGE) in the northwestern U.S. Among norovirus-positive individuals, we further describe the distribution of norovirus genotypes.
Methods: Starting November 2023 to October 2024, Oregon State Public Health lab received raw stool samples from Kaiser Permanente Northwest (KPNW) members of all ages presenting with MA-AGE symptoms who consented to participate in the study. Norovirus, astrovirus, sapovirus and rotavirus detection was assayed through a real-time polymerase chain reaction. Norovirus-positive samples were genotyped using Sanger sequencing and/or next generation sequencing (NGS) methodologies.
Results: A total of 960 stool specimens were tested, of which 152 (16%) were positive for norovirus (135 GII and 19 GI), 29 (3%) for sapovirus, 15 (1.5%) for rotavirus, and 13 (1.3%) for astrovirus. Stratified by age group, the norovirus positivity rate was highest among participants aged 5-17 years (45%), followed by those 0-4 years at 38%, and 18-44 years (17%). Norovirus GII genotyping was completed for 98 (72%): 43 (44%) GII.17, 20 (20%) GII.4, 11 (11 %) GII.2, 9 (9%) GII.6, 7 (7%) GII.3, 4 (4%) GII.14, and one each was GII.7, GII.8, GII.10, and GII.12. Out of the 98 norovirus GII samples genotyped, 60 (61%) produced full-length norovirus genomes. Norovirus GI genotyping was completed for 15 (78%): 6 (40%) GI.5, 5 (33%) GI.3, 3 (20%) GI.6, and one GI.7.
Conclusion: This study describes occurrence of norovirus and other viral pathogens among sporadic, all-age MA-AGE individuals in the Pacific Northwest, with norovirus being the most commonly identified of the four tested pathogens. In this study, 45% of specimens submitted by participants aged 5-17 years were norovirus positive. The high frequency of GII.17, paired with a decrease of

GII.4, found in sporadic MA-AGE is consistent with CDC CaliciNet data of genotype distribution of norovirus outbreaks that have occurred in the United Sates as of 2024. The findings from this study emphasize the importance of continued laboratory-based surveillance to provide data to assist in better understanding the burden of norovirus and to support the development of future preventative measures, such as vaccines.

Presenter: Laura Tsaknaridis, Oregon State Public Health Lab, Laura.j.tsaknaridis@OHA.oregon.gov

Poster 129
Manage Up: Lead from any Position in a Public Health Laboratory
C. Miranda, Oregon State Public Health Lab , H. Mims, Alabama Department of Public Health Bureau of Clinical Laboratories, B. Clemons, New York State Department of Health-Wadsworth Center

Bench scientists and first-line laboratory supervisors are often the individuals that identify and design new projects or initiatives to improve laboratory operations within their areas of technical expertise. However, the ability to implement these ideas and changes often relies on leadership buy-in at higher organizational levels. One barrier to implementing innovations is the communication of ideas across hierarchical levels. Because each level of public health laboratory leadership may have different backgrounds, informational needs, priorities, and communication styles, an understanding of how to present ideas in a way that engages, and influences leadership is essential. While “Managing up” is a critical professional skill, it is typically not taught in most scientific professional development curricula. To assist all levels of public health laboratory staff in mastering the art of “Managing Up” across multiple levels of leadership, the APHL Emerging Leader Program Cohort 18 has laid the groundwork for a practical, evidence-based interactive tool.

The ELP Cohort, in collaboration with APHL staff has developed and launched a survey to capture data from public health laboratory staff and leaders across the nation. The incentivized survey consists of 20 questions tailored for three organization levels of the public health laboratory (e.g., entry-level (bench scientists, non-supervisory staff), mid-level (manages or supervises entry level/senior level bench scientists) and high-level (manages other managers)) and has recently been distributed through multiple communication channels. The survey will collect demographic data and includes prompts to collect data on communication barriers and preferences, as well as informational priorities and needs.

Analysis of the collected survey data will be performed and a broad review of managing up, and related soft skills literature will be conducted. This information will be summarized within an operational report, including curated, publicly available content. This document will serve as a guidance tool for the development of the final product by future cohorts.

Cohort 18 envisions a final deliverable which provides hierarchal communication strategies and prepared examples and scenario-based resources. The Cohort recommends that communication, feedback, challenge anticipation, and strategic alignment of goals, are highlighted skills selected for inclusion in the tool, and that testing in multiple public health laboratories occurs. The final deliverable should include materials to assist public health laboratory staff and leaders in communicating ideas and maximizing their potential for implementation. ELP Cohort 18 believes that this tool will contribute to improved efficiency and productivity, increased trust across hierarchal levels, and better problem solving, to aid public health laboratories in innovation and continual improvement efforts to better address public health needs.

Presenter: Caitlin Miranda, Oregon State Public Health Lab, Caitlin.Miranda@oha.oregon.gov

Poster 130
Enhancing Courier Services for Preparedness and Laboratory Efficiency
S. King, Oregon State Public Health Laboratory, S. Wilcox, Oregon State Public Health Laboratory

Innovative Procurement

In 2022, the Oregon State Public Health Laboratory (OSPHL) was offered the opportunity to procure regional courier services in a unique and collaborative manner through a Request for Applications (RFA), an exception to typical state procurement methods. A Request for Proposals (RFP) was not feasible because OSPHL staff did not understand the courier and driving routes in the state well enough to define a regional framework within the RFP and determine how many vendors would be awarded contracts from the RFP. The RFA allowed OSPHL to engage in conversations with each applicant, learning directly from them about their implementation methods, maintenance processes, subcontractor relationships, inclement weather and continuity of operations plans, and more. Applicants appreciated the opportunity to engage with laboratory staff in this way and expressed an interest in more states engaging in procurement in this manner.

Laboratory Efficiency

After implementing courier services with two new, regional courier vendors, the frequency of transport errors dramatically decreased. As a result, when errors do occur the root cause is easier to identify. OSPHL staff can focus on core work, serving lab clients, and implementing efficiencies in the courier system rather than primarily responding to problems. In addition, the regional model allows for modification of the service regions if needed, creating a system responsive to surge needs or problem areas.

History

For over ten years, OSPHL's contracted courier system supported only communicable disease testing services through one vendor contract. Through multiple State RFP processes, the same vendor was selected over those 10 years. With this vendor, OSPHL staff spent countless hours coordinating the vendor's movements, tracking and managing errors, and following up on nonconforming events.

During the pandemic response, there was great strain placed on the single vendor. It also became increasingly clear that the single vendor was losing other clients and may not have continued capacity to serve OSPHL or remain solvent for a long duration.

While authorization for an emergency procurement to add a second vendor was granted by leadership during the pandemic response, the capacity to implement the contract and services during the response was not available. Later, the OSPHL Contracts Coordinator and Contract Administrator spent many hours explaining the courier services model, scope of services, regulatory framework, and the need for regional services. Ultimately the option to procure services through the RFA was offered to OSPHL.

During this time, Oregon also implemented a pilot project for the single vendor to pick up newborn screening (NBS) specimens at pilot facility hospitals, yielding great success. The team continues work to optimize this service area to add more NBS clients to courier routes in Oregon.

Presenter: Sarah King, Oregon State Public Health Laboratory, sarah.m.king@oha.oregon.gov

Poster 131

An End-to-end Sequencing Validation Approach for the Genomic Characterization of Bacterial Pathogens as Causative Agents of Healthcare-associated Infections

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Antimicrobial resistance (AMR) is now recognized as a major global health threat. Carbapenem antibiotics, among the most effective options for treating infections caused by Gram-negative bacteria, particularly healthcare-associated infections (HAIs), are increasingly losing their efficacy due to the emergence of carbapenem resistance, identified as an urgent threat. The genes responsible can be transferred rapidly between bacterial species, urging action for effective and rapid detection.

Methodology

Whole Genome Sequencing (WGS) has emerged as a powerful tool for AMR surveillance, enhancing the ability to predict bacterial traits when compared to PCR-based methods. In HAIs, AMR is often strongly correlated with the presence of known resistance genes and mutations. Here, we present a validation strategy that encompasses both wet and dry lab processes for Illumina sequencing data, aimed at achieving full genomic characterization of Gram-negative and Gram-positive bacteria—the primary agents of HAIs. Our strategy covers taxonomic assignment, detection of targeted antimicrobial resistance genes, and sequence typing, aligning with the regulatory standards required for laboratories under established accreditation bodies and quality systems.
Results
The validation panel (n=111) consisted of AMR bacterial pathogen isolates of public health significance, supplemented with in silico datasets and reference bacterial strains from the CDC & FDA Antibiotic Resistance Isolate Bank. Libraries were prepared using Illumina's DNA prep kit and sequenced on the MiSeq platform. Two bioinformatics pipelines—TheiaProk_Illumina_PE_PHB v2.2.0 and PHoeNIx v2.0.1—were selected for their ability to fully characterize bacterial genomes and compatibility with the Terra.bio platform.
Our validation strategy demonstrated high performance across multiple metrics when evaluated against reference samples, showing near-identical results for key performance indicators. PHoeNIx and TheiaProk performed as follows: Accuracy (99%/100%), Repeatability (96%/100%), Reproducibility (97%/100%), Analytical Sensitivity (99%/100%), and Analytical Specificity (100%/100%).
Both pipelines provided reliable and comparable results. While TheiaProk offers a slight advantage in taxonomic accuracy, both pipelines deliver comprehensive genomic characterizations suitable for routine use. Their integration into the Terra platform offers user-friendly interfaces, simplified training, and ease of implementation.
Conclusions
The results of this validation underscore the importance of routinely incorporating WGS technology for the diagnostic and characterization of HAI pathogens in public health laboratories. Our validation strategy and data can also be adapted for evaluating bioinformatics workflows or targeted assays for other pathogens, providing a versatile framework for laboratories with varying regulatory requirements.

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Poster 132
The Oregon State Public Health Laboratory LIMS Replacement RFP Story
M. Peckham, Oregon State Public Health Laboratory

The Oregon State Public Health Laboratory started a project to replace the four existing Laboratory Information Management Systems (LIMS) applications with a single LIMS solution. As part of this process, this project was required to follow State of Oregon Information Technologies and Administrative Services procurement regulations and processes for Requests for Proposal Evaluation. This is the story behind this adventure.

Problem statement – How could the project meet all the procurement regulations and processes while evaluating complex LIMS proposals from multiple LIMS vendors, while maintaining the same large team of RFP Evaluators over yearlong timeline?
First, we selected the RFP Evaluator Team members. These team members came from the OSPHL Laboratory Staff, including members from all lab sections, the OSPHL Informatics Team and the Information Services Team.
Second, we met monthly and trained on the RFP Evaluation process. We oriented all the team members to the regulatory and statue requirements concerning RFP evaluations. We also walked them through the RFP posting, system requirements, and the RFP evaluation tools that would be used so they were familiar with the information that was provided to and requested from the proposing vendors.

Next, we made plans regarding RFP Evaluator participation in the actual evaluation process. These plans required negotiating with laboratory managers to dedicate staff to the evaluation team and freeing them from their daily work, since most of the RFP evaluators are active laboratory staff who perform testing activities daily. To ensure that this team was fully engaged and not diverted, the LIMS project team developed novel RFP evaluation processes:

1. RFP Evaluators were able to review and score written proposals electronically during the initial evaluation.
2. The project team then scheduled the vendor demonstrations using in-person and Microsoft Teams with session recordings. This allowed RFP Evaluators who were unable to attend in-person to still participate and score the vendor demonstrations.
3. LIMS vendor scripts and evaluation criteria allowed for rapid orientation to the vendor demonstration recordings when reviewed later by team members who had absences.

Because of the preparation and training, the RFP Evaluators completed their evaluations and scoring without losing a single team member (out of a team of eleven individuals) over a four-month evaluation period. This included working around conference presentations and participation, illnesses, and family emergencies. Their work allowed OSPHL to complete the RFP process and select a single LIMS solution vendor fairly without any significant issues or delays.

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Poster 133

The L-SIP: Lessons Learned from a Public Health State Laboratory System Assessment

H. Nguyen, Oregon State Public Health Laboratory , S. Wilcox, Oregon State Public Health Laboratory, A. Saito, Oregon State Public Health Laboratory

On April 3rd, the Oregon State Public Health Laboratory, in partnership with the Association of Public Health Laboratories, will be hosting a Laboratory System Improvement Program (L-SIP) assessment. This assessment will bring together public health laboratory leaders from across the state to collaboratively evaluate Oregon's current performance in fulling the 10 Essential Services of Public Health Laboratories. The participants being considered are selected with Oregon Health Authority health equity goals in mind, including representatives from underserved communities, including tribal, rural, corrections, and migrant populations. The discussion will cover diverse topics related to health hazards, education, regulations, and trust. This poster will give an overview of the key points learned from the L-SIP as well as key action items the state can pursue to drive future improvements.

Presenter: Hanna Nguyen, Oregon State Public Health Laboratory,
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Poster 134

Optimizing Digital PCR Multiplexing for Wastewater Surveillance in Oregon

D. Mickle, Oregon State University, C. Kelly, Oregon State University, M. Sutton, Oregon Health Authority, R. Falender, Oregon State University, H. Dastgerdi, Oregon State University, T. Radniecki, Oregon State University

Wastewater surveillance, the process of identifying and quantifying pathogens in wastewater to monitor disease burden in communities, has emerged as a reliable and equitable epidemiological tool. Digital polymerase chain reaction (dPCR) platforms have revolutionized this field by increasing the sensitivity and reliability of viral pathogen detection in wastewater—a complex and challenging matrix. These advancements have made it possible for wastewater surveillance to provide critical data on viral prevalence, emerging pathogens, and broader communicable disease trends across Oregon.

In 2020, the Oregon Health Authority, in partnership with Oregon State University, launched a

wastewater surveillance program monitoring SARS-CoV-2 in approximately 40 communities across Oregon. Initially, the program utilized droplet digital PCR (ddPCR) on the Bio-Rad QX200 system, which had a maximum multiplexing capability of three targets. Initially, a single multiplex assay was sufficient to survey the N1 and N2 targets of SARS-CoV-2. However, expansion of the program to include influenza and Respiratory Syncytial Virus (RSV) required an additional three targets to be added (influenza A, influenza B and RSV). This required an additional set of plates (thus doubling supply costs) and increased the ddPCR hands on-time from roughly 6 hours a week to complete 2 plates to roughly 12 hours a week to complete 4 plates.

In July 2023, the program transitioned to the QIAcuity dPCR system to take advantage of its superior multiplexing capabilities, saving both time and supply costs. This is important as the program's wastewater surveillance needs have evolved to include year-round influenza testing as well as the inclusion of the H5 subtype, a priority target due to the emergence of highly pathogenic avian influenza A (H5N1) in North America. The QIAcuity dPCR system, with its capacity to handle up to 5 targets per assay, allowed for the implementation of a single multiplex assay to survey all 5 targets (N1, RSV, influenza A, influenza B and H5). This streamlined operations and saved on supplies. Furthermore, a recent software update has expanded the QIAcuity dPCR system's multiplexing capacity to an unprecedented 12 targets, allowing us to increase our targets by over 2-fold in a single run.

Thus, over time we have developed a comprehensive guide for laboratories seeking to leverage the QIAcuity dPCR system's enhanced multiplexing capabilities. Key considerations have included optimizing target combinations, selecting fluorophores based on relative target abundance and signal strength, and effectively managing "crosstalk" between fluorophores with overlapping excitation curves. By addressing these factors, we have created a roadmap for designing efficient, high-capacity assays that minimize thresholding challenges such as signal noise (i.e., "rain").

Presenter: David Mickle, Oregon State University, mickled@oregonstate.edu

Poster 135
Win-Win: Effective Laboratory Internships with Undergraduate Students
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Building a wastewater surveillance laboratory internship program for undergraduate students presents a unique opportunity for lab workforce development. Integrating students into a program involves a commitment to training, flexibility, assessment, teambuilding, and professionalism. This investment of time can result in an amply skilled intern, a rewarding mentor-mentee relationship, and new leadership skills for the mentor.

The Oregon Wastewater Surveillance Program consists of a partnership between the Oregon Health Authority, three labs at Oregon State University, and over 35 utilities across the state. Because OSU offers training programs in health, engineering, and biological sciences, it has the ability to employ students from a variety of disciplines to assist in the lab to both enrich their education and prepare for future employment. Lab skills learned include aseptic technique, biohazard and chemical handling, biosafety cabinet operation, data management, and nucleic acid extraction.

A primary component of a successful internship program is providing relevant opportunities to learn. Highly valued programs include hands-on and diverse opportunities that include some challenges to promote critical thinking. Safety training, central to any internship, should be communicated frequently in different modalities: online training modules, in-person lab training, reinforcement discussions at meetings, and as incidents occur. For more seasoned interns, providing leadership roles either through project management or newer intern training, can deepen understanding of

concepts and interpersonal skills.

A second component of a successful internship program is structure. A structured program sets clear expectations for lab behavior, including safety, and meeting internal and external deadlines. This structure is also defined by clearly written lab protocols, in-person guidance, and regular communication with interns. As students have varying class schedules, it is crucial to design programs with flexible hours but also require consistent participation.

Further components of an effective program include assessment and an expectation of professionalism. Adherence to protocols and an emphasis on technical precision instill job-ready skills and competence while producing valid lab results. Incorporating additional quality control measures help ensure proficient technique and provide practical feedback. Once knowledge gaps are identified, tailored training and group discussions can correct technical errors. In addition, an expectation of professionalism can help create a culture of accountability and integrity. These skills, while essential to lab operations, are also pertinent to a student's future employment.

A final and critical component of a lab internship program is team building. Including students in regular meetings, promoting a collaborative environment, and encouraging staff-to-student interactions all support progress toward joint goals and convey value to student members of the lab. These interactions promote interpersonal and communication skill development for both the student and mentor and can lead to networking opportunities for a student's future lab career.

In summary, a student lab internship program can provide valuable workforce development for both students and mentors while also contributing to daily lab operations.

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Poster 136
Fast and Efficient LC-MS/MS Analysis for PFAS Biomonitoring Using Microelution SPE
S. Marin, Phenomenex, S. Huq, Phenomenex, R. Jack, Phenomenex

INTRODUCTION: Per- and Polyfluoroalkyl Substances (PFAS) are chemicals that include more than 12,000 different compounds. PFAS compounds are present in all environmental matrices including water, soil, air, and living organisms. Due to the persistent nature of these compounds, as well as their ability to be easily transported through the environment, there is a significant concern for the presence of PFAS in the human body. Analysis of PFAS in serum is performed for clinical research and biomonitoring by public health labs. The National Academies of Sciences, Engineering, and Medicine identified seven PFAS important in clinical research and published guidelines for their analysis. In this study, a method for PFAS in bovine serum by LC-MS/MS for these seven analytes that meet sensitivity requirements in the National Academies clinical guidelines is demonstrated. The efficient LC method and microelution SPE extraction requiring no elution solvent evaporation is an elegant solution for high-throughput and automated workflows.

METHODS: Analytical reference standards and internal standards were purchased from Wellington Laboratories, LLC (Overland Park, KS, USA). Ultrapure D.I. water was obtained via Sartorius® Arium® Comfort II from Sartorius Corporation (Bohemia, NY, USA). Lyophilized bovine serum albumin and all other chemicals were obtained from Sigma-Aldrich® Company (St. Louis, MO, USA). LC separation used a Gemini™ 3.0 µm C18, 100 x 3.0 mm column with gradient elution using 5 mM Ammonium Acetate and Methanol. PFAS was extracted from bovine serum using polymeric mixed-mode weak anion exchange SPE (Strata™-X-AW, Phenomenex). Each sample consisted of 50 µL of bovine serum pretreated with 150 µL of 1% Formic Acid in Methanol, vortexed and centrifuged prior to load on the Strata-X-AW microelution plate. LC-MS/MS analysis was accomplished using an Agilent® 1290 Infinity UHPLC and a SCIEX® 5500 QTRAP®. Commercial kits are available to convert the LC system to a PFAS-free system, but none were used in this work.

RESULTS: The LC method for the seven PFAS analytes was based on the separation for a larger 30 analyte panel on a Gemini 3.0 µm C18 column with a 15-minute run time, including a four-minute column equilibration. The chromatographic separation of the 7-analyte panel used the same columns and conditions as the larger panel (TN-1325). The SPE method was developed utilizing a polymeric mixed-mode weak anion exchange sorbent, Strata-X-AW microelution plate. The absolute recoveries were 71-120 %, with % CVs whin 16 %. The dynamic range was 0.1 to 20 ng/mL. A quadratic fit with a 1/x weighting was applied. Correlation coefficients were ≥ 0.996 for all analytes. All analytes had an S/N ratio ≥3 at a concentration of 0.1 ng/mL except for PFOA, which had an S/N ratio of ≥3 at 0.25 ng/mL.

CONCLUSIONS: This study summarizes a method for quantitation of seven PFAS analytes from bovine serum listed in the National Academies of Science and Engineering recommendations for clinical laboratories. The LC-MS/MS method and microelution 96-well plate SPE extraction eliminating the evaporation step is a high-throughput workflow that can be easily automated and adapted for methods in biological matrices.

Presenter: Stephanie Marin, Phenomenex, stephaniem@phemomenex.com

Poster 137

Advancing OD2A Biosurveillance with Automated LC-MS/MS Workflows and Standardized Materials for Public Health Laboratories

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Biosurveillance plays a critical role in shaping public health policies aimed at addressing polysubstance abuse by analyzing clinical specimens to gather exposure data unavailable from traditional sources such as epidemiological studies, emergency medical services, and seized drug data. CDC's Overdose Data to Action (OD2A) initiative aims to address significant gaps in biosurveillance, leveraging the expertise of public health laboratories (PHLs). However, PHLs face substantial challenges. For example, Washington, D.C.'s overdose surveillance system currently relies on post-mortem toxicology results, which can take months to produce, limiting the ability to respond to overdose events in a timely and evidence-based manner.

Sustaining OD2A biosurveillance efforts necessitates a fully automated, standardized, and expandable LC-MS/MS confirmatory testing system. This study presents an innovative approach to developing standardized materials and foundational methods to support such systems while enhancing quality assurance. Key achievements include:

1. Analytical Reference Standards: Reformulating NIST-certified, ISO 17034 materials into ready-to-use test kits compliant with CLIA and ISO17025 validation requirements.
2. Streamlined Urine Toxicology Methods: Validating a single LC-MS/MS method using simplified validation kits to reduce sample preparation and analytical complexity and to improve efficiency.
3. Fortified Human Urine Specimens: Producing standardized specimens to enable inter-laboratory comparison studies and address the absence of accredited proficiency testing providers for OD2A.
4. Automation Technology Integration: Implementing suspended-state technology and nano dispersive agents to fully automate LC-MS/MS workflows, including sample aliquoting and hydrolysis, using only LC-MS/MS instrumentation commonly found at PHLs.

Results demonstrate significant improvements in laboratory efficiency and data quality. The validated LC-MS/MS method met CLIA and ISO17025 standards, ensuring applicability for both clinical and forensic testing. Fortified urine specimens proved effective for inter-laboratory comparison and alternative proficiency testing, filling critical gaps in regulatory compliance. LC-MS/MS automation strategies eliminate manual pipetting and sample handling, enabling scalable and accessible solutions for resource-limited PHLs while maintaining the accuracy required by their QA protocols.

These advancements are transformative, leading to the reduction of operational burdens and enhancing biosurveillance capabilities. The ability to generate high-quality, real-time data enables rapid, informed responses to the polysubstance use epidemic. Additionally, fortified specimens and alternative assessment models improve inter-laboratory consistency, support regulatory compliance, and foster collaborative biosurveillance networks.

As OD2A programs expand, the standardized materials and automated LC-MS/MS workflows outlined in this study provide a scalable framework for PHLs nationwide. These innovations will help meet the growing demands of polysubstance use surveillance and will help mitigate its impact on public health and safety.

Presenter: Isabel Ritter, PinPoint Testing LLC, Isabel.Ritter@pinpointtesting.com

Poster 138
Validation Study of GoTaq® *Legionella* Purification and qPCR Detection Workflow
N. Feirer, Promega Corporation, J. Yang, Promega Corporation, S. Mondal, Promega Corporation

Accurate detection and quantification of Legionella in water systems is paramount for ensuring public health and safety. This study evaluated the performance of Promega's GoTaq® Legionella pneumophila and GoTaq® Legionella spp/pneumophila/SG1 qPCR kits (in conjunction with Maxwell® automated nucleic acid purification) under ISO/TS 12869:2019 standards, with a focus on qPCR assay standard curve performance, internal qPCR controls, assay specificity, and DNA recovery efficiency.

5-log qPCR standard curves performed with both kits using the Legionella Quant Standard demonstrated efficiencies (>89%) and linearities (>0.99) well within the limits set in ISO/TS 12869. The internal positive control (IPC) qPCR target demonstrated consistent Cq values independent of assay or template DNA. Specificity (inclusivity and exclusivity) testing involved 65 isolates, combining ISO-recommended organisms with additional environmental strains to represent diverse contamination scenarios. At 100 GU/reaction, all inclusivity isolates, including L. pneumophila serogroups 1-15 and 20 non-pneumophila Legionella species were accurately detected in the proper qPCR channel without false negatives. 27 exclusivity isolates including diverse Pseudomonas, Mycobacterium, Staphylococcus, and fungal species showed no cross-reactivity at 10,000 GU/reaction. This data confirms the consistency and specificity of both qPCR assays.

DNA recovery was tested (two users, five independent replicates per user) using spiked water samples processed via the Maxwell® RSC PureWater Kit coupled with filter-based concentration. Results demonstrated conformance with ISO-defined recovery ranges (-0.6 to +0.3 log10) for both qPCR kits, highlighting the robustness of the extraction and amplification protocols. Incorporating the Viability PCR Reagent into the sample preparation resulted in recovery values outside of the acceptable range. This outcome is likely due to stress-induced permeability in bacterial membranes, highlighting the need to refine viability-specific sample preparation methods. Although ISO/TS 12869 does not cover viability testing, this study demonstrates the potential of the Viability PCR Reagent to improve pathogen viability assessments.

This validation study emphasizes the utility of the GoTaq® qPCR workflow for ISO-conformant Legionella detection. Moreover, it underscores the potential for incorporating molecular-based viability assessments of pathogens into routine water quality monitoring, enhancing public health protection with precise detection and quantification of viable pathogens. The GoTaq® Legionella workflow supports OneHealth initiatives by enabling a comprehensive approach to monitoring the interconnected health of the environment, animals, and humans. These findings support its application in regulatory compliance, research-driven advancements in water safety, and as a cornerstone technology in integrated health management systems.

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

Poster 139
Validation of Multiplex RT-qPCR Assays Detecting Respiratory Syncytial Virus (RSV), Influenza A Virus, Influenza B Virus and SARS-CoV-2 in Wastewater
D. Ma, Promega Corporation, S. Mondal, Promega Corporation

Respiratory viruses are a significant global health concern, contributing to acute respiratory diseases, which rank as the third leading cause of death worldwide. Prior to the COVID-19 pandemic, acute respiratory infections were responsible for approximately four million deaths annually. Monitoring respiratory viruses in environmental reservoirs, such as wastewater, presents a valuable opportunity for community-level surveillance of these pathogens, offering early detection and insight into public health trends. We developed and validated two novel multiplex RT-qPCR assays for respiratory virus detection. The first assay targets Influenza A (Flu A), Influenza B (Flu B), and SARS-CoV-2 (SC2), while the second expands detection to include Respiratory Syncytial Virus A and B (RSV A/B), along with, Flu (Influenza A and B subtypes), and SC2. Both assays exhibit high performance, with efficiencies exceeding 90% and correlation coefficients (R^2) greater than 0.99. Specificity testing against panels of non-target microorganism genomes confirmed no cross-reactivity.
For wastewater surveillance, we employed a direct capture method to concentrate and purify total nucleic acid (TNA) from municipal wastewater treatment samples. Using this approach, our assays successfully detected respiratory virus targets, including SC2, in environmental samples. To enhance reliability, both assays incorporate an internal process control targeting Pepper Mild Mottle Virus (PMMoV), enabling normalization of viral detection in wastewater matrices.
Notably, through bioinformatics analysis, our assays demonstrate the capability to detect Avian Influenza H5N1, a subtype of Flu A. Further molecular characterization is required for definitive confirmation of H5N1 presence when Flu A is detected.
These validated assays align with the One Health initiative by providing an integrative framework for monitoring the health interconnections between humans, animals, and the environment. Public health laboratories can leverage these tools to advance respiratory virus surveillance, improve outbreak preparedness, and contribute to the broader goal of safeguarding global health through comprehensive pathogen monitoring.

Presenter: Dongping Ma, Promega Corporation, dongping.ma@promega.com

Poster 140
Validation of a Novel Sample Collection-to-detection Workflow Using PrimeStore® MTM, Pathogen Total Nucleic Acid Kit, and PrimeMix® MTB
D. Wieczorek, Promega Corporation, B. Kosloff, Longhorn Vaccines and Diagnostics, K. Kroscher, Longhorn Vaccines and Diagnostics, C. Sei, Longhorn Vaccines and Diagnostics

Background: Rapid, accurate, and safe detection of Mycobacterium tuberculosis (MTB) is critical for effective disease management and control. Most institutions and facilities in the United States require that MTB diagnostic samples be handled in a Biological Safety Level 3 (BSL-3) laboratory to ensure that laboratory workers are not exposed to live strains of MTB. However, the limited accessibility of BSL-3 laboratories poses significant challenges to One Health research and disease surveillance for BSL-3 pathogens such as MTB as well as H5N1, and even BSL-2 pathogens like H3N2 and H1N1. This study describes the validation of an integrated workflow for use outside of BSL-3 facilities that combines sample collection in PrimeStore® Molecular Transport Medium (MTM), the only sample collection media FDA cleared for inactivating MTB and both DNA and RNA nucleic acid preservation, the Maxwell® CSC Pathogen Total Nucleic Acid Kit* for nucleic acid isolation, and PrimeMix® MTB Multiplex Amplification Solution for PCR-based detection. Additionally, H5N1, H3N2 and H1N1 were tested downstream of PrimeStore® MTM and Maxwell® CSC Pathogen Total Nucleic Acid Kit* using publicly available primer and probe sequences.

Methods: PrimeEQA® MTB (Longhorn Vaccines and Diagnostics Bethesda, MD), a five-tube panel comprising four MTB complex strains with varying drug resistance profiles and a negative (no DNA)

control inactivated and stabilized in PrimeStore® MTM (Longhorn Vaccines and Diagnostics), served as the blinded test samples. All work was performed in a BSL-2 laboratory. The samples were extracted using the Maxwell® CSC Pathogen Total Nucleic Extraction Kit* and the Maxwell CSC 48 instrument* (Promega, Madison, WI), following manufacturer protocols. PCR detection utilized PrimeMix® MTB Multiplex 1X Amplification Solution (Longhorn Vaccines and Diagnostics), targeting IS6110 and IS1081 gene regions of the MTB complex. For H5N1 testing, milk was used as sample source. For H3N2 and H1N1 testing, porcine oral fluid samples were used.

Results: The workflow demonstrated a sensitivity of 100% and specificity of 100% for MTB detection. PrimeMix® MTB provided robust amplification with consistent detection of IS6110 and IS1081 targets (Cq < 25). PrimeEQA® MTB validation showed a 100% concordance rate across five tests. Across all sample types and workflows, ease of use outside of a BSL-3 laboratory or biological safety hood and minimal hands-on time for the integrated workflow were reported as significant advantages, making it suitable for point-of-care and high-throughput diagnostic settings.

Conclusion: The PrimeStore® MTM- Maxwell® CSC Pathogen Total Nucleic Extraction Kit* - PrimeMix® MTB workflow offers a safe, streamlined, and validated solution for MTB detection outside of BSL-3 laboratories. This integrated system is poised to enhance diagnostic capabilities in both clinical and field settings. The inactivation of BSL-3 level pathogens, such as MTB and H5N1, and BSL-2 pathogens like H3N2 and H1N1, enables this workflow to be deployed in a variety of settings, facilitating rapid disease detection while ensuring safer laboratory working conditions.

Keywords: Tuberculosis, PrimeStore® MTM, PrimeMix® MTB, Pathogen Total Nucleic Acid Kit, PrimeEQA® MTB, Molecular Diagnostics, PCR, One Health

*For In Vitro Diagnostic Use.

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Poster 141

Characterization and Epidemiology of Non-Respiratory *Corynebacterium diphtheriae* in an Oregon Based Multi-Hospital System

V. Wilson, Providence Health & Services, G. Tallman, Providence St. Vincent Medical Center, E. Fox, Providence Portland Medical Center, M. Precit, Providence Health & Services

Corynebacterium diphtheriae is best known as the etiological agent of respiratory diphtheria, a severe upper respiratory tract infection rarely seen in the United States (US), due to widespread vaccination. However, within our hospital system, recovery of C. diphtheriae from non-respiratory sources has increased. Characterization and epidemiology of non-respiratory C. diphtheriae cases is understudied within our region. We conducted a retrospective multi-hospital study evaluating incidence, culture characteristics, clinical presentation, patient history, and clinical interventions to characterize C. diphtheriae isolates and cases. Culture results were reviewed for specimen type/source, co-infecting organisms, and if C. diphtheriae isolates produced toxin. Chart review was conducted to evaluate patient history and demographics, county of residence, co-morbidities, clinical presentation, and patient treatment course. From January 2022 – January 2024, 36 patient cases were identified with non-respiratory cultures positive for C. diphtheriae. All C. diphtheriae isolates were non-toxin producing. Most cases were in Multnomah County (25, 69.4%) followed by Washington County (4, 11.1%) then Clackamas County (3, 8.3%); the remaining were individual cases within Clark, Columbia, and Hood River Counties. Mean patient age was 46.7 years, 30 (83.3%) were men, 16 (55.6%) were experiencing housing insecurity, and there was no indication any patients traveled outside the state of Oregon within the last year. Baseline characteristics included: 19 (52.8%) used intravenous drugs, 8 (22.2%) had diabetes, 4 (11.1%) had heart failure, and 21 (58.3%) reported immunization. Most infections were polymicrobial (32, 88.9%). The majority had cutaneous sources (29, 80.6%). Interestingly, there were 7 C. diphtheriae bacteremia cases, and 3 had no identifiable wound source. Notable co-isolated pathogens included Staphylococcus aureus (20, 55.6%) and/or Streptococcus pyogenes (20, 55.6%). About half of the treatment

regimens ignored the presence of C. diphtheriae (17, 47.2%). The highest risk population for non-respiratory diphtheria were middle-aged men experiencing housing insecurity and/or intravenous drug use. C. diphtheriae isolates recovered from wound or blood sources were non-toxin producing, suggesting vaccination (which targets the diphtheria toxin) would be ineffective at protecting against infection in our patient population. C. diphtheriae is frequently isolated with S. aureus and/or S. pyogenes suggesting the clinical microbiology laboratory should identify any co-infecting coryneform organism to the species level to rule in/out C. diphtheriae. Less than half of patients were treated for C. diphtheriae, highlighting a knowledge gap among clinicians. Continue identification of C. diphtheriae and provider awareness of C. diphtheriae risk in our region is an important public health safety concern.

Presenter: Victoria Wilson, Providence Health & Services, victoria.wilson3@providence.org

Poster 142
Structure and Function Investigation of Transcription Factor AbmR from *Mycobacterium tuberculosis*
K. Fisher, Public Health Laboratory Fellowship Program: An APHL-CDC Initiative, J. Panmanee, Wadsworth Center, H. Sui, Wadsworth Center

ATP-binding mcr11-regulator (AbmR) is a transcription factor recently discovered from Mycobacterium tuberculosis (Mtb). It upregulates the expression of mcr11, a stress-responsive small RNA, during the latent growth phase. Most purified AbmR proteins assemble into 39S complexes containing large RNAs, while a tiny portion are present as soluble dimers. Our structural study revealed that the 39S complexes display a cage-like architecture formed by 24 AbmR dimers, with their DNA binding site facing the luminal side. To study AbmR-DNA and AbmR-RNA interactions in vitro, we needed a high concentration of stable AbmR dimers. We produced MBP-AbmR dimers by fusing maltose-binding protein (MBP) to AbmR, preventing 39S complex formation. With the MBP-AbmR dimers, we can study the interaction of AbmR with nucleic acids in vitro. Detailed characterization of AbmR-DNA and AbmR-RNA interactions and the influence of ATP in these interactions are anticipated to provide critical insight into the functional roles and mechanisms of AbmR and its role in the latent growth phase of Mtb.

Presenter: Kathryn Fisher, Public Health Laboratory Fellowship Program: An APHL-CDC Initiative, Kathryn.Fisher@HEALTH.ny.gov

Poster 143
Depletion of Host DNA Enables Deeper Sequencing of Microbial Gene Content and Antimicrobial Resistance
D. O'Neil, QIAGEN GmbH, J. Leinberger, QIAGEN GmbH, N. Scholle, QIAGEN GmbH, M. Sprenger-Haussels, QIAGEN GmbH

Tissue microbiomes are an important aspect of animal biology and increasing evidence highlights their impact on host health, behavior and development. Furthermore, recent studies have demonstrated that animal tissue microbiomes undergo significant changes during disturbances in the host environment and that the presence or absence of certain taxa correlates with factors such as temperature or anthropogenic antibiotic discharge.

Major challenges in tissue microbiome studies arise from low residual bacterial biomass and a therefore high host-to-bacterial cell ratio. To overcome these particular issues, we have developed a specialized workflow. Our protocol is designed to ensure reliable host DNA removal from mucosal, soft or fibrous tissue, while yielding high quality bacterial DNA in quantities that allow for subsequent metagenome sequencing.
Thorough and even tissue homogenization is the first essential step of our workflow and is ensured by a enzymatic dissociation step. Targeted eukaryotic cell lysis is followed by enzymatic depletion of host nucleic acids, while bacterial cells remain pristine. After microbial cell lysis via bead beating, the extracted DNA is bound to a silica-coated column to facilitate washing and purification.

To ensure conservation of the microbial community structure and to rule out discrimination for individual taxa during our extraction procedure, we have employed 16S rRNA amplicon sequencing in combination with mock microbial community standards. Multiple samples were analyzed by whole genome sequencing after extraction with the new protocol, revealing much deeper diversity of microbial genes, including antibiotic resistance genes, which were not visible when these samples were extracted without host depletion. Our protocol is characterized by its streamlined workflow yielding reliably reproducible results.

Presenter: Dominic O'Neil, QIAGEN GmbH, dominic.oneil@qiagen.com

Poster 144
High-throughput Automation of Diverse Sample Types on an Open Platform
D. O'Neil, QIAGEN GmbH, H. Block, QIAGEN GmbH, J. Schulz, QIAGEN GmbH, S. Magyar, QIAGEN GmbH, M. Sprenger-Haussels, QIAGEN GmbH

We have developed nucleic acid extraction workflows for multiple sample types on the upcoming QIAsprint Connect instrument, set to launch at the beginning of 2026. This instrument is designed for high-throughput automation for up to 192 samples with minimal hands-on time, in a 96 well format. The workflows developed here use magnetic bead purification methods, which enables rapid and flexible automation of a wide variety of workflows.

The workflows include DNA and RNA extraction chemistries for blood, tissue, saliva, stool, swabs, wastewater, plant, cell culture, clean-up and plasmid, and the chemistries are flexible to expand to many other sample types, such as soil or food. These sample extraction methods have been used in workflows including dPCR, qPCR and NGS. Here, we present the detailed results of extraction of microbial DNA from human samples such as stool and swab using this new instrument.

The QIAsprint connect system is an open and flexible platform, enabling easy customization of protocols. To reduce overall consumption of plastic and generation of waste, workflows have been streamlined and specialized plastic consumables have been developed which require much less plastic than standard 96-well plates. Tailored for academic, applied testing, and pharmaceutical labs, QIAsprint Connect provides exceptional workflow flexibility with both pre-programmed and customizable protocols. Its sustainable consumable concept reduces plastic waste while ensuring reliable results even with challenging starting materials.

Presenter: Dominic O'Neil, QIAGEN GmbH, dominic.oneil@qiagen.com

Poster 145
High-throughput Genomic DNA Extraction from Dried Blood Spots and Library Preparation for Newborn Sequencing Using Quantabio's sparQ DBS Solutions
S. Panja, Quantabio, S. Chung, Quantabio, M. Aitichou, Quantabio, B. Komorous, Quantabio, D. Schuster, Quantabio

Newborn screening systems have been monitoring clinically significant but treatable disorders since their introduction in 1960. Newborn blood is collected in a specialized paper to generate dried blood spots (DBS) to test metabolic, endocrine, hemoglobin, and other genetic disorders. Existing tandem mass spectrometry and molecular assays can detect only a finite number of genes associated with specific genetic disorders; therefore, novel disorders will be undiagnosed. In comparison, whole genome sequencing (WGS) of newborns can provide comprehensive genetic information regarding disorders present in newborns that is both reliable and fast. Next-generation sequencing is increasingly becoming affordable at the scale needed to be suitable for whole genome sequencing of newborns. However, existing extraction methods do not support quick and cost-effective extraction of high-quality genomic DNA (gDNA) from DBS samples.
With these challenges in mind, the sparQ DBS Library Prep Kit was developed to include a fast and robust genomic DNA (gDNA) extraction module as well as reagents for preparing PCR-free whole

genome libraries. The extraction process takes only 40 minutes to isolate high molecular weight (>50 kb) gDNA. From three 3.2 mm punches, the average yield obtained was 175 ng from cord blood samples and 210 ng from newborn samples. The extraction process is compatible with manual extraction with either a few samples or high throughput extraction of up to 96 samples using deep well plates. Extracted gDNA samples were used to generate PCR-free whole genome libraries within 90 minutes. The libraries were sequenced on an Illumina NovaSeq 6000 Xp with 150 bp pair-end reads to achieve 160 Gb reads from each sample. The sequencing data provided >30X coverage from each sample with a low duplication rate (< 8%). The variant calling from the WGS data was similar or better compared to other well-known kits available.
In addition to short-read sequencing applications, the extracted high molecular weight gDNA can be used for long-read sequencing and other applications. Further, the same kit can be used to quickly isolate gDNA from whole blood, saliva, and buccal swab samples, making it beneficial for sequencing adult samples alongside newborns and allowing for trio testing of families.
Overall, the sparQ DBS Library Prep Kit provides a rapid, high-throughput, cost-effective, and robust solution for both gDNA extraction and PCR-free whole genome library preparation for the sequencing of newborns.

Presenter: Subrata Panja, Quantabio, Subrata.Panja@quantabio.com

Poster 146
Indoor Air Monitoring of Respiratory Viruses Correlates with Clinical Percent Positivity
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Background: Environmental surveillance can support public health monitoring efforts for viral infections that are clinically unreported or to detect off-season emergence of respiratory viruses. Wastewater surveillance has proved valuable for monitoring viruses at the community level. Indoor air monitoring could be used for pathogen detection at a finer institution scale or pooled for citywide levels, but this remains largely untested.

Methods: Indoor air monitors (n=9) were deployed by the Chicago Department of Public Health (CDPH) across a range of healthcare and congregate settings in Chicago, IL. These include three acute care hospitals, two skilled nursing facilities, a long-term acute care hospital, a specialty clinic, a transitional housing facility, and a correctional facility. Air samples were collected weekly and transferred to the Regional Innovative Public Health Laboratory (RIPHL), a public health-academic partnership between CDPH and Rush University Medical Center (RUMC) for advanced molecular detection. Total nucleic acids were extracted from the air samples, and levels of SARS-CoV-2, influenza A, influenza B, and respiratory syncytial virus A/B (RSV) were assessed using quantitative PCR.

Clinical percent positivity data for HHS Region 5 (IL, IN, MI, MN, OH, and WI) were obtained from the CDC's COVID Data Tracker (SARS-CoV-2), FluView (influenza A/B), and the National Respiratory and Enteric Virus Surveillance System (RSV). Spearman's rank correlation coefficient and p-value ($\alpha = 0.05$) were applied to assess the correlation between air sample Ct and clinical percent positivity in the region. Additionally, CDC/NWSS wastewater analysis methods were adapted to create Ct value quintiles for influenza A using the Ct distribution from 169 air samples collected during the 2023-2024 period of elevated clinical activity for influenza (10/1/2023-3/2/2024).

Results: A total of 687 air samples were collected weekly across 88 weeks (3/26/2023-11/30/2024) from 9 sites (mean 7 per week). Correlations were found between mean weekly air sample Ct values and HHS Region 5 clinical percent positivity for all four pathogens (p = < 0.0001). Moderate correlation was seen for SARS-CoV-2 (Spearman r = -0.5667) and strong correlations were seen in

traditional respiratory viruses with larger variation in clinical percent positivity (RSV (Spearman r =-0.6667), influenza A (Spearman r = -0.7527), and influenza B (Spearman r = -0.8169).

Influenza A Ct values were categorized into 5 quintiles based on the 2023-2024 period of elevated clinical activity for influenza: minimal (Ct > 38.3), low (Ct 38.2-37.8), moderate (Ct 37.7-34.0), above average (Ct 33.9-32.1), and high (Ct < 32.1). Mean weekly Ct values for the influenza off-season (3/3/2024-9/28/2024) were in the minimal or low category for 19 and 4 of 30 off-season weeks, respectively. During the period of elevated clinical activity for influenza, mean weekly Ct values were in moderate, above average, and high categories for 11, 5, and 1 of 22 weeks, respectively. Above average weekly mean Ct values were observed from 12/30/2023 to 2/24/2024, corresponding to peak clinical percent positivity.

Conclusion: The significant correlations between air sampling Ct and clinical test positivity suggest that indoor air monitoring can serve as an additive tool for respiratory virus surveillance, offering a supplementary approach for public health departments to detect and respond to infectious diseases across varied settings.

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Poster 147

Establishing Genomic Epidemiology of Group A *Streptococcus* in Chicago

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Background: A 2023 outbreak of genomically linked emm44 GAS cases in a correctional facility led the Chicago Department of Public Health (CDPH) to initiate whole-genome sequencing (WGS) and emm typing of GAS cases in high-risk populations. CDPH engaged with the Regional Innovative Public Health Laboratory (RIPHL), a public health-academic partnership between CDPH and Rush University Medical Center for advanced molecular detection, to produce genomic data to characterize possible intra- and inter-facility transmission during outbreaks.

Methods: CDPH performs routine surveillance of invasive and wound GAS infections among high-risk populations (healthcare, correctional, or shelter facility residents and unsheltered homeless persons) and post-surgical and post-partum infection isolates. From February to November 2024, RIPHL performed WGS on isolates from 19 patients from high-risk populations including 6 isolates from 2 known clusters and 13 from non-cluster-associated cases. Draft genomes were assembled for emm typing and antibiotic resistance gene (ARG) detection using the TheiaProk pipeline. Phylogenetic trees were constructed, and single nucleotide polymorphism (SNP) analysis was conducted among isolates of the same emm type using the Snippy Streamline pipeline. Available epidemiologic data (e.g. collection date) were integrated into phylogenetic tree visualizations using Microreact.

Results: Among 19 patients with WGS, 15 had invasive GAS infections, 3 had non-invasive infections and 1 colonized individual was epi-linked to an invasive case. The most frequent emm types isolated were emm44 (n=4), emm76 (n=4), and emm1 (n=3); all other emm types (emm6, emm11, emm49, emm77, emm82, emm89, emm103, and emm149) were unique. The emm44 isolates were 4-15 SNPs diverged and were collected from patients at 3 different congregate facilities; two cases had epidemiologic links to the same facility. The four emm76 specimens clustered together and were 0-3 SNPs diverged from one another; 3 of 4 cases in this cluster were unsheltered and had known intravenous drug use. The emm1 specimens were 77-121 SNPs apart. The emm types of the epi-linked colonized individual and invasive case were discordant. Nine isolates carried antimicrobial resistance genes (ARGs), and isolates that were determined to be

closely related by SNP analysis carried the same ARG profiles.

Conclusions: Genomic sequencing data enhanced CDPH GAS surveillance and response. Close genetic clustering of emm44 GAS infections associated with three different facilities suggests possible unknown transmission pathways. Sequencing data has also allowed CDPH to assist healthcare facilities with their own investigations and provide supplemental laboratory evidence that a colonized healthcare worker, while epidemiologically linked was not genomically linked to a facility associated infection. This project demonstrates the value that WGS can provide to epidemiological investigations of GAS and other bacterial pathogens of public health concern.

Presenter: Erin P Newcomer, Rush University Medical Center, erinpnewcomer@gmail.com

Poster 148

Using Whole Genome Sequencing and Cluster Identification to Understand Transmission Patterns of *C. auris* Among Chicago Healthcare Facilities

E. Newcomer, Rush University Medical Center, K. Walblay, Chicago Department of Public Health, E. Newcomer, Rush University Medical Center, K. Clausell-Mobley, Chicago Department of Public Health, A. Kittner, Chicago Department of Public Health, C. Zelinski, Chicago Department of Public Health, S. Black, Chicago Department of Public Health, M. Hayden, Rush University Medical Center

Background: Candida auris emerged in Chicago in 2016 and has since become endemic. We used whole genome sequencing (WGS) and cluster identification to describe the genomic epidemiology and transmission of C. auris among Chicago healthcare facilities.

Methods: C. auris isolates (8/1/2018-12/15/2021) (n=494) were sequenced and analyzed using the MycoSNP pipeline. Phylogenetic trees were constructed with other available Illinois sequences and visualized using Microreact. Phylogenetic clustering (monophyletic clades with >80% bootstrap support) and genetic distance (< 8 SNPs) were used to identify clusters. Maximum cluster genetic distance was selected by examining the fraction of specimen pairs that were intrafacility at pairwise SNP distances. Sample metadata (e.g., specimen collection date, facility, floor and room) were used for cluster analysis.

Results: Of the 494 Chicago C. auris isolates newly sequenced, 491 (99.4%) were clade IV and three (0.6%) were clade III. All Chicago clade IV sequences fell into a single cluster with other publicly available Illinois C. auris sequences. Mean SNP distance among Illinois clade IV sequences increased from 4.3 in 2016 to 30.7 in 2021. A total of 77 clusters were identified with 196 of 572 (34%) Illinois sequences included in a cluster. Of these, most were small (mean 2.5 specimens per cluster, range: 2-7 specimens) and closely related (mean 2.4 pairwise SNP distance within clusters) with strong phylogenetic support (mean bootstrap 95.7%). Twenty-one facilities were involved in the 77 genomic clusters, including four long-term acute care hospitals, four ventilator-capable skilled nursing facilities (SNFs), two inpatient rehab facilities, one SNF and 10 acute care hospitals (two of which were outside Chicago). Evidence of intra-facility and interfacility transmission was seen in both large (5-7 isolate) and small (2-4 isolate) clusters.

Conclusions: Longitudinal genomic analysis of C. auris isolates identified a single introduction to Illinois with spread through several care facilities. Understanding transmission dynamics (intra-facility vs. interfacility) by integrating WGS, cluster detection, and epidemiologic metadata could be used to guide targeted interventions to prevent transmission.

Presenter: Erin Newcomer, Rush University Medical Center, Erin_P_Newcomer@rush.edu

Poster 149
Mobilizing Community-driven Public Health Response: Increasing Access to Diagnostic Testing for Underserved and Uninsured Individuals in Connecticut Through Lab-in-a-van Partnerships

By combining resources from local nonprofits and well-equipped medical or academic institutions with the on-the-ground expertise of community-centered organizations, we can work together to jointly address structural and systemic inequities key to realizing health equity. This pilot showcases how addressing barriers through community-driven public health can make a significant impact. Namely, establishing relationships with trusted community partners, embracing an iterative engagement process, building a testing program with target demographics in mind and ensuring there is value in what is being offered to the community.

Presenter: Brittany Choate, SalivaDirect, Inc., brittany@salivadirectinc.org

Poster 150
Validation of a Group A Strep emm Typing Pipeline
R. Cox, South Carolina Public Health Laboratory, G. Goodwin, South Carolina Department of Public Health Laboratory, J. Freeman, South Carolina Department of Public Health Laboratory, G. Godfrey, South Carolina Department of Public Health Laboratory, C. Coler, South Carolina Department of Public Health Laboratory, D. Hook, South Carolina Department of Public Health Laboratory, J. Meredith, South Carolina Department of Public Health Laboratory, C. Weaver, South Carolina Department of Public Health Laboratory

For the South Carolina Department of Public Health Laboratory (SCPHL), new test validations are categorized as clinical, falling under CLIA regulations, or surveillance. The purpose of this validation was to establish an in-house surveillance method of assigning emm types to Group A Strep (GAS) isolates using the Bactopia: Complete Analysis of Bacterial Genomes pipeline to analyze high quality sequences. The GAS emm Type Validation relied on the CDC's GAS Surveillance group for both additional GAS validation sequences and comparison results. A panel of 97 GAS validation samples representing 35 different emm types was sequenced by the SCPHL Advanced Molecular Laboratory or the CDC, both of which performed emm typing. A set of six non-GAS sequences was also processed by both the CDC and SCPHL: Streptococcus agalactiae, Streptococcus pneumoniae, Listeria monocytogenes, Campylobacter jejuni, Clostridium perfringens, Escherichia coli. Of the 97 GAS

sequences evaluated, SCPHL was able to confidently assign emm types to 92 using the Bactopia pipeline. The five other sequences were tagged by the pipeline as not being a 100% match to any known emm type in the database. Of these, two were confirmed by the CDC pipeline as new emm types that needed to be submitted to the surveillance database. The procedure for this validation states that any sequences that do not have a 100% match will be submitted to the GAS surveillance portal. The 100% match of emm types with a confident call (95% of the panel) supports the accuracy of the pipeline. The analysis was performed in triplicate both with and without an added parameter to the pipeline that automatically channels sequences to appropriate downstream analyses tools, which, in the case of GAS, is the emm typing tool emmtyper. When this parameter is not used, all sequences are manually channeled to emmtyper. Kraken2 was added as a contamination check. The variety of emm types represented and the consistency of the two sets of three replicates support the inclusivity and the precision of the pipeline, respectively. Non-GAS sequences produced non-results in both the CDC and Bactopia workflows. The SEQ2MGS pipeline was used to mix reads from two fastq files at a designated ratio, mimicking contamination in silico. For GAS:GAS combinations, a secondary set of reads was mixed with a primary set at 2%, 5%, 10%, 20%, 25%, 30%, 40%, and 50% for five GAS pairs. A drop in the N50 metric below 100,000 was the first indication of contamination between 5% and 30% contamination. In all but one pair, this drop occurred either at the same level or sooner than a change to the emm type call. Three GAS sequences were chosen for GAS:non-GAS combinations at 2%, 5%, 10%, and 50% contamination of each of the six non-GAS sequences. For GAS:Non-GAS combinations, Kraken2 was able to identify the contamination at 5% or 10% for four out of the six non-GAS sequences. For Streptococcus agalactiae and Escherichia coli, contamination went primarily undetected until 50% contamination. Nevertheless, the emm type call was not impacted by the contamination except for one of the three 50% C. perfringens contamination, where no emm type call could be made. We concluded that this validation met the in-house requirements for a surveillance procedure, though some limitations, such as with new emm types or the detectable levels of contamination, will need to be clearly communicated.

Presenter: Rachel Cox, South Carolina Public Health Laboratory, coxrs@dph.sc.gov

Poster 151
Pathogen Recovery from CIDT Positive Stool Samples in South Dakota 2023–2024
C. Carlson, South Dakota Public Health Laboratory, J. Bush, South Dakota State University, T. Southern, South Dakota Public Health Laboratory

As with many public health laboratories across the country, the South Dakota Public Health Laboratory has seen an increase in preserved stools samples received for surveillance testing in recent years. An evaluation of recovery rates of pathogens from all positive preserved stool samples received from 2023 and 2024 was conducted. Key data elements looked at were positivity rate by pathogen(s), specimen transport time, and clinical lab test method. Sample data was collected from the laboratory LIMS system for analysis. A total of 420 preserved stool specimens were received during the 2-year period. Samples were received within 3 days of collection on average, with a range of 1-26 days. Positive specimens were received from 18 different submitters with the primary clinical test method being Biofire ®. The second most common clinical method for Shiga toxin identification only was an ImmunoCard EHEC ® method. Recovery rates varied by organism with Salmonella being most successful 79%, Escherichia coli O157:H7 43%, Escherichia coli non-O157 43%, Campylobacter 58%, Shigella 21%, and Yersinia 17%. More than 1 positive pathogen was reported on 33% of the samples. This was passively collected data as it is not required on requisition form. While Salmonella recovery was quite good, the other pathogens could use a more thorough review of factors that could impact recovery. These factors could include recovery by submitter, shipping time and temperature conditions, multiple pathogens positive or type of transport medium. This evaluation was conducted to identify the recovery rates of reportable pathogens from CIDT positive stool specimens in South Dakota. This initial evaluation showed that further studies are needed to identify potential issues and how to best isolate pathogens from preserved stool samples.

Presenter: Christopher Carlson, South Dakota Public Health Laboratory, chris.carlson@state.sd.us

Poster 152

Effective Mosquito Surveillance and Mitigation Efforts to Control Arboviral Activity in Southern Nevada (2024)

H. Kan, Southern Nevada Health District, V. Raman, Southern Nevada Health District, C. DeHaan, Southern Nevada Health District, K. Del Rosario, Southern Nevada Public Health Lab, W. Chan, Southern Nevada Public Health Lab, L. Reyes, Southern Nevada Public Health Lab, A. Figueredo-Perello, Southern Nevada Public Health Lab, M. Mendy, Southern Nevada Public Health Lab, E. Nmani, Southern Nevada Public Health Lab, A. Young, Southern Nevada Public Health Lab, B. Northam, Southern Nevada Health District, W. Bendik, Southern Nevada Public Health Lab, C. lockett, Southern Nevada Health District, H. Kan, Southern Nevada Public Health Lab

The Environmental Health (EH) Division of the Southern Nevada Health District (SNHD), in partnership with the Southern Nevada Public Health Laboratory (SNPHL), conducted an extensive mosquito surveillance program from April to November 2024, aimed at monitoring arboviral diseases, including West Nile Virus (WNV) and St. Louis Encephalitis (SLE). Over the course of the year, the program sets over 3,450 traps across Clark County, with peak trapping activity occurring in May and June. During these months, mosquito pool submissions reached over 1,400 samples, each containing up to 50 mosquitoes of the same species from a single location.

The first detection of WNV occurred on May 20th, with positivity rates surging to 43.65% by June 10th, sparking immediate action from the Health District. A Health Alert Notice (HAN) was issued to healthcare providers, emphasizing the rapid increase in WNV activity. Additionally, several pools tested positive for SLE, with co-infections of WNV and SLE also observed. The highest levels of positive mosquito pools were recorded in May and June, with combined WNV and SLE cases reaching 11,379 mosquitoes. The distribution of these positives was widespread across Clark County, suggesting significant amplification and transmission of WNV within local bird populations. Notably, some areas did not report any WNV positives, highlighting geographic variability. In response to the elevated threat, the EH team collaborated closely with local municipalities to implement targeted mitigation measures, which included mosquito control programs, public education campaigns, and outreach to at-risk populations. These proactive efforts, combined with ongoing surveillance, led to a decline in positivity rates. By early July, WNV positivity dropped to 1,203 positive mosquitoes, a significant decrease from June's 7,644. Notably, the temperature spikes in Las Vegas, with consecutive days exceeding 110°F in early July, likely contributed to reduced mosquito activity, resulting in zero positive mosquito pools by August 12th.

By the end of the surveillance season, a total of 12,033 WNV-positive mosquitoes and 583 SLE-positive mosquitoes had been recorded from a total of 51,307 mosquitoes trapped. Compared to CDC 2024 data, Clark County, NV saw a higher human disease incidence (26 cases) than Maricopa, AZ (20) and Los Angeles, CA (23). The highest human disease incidence was 20 cases in June and decreased to 5 cases in July and by August, only 1 was reported. The findings highlight the success of the joint initiatives undertaken by the EH Division and SNPHL in managing arboviral transmission. In the absence of these collaborative actions, the incidence of human disease cases could have been considerably elevated.

This presentation will highlight the successes and lessons learned from Southern Nevada's 2024 mosquito surveillance and mitigation response, demonstrating the value of a robust public health infrastructure in protecting communities from emerging vector-borne threats.

Presenter: Horng Yuan Kan, Southern Nevada Health District, kan@snhd.org

Poster 153

Assessing Aerobic Treatment Units for Mosquito Habitat Compatibility and Pollutants

H. Penton, St. Tammany Parish Mosquito Abatement District, N. DeLisi, St. Tammany Parish Mosquito Abatement District, K. Caillouet, St. Tammany Parish Mosquito Abatement District

There are 600+ miles of roadside ditches in St. Tammany Parish, LA that individual household sewage is discharged into. Estimates from prior years indicate ~60% of the aerobic treatment units in these

onsite systems may be nonfunctioning, creating habitats attractive to ovipositing Culex quinquefasciatus. In collaboration with the parish's Department of Environmental Services, we investigated whether onsite sewage system functionality has an impact on mosquito/mosquitofish presence, and its correlation to water quality parameters.

The Department of Environmental Services visited ~1000 properties in 2024 and determined the type of system installed, the functioning status, and where effluent was discharged. Water quality (pH, dissolved oxygen, dissolved solids, oxidative reduction potential) and the presence of mosquitoes/mosquitofish were observed and measured from ditches at each. The effect of covariates on fish presence, mosquito presence, and water quality will be compared upon completion in late 2024

Presenter: Heather Penton, St. Tammany Parish Mosquito Abatement District, penton.heather@gmail.com

Poster 154
Timely Detection and Subtyping of Upper Respiratory Pathogens Using a Microfluidics-based Workflow
B. Mire, Standard BioTools, B. Hunt, Standard BioTools, L. Stewart, Standard BioTools

Timely detection and accurate identification of infectious pathogens including subtypes is key in developing tools used by public health programs to conduct outbreak surveillance and management. This can help shorten outbreak duration and support identification of the causative agent(s) so that appropriate corrective measures can be designed and implemented. Molecular methods such as polymerase chain reaction (PCR) and next-generation sequencing (NGS) routinely contribute to the effort by allowing multiple pathogens to be genomically profiled at the same time, and, in some cases, without a dependency on isolate culture. This effectively reduces the time and testing needed to identify pathogens present in samples collected from an outbreak.

In this study, we demonstrate proof of concept and describe a microfluidics-based protocol designed to detect and identify subtypes of 11 common upper respiratory pathogens in up to 48 samples from a single run. The protocol employs an automated workflow starting with nucleic acid mixed with or derived from saliva (extraction free). The use of nanoliter-scale microfluidic reactions conserves precious reagents while reducing plastic waste and enabling sustainable lab operations.

Following preparation of sample and assay mixes, which are dispensed into a microfluidic chip that is subsequently loaded onto the Biomark™ X9 System for processing, results are available in two hours without manual intervention. Each of the chip's 48 assay inlets connects with an independent reaction chamber, which enables all assays to share a common fluorophore and thermal profile while preventing assay-to-assay interference associated with multiplex reactions. Based on open architecture of the chip design, assays can be added to multiple inlets to generate replicate data points per sample which can increase confidence in results. Additional assays can be added easily to detect more pathogens or to further subtype pathogens of particular interest.

The current list of targeted pathogens tested includes Influenza A, Influenza B, Respiratory Syncytial Virus (RSV), SARS-CoV-2, Human Coronaviruses HKU1, NL63, OC43, and 229E, Human Metapneumovirus, Enterovirus, Human Parechovirus. Influenza A subtypes H1N1 and H3N2, and RSV subtypes A and B, were also included for additional subtyping. Commercially available control material from ZeptoMetrix was tested and accurate detection of expected targets was demonstrated, including further subtyping of Influenza A and RSV.

The microfluidics-based workflow described offers a rapid, scalable, and environmentally sustainable solution for pathogen detection and subtyping, with significant potential to enhance public health response during outbreaks.

Presenter: Brad Mire, Standard BioTools, brad.mire@standardbio.com

Poster 155
Time to Unbox a New Assay for Mpox
M. Ahmann, State Hygienic Laboratory at the University of Iowa, J. Benfer, State Hygienic Laboratory at the University of Iowa

Mpox is a double-stranded DNA virus belonging to the Orthopoxvirus genus. There are two types of the virus that cause infection, clade I and clade II. Until recently, clade II Mpox captured most of public health's attention starting in early May 2022, when clade II was reported outside of Central/Western Africa for the first time since 2003, causing a global outbreak in which the U.S. led in cases and deaths. Since then, clade II Mpox has continued to circulate at low levels in many countries. More recently, clade I Mpox has caused outbreaks throughout the Democratic Republic of the Congo and Central/Eastern Africa since January 2024, and there have been several travel-associated clade I Mpox cases reported in neighboring countries as well as Europe and Asia. More pressingly, the first travel-associated clade I Mpox case in the U.S. was detected in November 2024 and was related to the ongoing outbreaks in Central/Eastern Africa. The CDC has determined the risk to the U.S. posed by the clade I Mpox outbreaks as low for the general population. However, given the evolving nature of Mpox, public health laboratories must be prepared to detect beyond clade II Mpox cases in the event of future outbreaks. Here, we describe the process for developing and optimizing an updated laboratory-developed assay (LDA) targeting clade II Mpox as well as Non-variola Orthopoxvirus (NVO) for detection by PCR on the fully automated Panther Fusion® Open Access™ system.
For optimization, various concentrations of select reagents in a primer probe mix were evaluated following Hologic's Open Access™ training recommendations. The LDA was validated using inactivated material from the CDC Poxvirus Branch to assess accuracy, precision across two Panther Fusion® instruments and two days, sensitivity, and specificity among clade I Mpox, Vaccinia virus, HSV1, HSV2, and VZV.
We optimized the concentrations of KCl, MgCl2, Tris, primers, and probes to achieve the most robust, reliable, and accurate assay. The LDA achieved the standards for CLIA validation with 100% accuracy, 100% precision within an acceptable range, a sensitive limit-of-detection (LOD) of 13 copies/ul, and 100% specificity among specimens evaluated.
The methods outlined in this poster for developing an LDA using the Hologic Panther Fusion® Open Access™ system are great resources to help prepare public health laboratories for increased testing volume and to improve current workflows. The automated extraction reduces turn-around time as well as labor costs and increases reproducibility of results. Furthermore, the LDA does not require specimens to be processed under BSL-3 conditions, improving workflow efficiency and enhancing convenience of results. Lastly, the LDA detects clade II Mpox as well as NVO, ensuring detection of all Mpox cases. These methods can be easily applied to the rapid development and optimization of LDAs for many pathogens of public health significance.

Presenter: Megan Ahmann, State Hygienic Laboratory at the University of Iowa, megan-ahmann@uiowa.edu

Poster 156
Real-time Detection of *Candida auris* in Hospital Wastewater Using Digital PCR
L. Kloft, State Hygienic Laboratory at the University of Iowa, B. Bedell, State Hygienic Laboratory at the University of Iowa, R. Jepson, State Hygienic Laboratory at the University of Iowa, C. Cass, State Hygienic Laboratory, M. Pentella, State Hygienic Laboratory at the University of Iowa

Candida auris is an emerging multidrug-resistant yeast that poses significant challenges in healthcare settings. Given the persistence of C. auris in healthcare environments and the potential to cause severe infections, monitoring spread is critical. The primary aim of this study is to collect wastewater from a single healthcare facility, measure the concentration of C. auris using digital PCR, and correlate wastewater testing results to the number of positive clinical cases of C. auris. The secondary aim of this study is to evaluate wastewater testing as a tool to complement clinical screening by measuring the spread of C. auris in a hospital wastewater system.
24-hour composite wastewater samples were collected twice weekly from a local hospital using an

ISCO GLS sampler to provide a representative wastewater sample. Wastewater samples were concentrated using centrifugation and extracted using the Qiagen PowerFecal Pro Kit. DNA amplification was performed using C. auris primers from GT Molecular. The goal of this study is to evaluate the potential of wastewater testing as an early detection tool for C. auris and to assess the feasibility for use in routine monitoring at healthcare facilities.
The results from this study will contribute to the development of a wastewater-based epidemiology (WBE) tool for tracking healthcare-associated pathogens and to refine infection prevention strategies.

Presenter: Lauren Kloft, State Hygienic Laboratory at the University of Iowa, lauren-kloft@uiowa.edu

Poster 157
Evaluation of *Legionella* Multiplex PCR Assay as Screening Tool to Detect *Legionella* in Environmental Samples
V. Reeb, State Hygienic Laboratory at the University of Iowa, J. Benfer, State Hygienic Laboratory at the University of Iowa, R. Jepson, State Hygienic Laboratory at the University of Iowa, A. Yakos, State Hygienic Laboratory at the University of Iowa, C. Lueck, State Hygienic Laboratory at the University of Iowa, M. Nelson, State Hygienic Laboratory at the University of Iowa, L. Cooper, State Hygienic Laboratory at the University of Iowa, C. Cass, State Hygienic Laboratory, M. Pentella, State Hygienic Laboratory at the University of Iowa

Background
Legionella presence in healthcare and long-term care facilities' water systems is an increasing concern. To protect at-risk populations, facilities can develop a water management plan and perform routine environmental testing for Legionella to validate the plan is working. Testing can also be used during outbreaks to identify potential source of infection. The gold standard for detecting the presence of Legionella in water systems is the culture of environmental samples on selective media. However, the culture of Legionella is labor intensive and requires expertise to identify suspected colonies. In 2023, SHL developed a multiplex PCR based on the CDC assay to simultaneously detect Legionella pneumophila serogroup 1 (Lp1), L. pneumophila non-serogroup 1 (Lp) and L. species (Lsp) in clinical specimens and isolates. SHL wanted to determine whether the assay could be used as a screening tool, so that only PCR-positive environmental samples are cultured, thus reducing labor and saving resources.

Experiment
SHL performed multiplex PCR on 264 environmental samples submitted to the Legionella Reference Center between October 2023 and December 2024. Aliquots from low bacterial count water filtrates, biofilm swab resuspension, and high bacteria count water were both plated on BCYE and MWY and sent for PCR testing. All isolates recovered from those samples were also tested by multiplex PCR. When isolates were recovered for a sample, but no Legionella was detected by PCR, the PCR assay was repeated using 1/10 and 1/100 dilutions of the input material, if possible, to check for inhibition.

Results
Legionella was detected by multiplex PCR in 39.4% of the 264 environmental samples tested but was only isolated from 16.3% of those. However, Legionella was isolated from 7.2% of the 152 samples that were not detected for Legionella. When PCR was repeated using dilutions of the input material, Legionella was detected. Comparison between which Legionella types (Lp1, Lp, or Lsp) were detected in the samples by PCR versus isolates recovered from the same sample, the correlation was 63.6% for Lp1, 32.1% for Lp, and 9.2% for Lsp.

Conclusion
SHL determined our Legionella multiplex PCR assay would not be a useful screening tool for testing environmental samples. As expected, PCR had a higher detection rate than culture, due to increased sensitivity and the ability to amplify dead bacteria (free DNA) circulating in water. However, culture would still be necessary to give an accurate risk evaluation of what currently resides in the water system. The surprising result was to recover isolates from PCR-negative samples. Those samples

were associated with high bacterial load and required dilutions to be detected by PCR, thus making our pre-screening algorithm more complicated and costly. Finally, Legionella type (Lp1, Lp, and Lsp) recovered by multiplex PCR did not correlate well with the type recovered by culture. The presence of several Legionella types within one sample cannot consistently be distinguished by our PCR assay, which could result in misidentification for remediation.

Presenter: Valerie Reeb, State Hygienic Laboratory at the University of Iowa, valerie-reeb@uiowa.edu

Poster 158
Performance Quality Validation of Autoclave Vacuum, Liquid and Gravity Displacement Cycles for Sterilization of Biohazardous Waste
I. Vore, State Hygienic Laboratory at the University of Iowa, A. Morris, State Hygienic Laboratory at the University of Iowa

The development of a standardized autoclave validation procedure ensures reliable sterilization of complex biohazardous waste generated in public health laboratories, aligning with international biosafety standards. This study evaluates the specific functions, strengths, and limitations of pre-vacuum, liquid, and gravity displacement autoclave cycles to validate their appropriateness for routine sterilization. The results support optimization of cycle parameters and model waste loads to achieve consistent sterilization across diverse waste types and cycle settings.

Presenter: Ivan Vore, State Hygienic Laboratory at the University of Iowa, ivore@uiowa.edu

Poster 159
Detection and Differentiation of 9 CHDL β-lactamase Gene Families (OXA/oxacillinases) Using a Multi-platform Compatible Multiplex PCR Panel
S. Cossette, Streck, C. Connelly, Streck

BACKGROUND
As new antimicrobial resistance (AMR) genes continue to emerge, those encoding carbapenem-hydrolyzing class D (CHDL) enzymes, such as oxacillinases (OXA), are highly concerning, as they impart bacteria with resistance to antibiotics of last resort. In 2019, the CDC classified carbapenem-resistant Acinetobacter spp. and carbapenem-resistant Enterobacteriaceae (CRE) as urgent threats with a predicted increase in hospital infection rates. Because these resistance mechanisms can be acquired via horizontal gene transfer, which often leads to new mechanisms or phenotypes, it is imperative that AMR surveillance technology evolves to prevent outbreaks and promote antimicrobial stewardship. This study describes the expansion of a multi-platform compatible multiplex panel to include detection of 9 OXA β-Lactamase gene families.

METHODS
Oligo Design: Carbapenem-resistant OXA-143, OXA-48, OXA-24/40, OXA-58, OXA-51, OXA-23, OXA-134-like, OXA-198-like, and OXA-372-like gene variant sequences were downloaded from the NIH Pathogen Detection Reference Gene Catalog and aligned in UGENE software (UniPro) to facilitate oligo design and/or to perform in silico analysis. Oligo sets were designed to capture variants within each of the 9 gene families, while differentiating variants between the 9 groups.

Assay Optimization: Assays were optimized using synthetic target sequences then multiplexed together with an internal control (IC) sequence targeting a conserved region in Gram-negative bacteria to prevent false negative results.

Assay Evaluation: Performance of the multiplex assays was evaluated via in silico analysis and with a set of previously characterized clinical isolates and contrived specimens. Clinical isolates were cultured overnight, then DNA was extracted using the DNeasy Blood and Tissue Kit, Gram-Negative Bacteria protocol (QIAGEN). Contrived specimens were composed of target sequences in negative background DNA. Reactions were run on the 7500 Fast Real-time PCR platform (Applied Biosystems (ABi)), using SuperMix 2x (Streck) per the ARM-D® Kit, OXA (Streck) instructions for use.

RESULTS
Assay Performance: The ARM-D Kit, OXA is compatible with multiple PCR platforms including the QuantStudio™ 5 Real-Time PCR System (ThermoFisher Scientific) and the QIAcuity Digital PCR System (QIAGEN).

Variant Coverage:
ARM-D Kit, OXA PCR Mix #1 detects 83 of the 84 reported gene variants in the families included (99% agreement).

ARM-D Kit, OXA PCR Mix #2 detects 439 of the 439 reported gene variants in the families included (100% agreement).

ARM-D Kit, OXA PCR Mix #3 detects 35 of the 35 reported gene variants in the families included, including variants OXA-235, OXA-236, and OXA-237 (100% agreement).

CONCLUSION
New AMR strains and genetic variants can go undetected if existing assays are not designed to target the sequence differences associated with these variants. As such, updated assays are crucial to identify emerging pathogens of interest and to combat the spread of AMR. Here, we described how the ARM-D Kit, OXA was updated to detect new high priority carbapenem-resistant AMR variants in the OXA gene families. This kit supports the need for comprehensive tests capable of detecting critically important AMR mechanisms for epidemiologic surveillance and improved infection control practices.

Presenter: Stephanie Cossette, Streck, scossette@streck.com

Poster 160
How Much Does It Actually Cost to Do That Test? Developing Cost Studies for Public Health Labs
B. Cartmell, Tennessee Department of Health, B. Dalton, Tennessee Department of Health

Effective laboratory management hinges on a comprehensive understanding of operational costs. Cost studies serve as an essential tool to identify, evaluate, and optimize laboratory expenditures while ensuring the sustainability of high-quality testing services. This poster highlights cost studies' methods, challenges, and applications in Tennessee's public health laboratories. These labs serve a wide range of communities, each with different needs. Cost studies help address gaps in understanding how resources are used, ensuring funding is distributed equitably, identifying ways to save money without reducing quality, and planning for future needs. These studies allow Tennessee public health laboratories to better serve its residents, manage budgets effectively, and prepare for new public health challenges.

The Division of Laboratory Services at the Tennessee Department of Health developed the cost studies and aimed to develop a systematic approach to assess costs associated with routine laboratory operations for federal and state-funded testing. Specific categories included personnel, equipment, supplies, and facility overheads. These expenses were added together to determine an overall cost for each test. By integrating data from financial records, operational workflows, and benchmarking analyses, the studies provide actionable insights for resource allocation and budget planning.

Key findings from the cost studies included significant cost variances across testing sections, such as Aquatics, Newborn Screening, and Serology, emphasizing the need for granular cost-tracking mechanisms. One of the most common items that adds to the increased expense is labor. Additionally, the studies identified opportunities to improve efficiency by consolidating high-cost processes and leveraging shared resources. These cost studies will be completed annually to account for changes in testing costs over time. These findings underscore the importance of aligning laboratory operations with broader institutional goals, particularly in resource-constrained settings.

The step-by-step methodology for conducting cost studies, including data collection tools, analysis techniques, and reporting frameworks, can be valuable for public health laboratories seeking to improve their operations. These studies support decision-making processes, enhance transparency, and promote fiscal responsibility, ensuring resources are used effectively. Sharing insights and fostering collaboration among laboratory professionals can drive innovation and efficiency, benefiting laboratory operations and administration in diverse public health settings.

Presenter: Brandy Cartmell, Tennessee Department of Health, brandy.d.cartmell@tn.gov

Poster 161
Trends in Arboviral Infections in East Tennessee: Insights from Five Years of Diagnostic Data on La Crosse Virus
K. Jones, Tennessee Department of Health, S. Shama, Tennessee Department of Health, A. Moncayo, TN Department of Health, Communicable and Environmental Diseases and Emergency Preparedness

Background: Arboviruses are a diverse group of viruses that are transmitted by arthropods such as mosquitoes and ticks. These viruses are found globally, with a variety of climate zones supporting their transmission. There are approximately 500 arboviruses, and around 100 of these can cause human diseases. In recent decades, the frequency and severity of arboviral epidemics have risen dramatically, reflecting broader ecological and environmental changes. La Crosse Virus (LACV) is an arbovirus transmitted by Aedes triseriatus, the eastern tree hole mosquito, and is endemic to the Midwest, Appalachian, Mid-Atlantic, and Southeast regions of the United States. While majority of LACV infections remain asymptomatic, severe cases can lead to neuroinvasive disease, particularly in children under 16 years of age. The Knoxville Regional Laboratory has been actively involved in monitoring arboviral infections since 1998, focusing primarily on testing samples from patients at East Tennessee Children's Hospital presenting with neuroinvasive symptoms such as seizures or meningitis. This study evaluates trends in arboviral infections, specifically LACV, from 2020 to 2024, aiming to better understand the virus's impact on local populations and inform targeted public health interventions.
Methods: The Focus Diagnostic Arbovirus IFA IgG and IgM kits are used to detect La Crosse virus in serum specimens. This indirect immunofluorescent assay (IFA) detects human serum antibodies to arbovirus infections. Data for 5 years (2020-2024) were reviewed to identify trends in infection rates and to compare the geographic distribution of cases with mosquito activity in the corresponding counties.
Results: Over the five-year period, the Knoxville Regional Laboratory tested a total of 141 specimens for arboviral infections. All positive cases were for LACV. In 2024, 45 specimens were tested, with a positivity rate of 29%. The positivity rate in 2023 was also 29% (24 specimens), while 2022 saw a lower positivity rate of 11% from 18 specimens. The positivity rates in 2021 (25%, 28 specimens) and 2020 (54%, 26 specimens) were also notable. In 2024, the average age of positive patients was 8 years, with most cases reported in Knox County (n=6). Of the 13 positive cases, 12 were associated with a diagnosis of neuroinvasive disease, including 9 cases of meningitis or encephalitis.
Conclusion: Despite an increase in the total number of specimens tested in 2024, the overall positivity rate for LACV remained consistent with 2023 levels, underscoring the persistence of LACV transmission in the region. The data highlights the continued risk of neuroinvasive arboviral diseases, particularly among children, reinforcing the need for sustained surveillance efforts and public health strategies aimed at reducing the burden of mosquito-borne diseases. This study also demonstrates the critical role of public health laboratories in monitoring arboviral trends and guiding appropriate interventions to mitigate the health risks posed by these viruses. Ongoing testing, coupled with data analysis, will be pivotal in shaping future public health strategies for preventing and managing arboviral infections in Tennessee and other regions affected by similar ecological conditions.

Presenter: Katie Jones, Tennessee Department of Health, Katie.jones@tn.gov

Poster 162
Enhancing Laboratory Partnerships to Isolate Shiga Toxin-producing *Escherichia coli* in

Outbreak Investigations — Tennessee, 2013–2024
K. Orejuela, Tennessee Department of Health, L. Durso, USDA, L. Thomas, Tennessee Department of Health, D. Irving, Tennessee Department of Health, K. Orejuela, Tennessee Department of Health, M. Golwalkar, Tennessee Department of Health

Background: From 2013–2024, the Tennessee Department of Health (TDH) investigated 20 Shiga Toxin-Producing Escherichia coli (STEC) outbreaks. Most outbreaks (N&#3f11) were associated with food; three were associated with animal contact, two with person-to-person transmission, one with waterborne transmission, one with environmental contamination, and two outbreaks with indeterminate transmission mode. TDH attempts to collect environmental and food samples for outbreaks; however, isolating STEC from environmental samples and food (particularly raw milk) is challenging. This presentation aims to showcase how partnering with a laboratory that routinely conducts STEC environmental and food testing can be beneficial during an outbreak investigation.

Methods: Since 2013, TDH has partnered with the U.S. Department of Agriculture's (USDA) Agriculture Research Service (ARS) laboratory to assist with testing environmental and raw milk samples during STEC outbreak investigations. Environmental and raw milk samples from six STEC O157 outbreak investigations since 2013 were sent to USDA-ARS. Samples were tested for STEC O157:H7 using brilliant green agar, anti- O157 immunomagnetic beads, and CHROMagar O157. If STEC O157:H7 was isolated, the isolates were sent to the TDH Division of Laboratory Services (DLS) to determine genetic relatedness and characterization by pulse-field gel electrophoresis (PFGE) or whole genome sequencing (WGS).

Results: A combined 164 samples were collected in response to six STEC outbreak investigations: 132 environmental samples and 32 raw milk samples. Of these, 113 environmental and 32 raw milk samples were sent to USDA-ARS for isolation of STEC O157. STEC O157 was not isolated from any raw milk samples but was isolated from at least one environmental sample per outbreak, 31 (27%) environmental samples in total. The TDH DLS identified the outbreak strain by PFGE/WGS analyses from samples associated with five (84%) STEC O157 outbreak investigations.

Conclusions: Recovery of STEC from environmental samples and raw milk is complicated, especially for laboratories that do not routinely test these sample types. The collaboration with USDA-ARS allowed us to leverage their experience, specialized lab processes, and procedures for isolating STEC from samples not routinely tested by TDH DLS. Partnering with other public health laboratories routinely testing environmental samples and raw milk for STEC is important during outbreak investigations. State and local health departments should consider partnering with public health laboratories with environmental testing expertise to identify the outbreak strain.

Presenter: Kelly Orejuela, Tennessee Department of Health, Kelly.Orejuela@tn.gov

Poster 163
From Surveillance to Solutions: The Power of Epidemiology and Lab Partnerships
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Collaboration between epidemiology and laboratory teams plays a crucial role in effective public health surveillance and outbreak response. The successes of strong interdisciplinary relationships between the Division of Laboratory Services (DLS) and the Foodborne and Enteric Diseases Program (FED) at the Tennessee Department of Health (TDH) are exemplified through key dynamics such as communication and coordination. TDH-DLS and FED staff collaborate on laboratory data quality evaluation, timely coordination of testing during outbreaks, and increasing TDH-DLS efficiency and capacity. Strong cross-interagency communications between these teams are particularly valuable during outbreak responses, where clear communication about lab capabilities, capacity, turnaround

times, and specimen collection needs is essential for rapid intervention. The laboratory's work in testing a variety of sample types is critical in helping FED solve and prevent foodborne outbreaks including those related to raw milk, ruminant animals, and vegan cheese.

Cross-team communication is fostered through active involvement in weekly joint lab/epi staff meetings and active discussions during outbreak investigations and grant writing periods. Routine opportunities to share updates and insights on ongoing work and projects allow teams to understand each programs' goals, contribute to more accurate data collection and analysis, and improve public health surveillance. Furthermore, regular e-mail communications with the TDH-DLS Sequencing section and FED team have helped identify and track foodborne and enteric clusters more efficiently as well as identify issues with specimen submission earlier. Epidemiologists use data provided by the Sequencing team to share cluster findings via platforms like the PulseNet SharePoint site, ensuring timely data dissemination and coordination with the Center for Disease Control and Prevention's PulseNet and Outbreak Response and Prevention teams. Moreover, the FED team's opportunity to work directly with the Sequencing team and gain firsthand experience in reviewing whole genome sequencing (WGS) data has proven invaluable. This experience has enriched discussions in the TN Food Safety Centers of Excellence-hosted Community of Practice forum, where epidemiologists share their insights on WGS and cluster identification, promoting a deeper understanding and more effective application of lab findings in public health efforts.

Additionally, FED staff's initiative to track and review samples received at the TDH-DLS resulting in a "Test Not Performed" (TNP) have helped support laboratory capacity to ensure more specimens are tested when feasible. This has helped laboratories manage their workload while enhancing public health response and meeting submission requirements. Routinely working with laboratory data enables the identification of increases of TNPs and active collaboration between TDH-DLS and FED helped decrease the number of TNP samples, benefiting both teams.

Our laboratory and epidemiology teams demonstrate how this synergistic partnership strengthens disease surveillance, cluster detection, and outbreak management, ultimately contributing to improved public health outcomes.

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Protecting Our Waters: Assessing Tennessee's Water Testing Landscape after Hurricane Helene
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Background: Hurricane Helene caused unprecedented damage in regions not typically affected by catastrophic weather events. Prolonged rainfall and subsequent flooding raised concerns over the safety of drinking water in affected communities, particularly those relying on private wells. There are thousands of wells in East Tennessee that serve Tennesseans their drinking water. The Knoxville Regional Laboratory (KRL) routinely performs over 2,500 drinking water and 1,400 environmental water tests a year, while maintaining readiness to support response efforts for outbreaks and natural disasters. With transportation challenges, and strict sample collection timeframes, not all wells could be tested by Tennessee Division of Public Health Laboratories. To address these limitations for sampling waters, the Communicable and Environmental Disease and Emergency Preparedness Division (CEDEP) within the Tennessee Department of Health and with the Tennessee Department of Environment and Conservation (TDEC) determined Tennesseans were able to travel to their nearby county health department to receive an at-home well water testing kit. Tennesseans could sample their well's drinking water for total coliforms (TC) and interpret the test at home.
Method: KRL utilized the IDEXX Colilert-18 Test for simultaneous detection of total coliform bacteria and Escherichia coli in water. CEDEP distributed Water Works™ Bacteria Check bottles for at-home

testing the detection of TC in water. To perform the at home test, water is added to the container ensuring that the inside of the test bottle is not touched, and there is no spillage of the powder inside the bottle. After closing the test container, the bottle is shaken vigorously for 20 seconds then left undisturbed for 48 hours at room temperature without direct sunlight. A yellow color change indicated a positive result for total coliform bacteria.
Result: Although there was no mandated reporting for positive at-home test results, CEDEP received over twenty calls from flood affected residents seeking assistance with interpreting their results and positive water result questions. Over 3,000 at-home test kits were distributed through local county health departments, empowering community members to test their own well water. In addition to the self-testing efforts, both the Knoxville Regional Laboratory and Nashville Central Laboratory conducted 14 well water tests for TC. Eight of these water samples were positive for both TC and E. coli, three were positive for TC, two were negative for both analytes, and one sample was unable to be tested as it fell outside holding time requirements.
Conclusion: In response to the flooding, residents were provided with clear instructions in both English and Spanish on how to use the at-home testing kits and disinfect their wells. They were also given contact information for environmental health specialists from the Tennessee Department of Health for further support. In addition to the free testing kits, residents had the option to pursue laboratory testing through KRL or private labs. The Tennessee Department of Health is committed to ensuring the safety of drinking water and recreational use in Tennessee by providing both laboratory and at home testing options, ensuring high standards of water safety and quality for Tennessee residents.

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Poster 165
Importance of the APHL Internship Program
M. Stone, Tennessee Department of Health Laboratory Services

Internships are critical in preparing aspiring scientists to transition from classrooms to professional careers. They provide invaluable opportunities to develop technical and professional skills, engage in hands-on learning, and apply academic knowledge to real-life settings. They also lay the groundwork for professional networks, mentorship, and future collaborations.

The internship experience at the Tennessee Department of Health Laboratory Services (TDOH-DLS) focused on wastewater surveillance, environmental microbiology water testing, technical writing, and diagram creation. Examples of laboratory experience included the preparation of wastewater samples for analysis. The concentration process is critical for detecting low levels of target analytes and biomarkers. This step reduces the sample size while retaining the analytes, ensuring they remain detectable. Environmental water testing included testing drinking and well water for Total Coliform and Escherichia coli.

The internship experience at TDOH-DLS also provided opportunities to enhance technical writing skills. Technical writing requires communicating necessary technical information in an accessible format, making it easier for the intended audience to apply. During the internship, two standard operating procedures (SOPs) were created, one for environmental microbiology water testing and one for wastewater surveillance. Additionally, efforts were made to develop strategies to improve efficiency and simplify workflows in the lab. Diagrams were generated to enhance and standardize workflows for drinking water testing, beginning with sample receipt and pre-analytical processes and continuing through to the completion of water sample testing.

The benefits of internships extend beyond gaining new skills, offering invaluable experiences that foster personal growth and provide opportunities to apply academic knowledge. Internships within public health laboratories provide opportunities to gain knowledge, build professional networks, and receive mentorship, ultimately setting individuals on a path toward a positive career trajectory in public health.

Presenter: Mytasia Stone, Tennessee Department of Health Laboratory Services,

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Poster 166

Enhanced Surveillance for TB and Latent TB Infection in High-risk Tennessee State Prison Populations, 2015–2024

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Background

Tuberculosis (TB) is a disease that does not discriminate; it can affect anyone. Identifying and treating TB in the population presents challenges to public health systems worldwide. TB is especially difficult to identify, treat, and control when a source case is identified in a congregate setting such as prisons, due to overcrowding and the transient nature of the population. Prior to 2015, testing of the Tennessee prison population for active or latent TB infections (LTBI) was done using tuberculin skin tests (TSTs) by prison medical staff or IGRA tests by an out- of- state private laboratory. Positive LTBI results were not reported or readily accessible to the Tennessee Department of Health (TDH). TDH was only notified if an inmate had suspected or confirmed TB disease. The lack of data and subjectivity of reading TSTs presented significant challenges in identifying and monitoring TB in TN prisons. To identify individuals more accurately with TB or LTBI in congregate settings, TDH implemented a screening program to detect TB infection in TN prisons using the QuantiFERON-TB Gold Assay.

Methods

In 2015, TDH TB Elimination Program (TTBEP), Division of Laboratory Services (DLS), and TDC partnered to provide QuantiFERON®-TB Gold (QFT) testing during prisoner intake for the two intake facilities in TN. In 2022, this partnership expanded to replace annual TST testing with QFT testing. With this, inmates receive an initial QFT test upon intake and are screened annually. Additional QFT testing is performed if inmates have signs/symptoms of active TB or if a known exposure to active TB has occurred.

Results

From 2015 to November 26, 2024, 48,600 QFT tests were drawn on 47,617 inmates upon intake. Of these, 3,105 (6.52%) had a positive QFT result. The annual percent positivity for QFT testing at intake was 3.8-5.0% between 2015-2019 and increased to 6.6-10.4% between 2020-2024. Beginning 2022 to November 26, 2024, 852 QFT annual tests were collected on 819 inmates. Of those, 51 (6.23%) inmates were positive. The annual percent positivity for annual QFT testing ranged from 4.8-13% between 2022-2024.

Conclusion

Early identification and treatment of LTBI is key in preventing progression to active TB disease. In prison, active TB disease can easily be transmitted resulting in large outbreaks and contact investigations. Prior to 2015, TB testing protocols for TN prisons were not consistent or reportable. Additionally, testing methodology was subjective, resulting in many false positive cases and subsequent unnecessary treatments. Through the partnership between the TDH TTBEP, DLS, and TDC, QFT testing at intake and annual testing has been beneficial in accurately identifying TB in TN prison populations enabling treatment to reduce the progression and spread of the disease.

Presenter: Tanya Cooper, Tennessee Department of Health, Division of Laboratory Services, tanya.cooper@tn.gov

Poster 167

EQIP Your Team: Advancing Workforce Development in the Laboratory Field

J. Viruez, Tennessee Department of Health, Division of Laboratory Services , K. Levinson, Tennessee Dept of Health, Division of Laboratory Services, L. Jolly, Tennessee Dept of Health, Division of Laboratory Services

Introduction:
Recruiting and retaining skilled personnel remain universal hurdles for employers. While recruitment is an initial challenge, retaining a high-quality workforce is a long-term challenge. Peer-reviewed studies highlight the importance of a quality onboarding program as a retention strategy; however, laboratories encounter unique obstacles in delivering effective onboarding due to their staff's diverse educational backgrounds, regulatory requirements, roles, and experience levels. The Tennessee Department of Health, Division of Laboratory Services (TDH-DLS), has addressed these challenges by launching the Employee Quality Initiative Program (EQIP), a comprehensive onboarding program designed to prepare new employees for their career at TDH-DLS. EQIP focuses on providing staff with the foundational knowledge, skills, and connections to succeed in their roles.

Methods:
The daily operations and functions of TDH-DLS were carefully considered to develop a comprehensive onboarding program applicable to new employees across the laboratory. The program spans 2 days is conducted in person and is presented by subject matter experts. Day 1 is designed for all new employees. The curriculum includes an introductory series facilitated by laboratory directors covering topics such as key state government partnerships, regulatory requirements and laboratory administrative departments such as human resources and procurement. In addition, sessions on informatics, communications, quality assurance, laboratory safety, and the fundamentals of good laboratory practices are included. Day 2 focuses on more hands-on training tailored for technical employees. The day two courses include modules such as specimen receiving, media preparation, pipette boot camp, an overview of LRN, and commonly used math formulas and measurements. During the planning phases, administrative tools were utilized for content development and communication. Each day concludes with an evaluation to ensure the onboarding process remains effective.

Results:
The onboarding program was initially piloted in October 2024, with 16 participants: 5 in non-technical roles and 11 in technical roles. The pilot program spanned 4 days, and evaluations were collected from participants and reviewed. Feedback was overwhelmingly positive, with participants praising the content. Suggestions for improvement were considered, leading to enhancements and adjustments. These included condensing the program into 2 full days, incorporating materials such as binders and engagement tools. Based on participant feedback and human resources recommendations, EQIP will occur monthly throughout 2025. It is offered on the third Wednesday and Thursday of each month.

Conclusions:
The EQIP onboarding program has demonstrated its value as an essential tool for training a high-quality workforce in the laboratory field. This program addresses the challenges of diverse roles, technical requirements, and varying experience levels by providing a structured, hands-on approach to equipping new employees for success. The program also emphasizes inclusivity, fostering an environment where individuals from diverse backgrounds and roles feel welcomed, supported, and connected to the laboratory’s mission. Moving forward, our laboratory team is confidently equipped to meet the ever-evolving demands of the laboratory field.

Presenter: Julie Viruez, Tennessee Department of Health, Division of Laboratory Services, julie.viruez@tn.gov

Poster 168
Impact of Temperature Monitoring on Pre-analytical Laboratory Processes
K. Priester, Tennessee Dept of Health, Division of Laboratory Services, L. Jolly, Tennessee Dept of Health, Division of Laboratory Services, K. Levinson, Tennessee Dept of Health, Division of Laboratory Services

Introduction: Specimen integrity was highlighted during the recent CLIA inspection at the Tennessee Department of Health, Division of Laboratory Services (TDH-DLS). The Code of Federal Regulations citation 493.1242(a)(5) specifies that the laboratory must establish and follow written policies and

procedures for each of the following, if applicable: conditions for specimen transportation. Through collaboration with the state courier and laboratory submitters, TDH- DLS implemented protocols to improve specimen handling and transport practices from the time of collection to receipt at the laboratory.

Methods: To maintain regulatory compliance, TDH-DLS collaborated with public health partners within the state to ensure submission guidance provided on the Directory of Services was communicated to all clinical submitters. The laboratory developed guidance documents for submitters and couriers outlining processes for specimen storage before and during transport including an added centrifugation step for QuantiFERON specimens prior to transport. In addition to education and communication, TDH-DLS implemented a specimen receipt log to document specimen temperature upon arrival at the laboratory. Stability studies were also performed to expand the acceptable temperature range of multiple specimen types for various assays across the laboratory.

Results: Following the initial implementation of temperature monitoring protocols, from January 2023 to November 2024, the laboratory identified an overall increase in tests reported as Unsatisfactory (Unsat) or Test Not Performed (TNP) due to temperature excursions in various clinical departments. Special Microbiology had an increase of approximately 33.3% in Unsat/TNP results and Serology rates increased by 4.5%, while Bacteriology and Virology rates did not change. Bacteriology did see an increase (36.4%) following initiation of temperature monitoring for FedEx/UPS/USPS package, as did Special Microbiology (62.2%) and Virology (6.4%). To mitigate the high rates of unsatisfactory QuantiFERON samples, the laboratory adjusted specimen handling protocols to encourage centrifugation post-transport. Rates of unsatisfactory and TNP results have decreased to approximately 5.8% overall as of November 2024 in response to the expanded acceptable temperature ranges and addition of centrifugation post-transport guidance for QuantiFERON submissions.

Conclusions: TDH-DLS continues to educate submitting facilities on proper specimen submission, especially during winter and summer when temperature extremes are more likely to impact specimen integrity. In addition, TDH-DLS communicates with the courier routinely in effort to maintain appropriate specimen transport conditions.

Presenter: Kala Priester, Tennessee Dept of Health, Division of Laboratory Services, kala.priester@tn.gov

Poster 169

Genomic Epidemiology Reveals Multiple SARS-CoV-2 Introductions in a Long-term Care Facility Outbreak

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The COVID-19 pandemic significantly advanced whole genome sequencing (WGS) technologies and expanded the application of genomic epidemiology. Analyzing SARS-CoV-2 genomic data offers valuable insights into outbreaks, including viral introductions and transmission patterns. Combined with traditional epidemiological methods, this data enhances disease prevention and control strategies. This study examines SARS-CoV-2 genetic sequences from a COVID-19 outbreak in a long-term care facility (LTCF) in Tennessee.

Between December 2023 and January 2024, an LTCF experienced a COVID-19 outbreak involving 46 cases. Collaboration between outbreak response and genomic epidemiology teams facilitated timely specimen retrieval, sequencing, and analysis. Nine remnant SARS-CoV-2-positive specimens were sent to the state laboratory for WGS; seven were successfully sequenced. Lineage assignment using Pangolin and GISAID databases, followed by NextStrain phylogenetic analysis, revealed two distinct

SARS-CoV-2 lineages circulating during the outbreak.
Four cases belonged to the HV.1 parent lineage, including three from sublineage EG.5.1.6, while three cases were associated with the JN.1 parent lineage, all from sublineage JN.1.8.1. The presence of two lineages indicates multiple virus introductions rather than a single-source transmission event. These findings align with the lineages circulating in the surrounding community, underscoring the role of community transmission in seeding LTCF outbreaks.
This study emphasizes the critical role of integrating genomic surveillance with public health measures to manage outbreaks in high-risk settings like LTCFs. Combining genomic and epidemiological data clarified transmission dynamics and informed interventions such as testing, patient cohorting, and infection control measures. Limited sample availability remains a challenge, highlighting the need for active surveillance and robust support systems to enhance data collection. Continuous genomic monitoring is essential for providing insights into outbreak responses and enabling LTCFs to adapt effectively as SARS-CoV-2 reporting guidelines evolve.

Presenter: Jyoti Narayana, Tennessee Health Department, jyoti.narayana@tn.gov

Poster 170
What Does Gen Z want?! How Can we Deliver?!
A. Keese, Texas Department of State Health Services - Public Health Laboratory Division

There are now an unprecedented five generations in the current workforce, with the oldest Gen Alphas (those born between 2010-2024) on the verge of embarking on their first professional experiences. With such a complex, multigenerational workspace, laboratories must evolve in both culture and policies to prepare for the expectations of the next generation of laboratorians. The rapid advances in technologies and emergence of new career paths over the past 25 years demand greater freedom by staff to make independent decisions in the face of the rapid pace of change. Gen Z will soon represent about one-third of the workforce, making it vital for laboratories to consider shifts in workplace priorities to attract and retain new talent. As the first generation to have been fully immersed in the internet since birth, their experience will set a precedent for the future workforce. Gen Z places higher emphasis on priorities such as purpose, work/life balance, transparency, collaboration and authenticity. Adaptation to cultural shifts can take time and require buy-in from all those involved. We have acknowledged the need for such changes and are making strides to recognize the priorities of the younger workforce to maintain and foster the best talent. We plan to present the following concepts and how we are championing changes to our work environment to match the needs and desires of this changing workforce.

Presenter: Anita Keese, Texas Department of State Health Services - Public Health Laboratory Division, anita.keese@dshs.texas.gov

Poster 171
EUROIMMUN® Anti-*Trypanosoma cruzi* ELISA (IgG) Test Performance in Diagnosis of Chagas Disease
N. Latif, TEXAS DSHS, L. Cao, TEXAS DSHS, M. Nolen, TEXAS DSHS, N. Latif, TEXAS DSHS

Chagas disease is a global illness caused by the parasite Trypanosoma cruzi, primarily found in Central and South America. Recently, however, due to increased migration and the presence of refugees and asylum seekers from regions where Chagas disease is endemic, cases of the disease are being increasingly detected in the United States. Without treatment, Chagas disease can lead to life-threatening cardiac or gastrointestinal complications. Accurate and specific screening assays to detect Trypanosoma cruzi antibodies in positive serum samples are essential for managing patients with chronic Chagas disease. Since no single serologic test is sensitive or specific enough to confirm diagnosis, screening for the chronic phase of Chagas disease at the TX DSHS Laboratory employs two different serologic tests, the Wiener Lab Chagatest ELISA recombinante v.3.0 and the Hemagen Chagas' ELISA. However, our current diagnostic system has yielded a high number of inconclusive results. To overcome this limitation, we evaluated the performance of the EUROIMMUN® Anti-Trypanosoma cruzi ELISA (IgG) assay (EUROIMMUN Medizinische Labordiagnostika, Germany) for

qualitative detection of IgG antibodies to Trypanosoma cruzi. We evaluated 33 serum samples from patients suspected to have Chagas disease using the Euroimmun® ELISA assay. Compared to the CDC confirmatory assays, the Euroimmun® Anti-Trypanosoma cruzi ELISA (IgG) assay demonstrates an overall 90.9% sensitivity (95% confidence interval [CI], 73.9% to 100%) and 77.3% specificity (95% confidence interval [CI], 59.8 % to 94.8%). For comparison, the Wiener Lab Chagatest ELISA recombinante v.3.0 demonstrates 90.9% sensitivity (95% confidence interval [CI], 81.1% to 100%) and 95.5% specificity (95% confidence interval [CI], 84.4 % to 100%), and the Hemagen Chagas' ELISA demonstrates 100% sensitivity (95% confidence interval [CI], 65.9% to 100%) and 50% specificity (95% confidence interval [CI], 32.9 % to 67.1%). One-Way ANOVA analysis reveals no significant difference between the CDC versus the Weiner and Euroimmun assays, $p = .742$ ($p>.05$); however, there are significant differences between the CDC versus the Hemagen assay, $p = .018$ ($p<.05$). These results indicate that the Euroimmun® Anti-Trypanosoma cruzi ELISA (IgG) assay performed significantly better than the Hemagen Chagas' ELISA assay in detecting Chagas disease, which would ultimately reduce the number of inconclusive specimens sent to the CDC for confirmatory testing if included in the testing algorithm as an alternative.

Presenter: Nabil Latif, TEXAS DSHS, nabil.Latif@dshs.texas.gov

Poster 172
Fostering Collaborative Relationships Between Academic Partners and Public Health Laboratories
D. Baker, The Association of Public Health Laboratories, A. Handzic, Association of Public Health Laboratories, H. Reiss, Association of Public Health Laboratories (APHL)

In his book To Repair the World, the late Dr. Paul Farmer reflected that "with rare exceptions, all of your most important achievements on this planet will come from working with others—or, in a word, partnership." APHL's efforts to strengthen strategic partnerships also recognized the importance of engaging new partnerships to support the workforce development needs of public health laboratories. In 2022, the APHL Academic Partnerships program was launched to support the recruitment and retention efforts of the public health laboratory workforce. Since its inception, the primary role of the Academic Partnerships Program has been to foster collaborative partnerships between public health laboratories and academic institutions in the promotion of Career Pathways in Public Health Laboratory Science: an APHL-CDC Initiative. In addition to developing an outreach strategy to increase the number of qualified applicants for the public health laboratory fellowship and internship programs, the Academic Partnerships program team has worked to strengthen partnerships with organizations that prioritize initiatives aligned with APHL's Core Values. Some of the outreach activities performed by this program area include exhibiting at career fairs and conferences, promoting fellowship and internship opportunities on job boards to ensure equitable access for all applicants, participating in annual meetings through poster and oral presentations, engaging in national STEM advocacy initiatives, and building connections with educational institutions. A new quarterly newsletter was created to disseminate information about career paths in public health laboratory science to academic partners throughout the country. In 2024, the Public Health Laboratory Ambassadors program was officially launched to engage public health professionals in outreach and promotion of the profession to students in their local communities. After its initial two years, the role of the APHL Academic Partnerships program continues to evolve in its efforts to support the public health laboratory workforce through enhanced visibility and awareness of the essential work performed in public health laboratories across the US. Creating a collaborative network has been instrumental in addressing the needs of the public health laboratory workforce while highlighting the opportunities for careers in public health laboratories. On this poster, we plan to share notable accomplishments and metrics that demonstrate the positive impact of building partnerships to foster collaboration and support in promoting the profession of public health laboratory science. Data insights from key academic partners, such as Handshake, will be used in the poster content focused on best practices in outreach and recruitment.

Presenter: Dana Baker, The Association of Public Health Laboratories, dana.baker@aphl.org

Poster 173
Are You Reporting to the National Respiratory and Enteric Virus Surveillance System (NREVSS)? The Informatics Technical Assistance Team Can Help!
D. Prettyman, The St. John Group, S. Abrams, Association of Public Health Laboratories, A. Liu, InductiveHealth

The National Respiratory and Enteric Virus Surveillance System (NREVSS) plays a critical role in monitoring viral activity across the United States. Participating laboratories voluntarily report weekly data to the US Centers for Disease Control and Prevention (CDC), including the total number of tests performed for various viral pathogens, the number of positive tests, specimen types, test locations, and week of collection. This surveillance system enables the timely analysis of data, which is essential for tracking viral seasons and circulation patterns.

To further expand its reach, CDC and the Association of Public Health Laboratories (APHL) are working in collaboration to include project-specific pathogens from all jurisdictions in reporting to NREVSS. Beginning January 1, 2024, the APHL Informatics Public Health Laboratory Interoperability Project (PHLIP) Technical Assistance (TA) team has focused on identifying laboratories that are not yet reporting, targeting engagement based on geographic and demographic needs.

By participating in NREVSS, laboratories contribute valuable information that aids in monitoring seasonal trends, clinical risk factors, hospitalization rates, and patient demographics for specific pathogens. While submission is voluntary, the increasing prevalence of many pathogens makes this initiative especially important. With the assistance of the APHL TA Team, laboratories can submit data electronically with minimal ongoing effort once initial setup is completed.

Presenter: DeAnn Prettyman, The St. John Group, dprettyman@tsjg.com

Poster 174
Fast, Robust and Accurate Analysis of 43 Elements in Whole Blood Using Triple Quadrupole Inductively Coupled Plasma Mass Spectrometry (ICP-MS)
J. Guo, Thermo Fisher Scientific, J. Guo, Thermo Fisher Scientific, K. Hassell, Thermo Fisher Scientific, D. Kutscher, Thermo Fisher Scientific

The exposure to toxic metals is a known risk factor for several diseases, whereas other trace elements, at appropriate levels, support regular biological function. Concentrations of elements within the blood of a population of subjects can correlate elemental exposure to geographical area, lifestyle, and socio-demographics. Inductively coupled plasma mass spectrometry (ICP-MS) is a highly sensitive technique that can work with low sample volumes while enabling high sample throughput and robust analysis even in the most demanding sample types. However, the analysis of samples containing higher levels of salts and biomolecules, such as proteins or metabolites, is a known challenge in ICP-MS. The complexity of the sample matrix can significantly affect the sensitivity of the instrument, and lead to increased system maintenance. In addition, the development of a multi-element method is challenging due to the wide concentration ranges that need to be covered across essential and toxic elements and the wide range of potential interferences from the sample matrix. These complex prerequisites often mean that triple quadrupole ICP-MS instruments are the best choice for analysis.

A Thermo Scientific™ iCAP™ MTX ICP-MS, operated together with a Thermo Scientific™ iSC-65 Autosampler, was used for all analyses. The autosampler was optimized for increased uptime using the Step Ahead function. Thermo Scientific™ Qtegra™ Intelligent Scientific Data Solution™ automatically selected the most appropriate isotopes, and the optimum analysis conditions (kinetic energy discrimination mode versus reactive gas, on mass mode vs. mass shift reaction mode). The certified reference materials (CRMs, Seronorm™ Trace Elements in Whole blood L-1, L-2 and L-3, SERO, Norway) and three types of whole blood (human, horse, and pig), a series standards (ranging from 2 ng/L and up to 25,000-fold), and a quality control sample were spiked with an internal standard (IS) mix (Sc, Ge, Y, Rh, and Ir) and processed.

All 43 elements were first analyzed for the detection limits and linearity. The polyatomic interferences were removed using the mass-shift reaction with O2. Low mass analytes detection limits in the ng/L range, even sub ng/L for some elements, were achieved. The coefficient of determination (R2) for all analytes were greater than 0.9995, indicating excellent linear response. The accuracy and precision of the method was assessed by analyzing CRMs. The results show that the calculated concentrations for most of the target elements matched the certified values. Three blood samples were analyzed to assess the repeatability of the results over longer analysis times. All 43 elements showed reliable and predictable recovery (88 – 124%), and IS (75 - 112%), over the 15 hr of the 287 samples, demonstrating robust analytical performance.

This work focuses on the development of a fast, robust, and accurate method for the analysis of 43 toxic and essential elements in whole blood using ICP-MS. The analytical method was rigorously tested, and the results obtained clearly demonstrated the analytical advantages of utilizing the capability of triple quadrupole ICP-MS to provide fast and accurate data in a true multi-element analysis manner for larger studies of assessing biological variation or occupational health.

Presenter: Jingshu Guo, Thermo Fisher Scientific, jingshu.guo@thermofisher.com

Poster 175
Discovering Unknown Analytes in City Wastewater Utilizing High-resolution Orbitrap Mass Spectrometry
C. Patterson, Thermo Fisher Scientific, J. Bowden, University of Florida, T. Sabo-Attwood, University of South Carolina, C. Patterson, Thermo Fisher

Introduction
With the rise of new psychoactive substances (NPS) and drug analogues, it is key to quickly identify novel drugs targets to drive public health interventions and improve community safety. Wastewater surveillance can serve as a tool to provide rapid information on illicit drug use to public health officials. Untargeted analysis by Orbitrap mass spectrometry provides labs with sensitivity and accuracy to find compounds in a sample beyond a targeted list. Coupled with Compound Discoverer software, unknown compound can be matched with lab-curated libraries as well as the online spectral database mzCloud. The goal of this study is to survey 10 wastewater treatment facility samples from 4 cities in hopes of identify drugs of abuse and NPS from each collection site.

Experiment
10 wastewater samples were extracted by SPE using OASIS MCX Extracted samples were separated by a Vanquish ultra-high performance liquid chromatography (UHPLC) using a 7-min gradient and Hypersil Gold C18 (150 x 2.1 mm, 5 µm) column. An Orbitrap Mass Spectrometer was used for untargeted screening using data-dependent MS2 with Top N&#3f10. This method was run in both positive and negative polarities. Data was analyzed using Compound Discoverer Software and mzCloud online spectral database which includes over 30,000 compounds.

Preliminary data
This untargeted screening method on an Orbitrap mass spectrometer was able to detect over 100 positive and negative compounds in each city’s wastewater. By using the mzCloud database scoring (>70%) and mass accuracy (+/- 5 ppm), we were able to filter down the hits. Using the compound class scoring node for fentanyl's, common fentanyl metabolites and unknown fentanyl analogues can be detected. Some of the drugs detected included benzoylecgonine, cocaine, gabapentin, ketamine, MDEA, as well as several unidentified NPS compounds.

Conclusion
The untargeted method was rigorously tested, and the results obtained clearly demonstrated the analytical advantages of utilizing the Orbitrap mass spectrometer with Compound Discoverer to accurately identify NPS and more commonly found drugs of abuse. The opportunity to broaden this study to more cities can provide a wealth of public health knowledge.

Presenter: Courtney Patterson, Thermo Fisher Scientific, courtney.patterson@thermofisher.com

Poster 176
West Nile Virus Lineage 3 Not Found in *Culex* Species Mosquitos in Nebraska Collected from 2012 to 2024
S. Freed, Jr., University of Nebraska Medical Center, S. Chandler, University of Nebraska Medical Center, K. Herzog, University of Nebraska Medical Center, J. Broadhurst, University of Nebraska Medical Center, J. Fauver, University of Nebraska Medical Center

West Nile virus (WNV) is the most prevalent arthropod-borne virus in the US. While the virus has multiple lineages capable of causing disease, West Nile virus lineage 1 (WNVL1) is the only lineage circulating within the US. Most cases are asymptomatic, but roughly 20% present with febrile illness. In around < 1% of cases, patients develop neuroinvasive disease. In 2023, a patient in Nebraska presented with neuroinvasive WNV disease, but also had unusually high viremia. Upon genomic investigation, it was reported that the patient serum sample contained both WNVL1 and lineage 3. West Nile virus lineage 3 (WNVL3) has only been detected in mosquitos within Czechia and previously has never been identified to cause disease in humans. The current health implications are unknown.

We used WNVL3 specific primers to screen our repository of mosquito pools using RT-qPCR. As part of entomological surveillance for WNV, Culex spp. mosquitos are routinely trapped and pooled during the summer, then tested for the presence of WNVL1. These pools were collected between the years 2012 and 2024 during the WNV epidemiological season and have tested positive for WNVL1. The pools were separated by Culex species and total nucleic acid had been extracted prior to this study. A standard curve using known quantities of WNVL3 was developed alongside this assay to quantify the number of viral copies upon a WNVL3 positive pool and to serve as a positive control.

847 mosquito pools were tested, representing 33,085 mosquitos, collected across 12 years. We observed no positive samples for WNVL3. Two samples displayed amplification with high cycle-threshold values of 34.95 and 38.00hat fell below our limit of detection determined by the standard curve. When these samples were tested again, there was no signal detected. Assuming the WNVL3 that was isolated from patient serum came from a mosquito, the minimum infection rate for WNVL3 in Culex spp. is 0.03 per 1000.

This study represents a robust sampling of mosquitos most important for WNVL1 disease transmission and management. These data suggest that if WNVL3 is circulating within Nebraska Culex spp., it is at extremely low levels. It is possible that WNVL3 may exist within a separate transmission cycle from Culex spp. and the bird species that they feed upon. However, establishing WNVL3 surveillance procedures within the same mosquito populations tested for WNVL1 will be unlikely to detection of WNVL3.

Presenter: Shawn Freed, Jr., University of Nebraska Medical Center, shawnfreed@creighton.edu

Poster 177
Challenges and Strategies for Implementing Wastewater-Based Epidemiology in Rural Communities
G. Dickey, University of Texas Health Science Center at Tyler, G. Dickey, The University of Texas Health Science Center at Tyler, M. Crum, UT Tyler Health Science Center

Introduction. Wastewater-based epidemiology (WBE) has emerged as an effective and valuable tool for monitoring public health, particularly in the detection of infectious diseases and the assessment of community health trends. While WBE is an extremely valuable tool for predicting trends in infectious disease, implementing WBE in rural communities, like Northeast Texas, presents unique challenges that require tailored strategies to create a sustainable WBE project.
Background.
In 2021, the Public Health Laboratory of East Texas (PHLET) initiated a WBE program to track SARS-

CoV-2 levels in Gregg and Smith Counties. Public Health Labs (PHLs) face multiple challenges in implementing these programs, from the pre-analytical stage to public dissemination. Issues of privacy, communication, and the lack of accessible public health data exacerbate community disparities, leading to a decline in overall health outcomes.
Objective.
This report seeks to elucidate challenges faced in WBE by a regional PHL. The WBE project proposes to address public health information availability for the Northeast Texas region through collaboration with The Northeast Texas Public Health District (NET Health), with a focus on surveillance of respiratory viruses. Utilizing data from the WBE program, the aim is to provide information for the creation of a user-friendly multi-level dashboard. Using WBE, our aim is to develop a user-friendly community dashboard.
Methods.
This report discusses how an intensive wastewater site analysis was performed to determine the starting location for sample collection. The selection of wastewater treatment plants (WWTPs) as sample locations and how access was determined by selecting sites that met criteria of under 1 MGD (million gallons per day). Throughout this process, a development of laboratory workflow for processing and resulting of samples has been completed. Lastly, this report describes the analysis of WBE data by using Microsoft PowerBI to create a multi-level dashboard.
Conclusion. In conclusion, while WBE offers significant potential for enhancing public health monitoring, its implementation in predominantly rural areas like Northeast Texas requires addressing unique challenges. By focusing on ethical considerations, infrastructure development, and resource allocation, and using a multi-level dashboard for data integration and community engagement, sustainable WBE projects can be successfully established. These strategies not only overcome the specific obstacles faced by rural communities but also pave the way for more effective and inclusive public health initiatives.

Presenter: Grace Dickey, University of Texas Health Science Center at Tyler, Grace.Dickey@uthct.edu

Poster 178
Making Genomic Data Actionable: NGS Report Content and Usability
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Background:
The interpretation and communication of next-generation sequencing (NGS) data are critical to public health responses, yet variability in report structure and content can impact utility. In our recent Pathogen Genomics Centers of Excellence (PGCoE) Ring Trial, we assessed how genomic reports were structured and shared across public health laboratories. Our objective was to identify key report elements, evaluate how findings were communicated to stakeholders, and explore the extent to which reports support actionable decision-making in outbreak investigations.
Methods:
The trial involved 11 clinical isolates of carbapenemase-producing Klebsiella pneumoniae from a simulated nosocomial outbreak. Seven public health laboratories participated, receiving genomic files, a detailed outbreak case stem, and a 40-question survey on NGS workflows and reporting practices. Reports were analyzed for elements such as organism identification, phylogenetic relatedness, and antimicrobial resistance profiles. We assessed how reports framed findings for diverse audiences, including infection prevention teams, epidemiologists, and hospital leadership. Visualizations were reviewed for clarity and utility, and data-sharing workflows between public health labs, healthcare

facilities, and stakeholders were examined to identify communication gaps.
Results:
All seven sites submitted reports with detailed genomic findings, but structures and content varied significantly. Only 29% (2/7) of laboratories offered genomic outbreak analysis as a routine public health service, and 43% (3/7) did not have a pre-existing outbreak report template. Among reports, 86% (6/7) included a method or pipeline description, and 86% (6/7) included single-nucleotide variant (SNV) matrices. However, only 57% (4/7) included result interpretation, ranging from brief summaries to detailed explanations. The majority of reports (71%) included a phylogenetic tree, though it was not always clear if this feature added value to conclusions or decision-making. Survey feedback indicated that 86% of participants routinely perform NGS-based outbreak investigations, though most highlighted challenges in balancing technical detail with usability for broader audiences. Data-sharing limitations, particularly in collaborations with external partners, were noted as barriers to communication.
Conclusion:
This ring trial revealed variability in NGS report structure, content, and communication, underscoring the need for standardized frameworks that support scientific rigor and usability. By aligning report elements with the needs of diverse stakeholders—including public health teams, care facilities, and leadership—genomic findings can be made more actionable. Future iterations will refine report templates, improve data-sharing workflows, and expand collaboration to enhance genomic reporting in outbreak investigations.

Presenter: Emily Snavely, University of Virginia Health, xjx4nh@uvahealth.org

Poster 179
Carbapenem Resistance Genes Associated with Various Human and Environmental Microbial Communities in Wastewater
M. Schussman, University of Wisconsin - Milwaukee, School of Freshwater Sciences , S. McLellan, University of Wisconsin-Milwaukee, K. Jansen, Wisconsin State Laboratory of Hygiene, E. Doolittle, Wisconsin State Laboratory of Hygiene, G. Knuth, Wisconsin State Laboratory of Hygiene

Carbapenem-resistant bacteria are a growing threat to human populations globally, but their distribution in human and environmental reservoirs is not well understood. Wastewater surveillance has moved to the forefront as a practical and informative public health tool for assessing viral and bacterial pathogens in the human population, however, the usefulness of tracking antibiotic resistant genes (ARGs) of clinical concern is not straightforward given the large reservoir of free-living bacteria within sewer systems. In this study we examined 122 samples for the quantity and diversity of six high-priority carbapenem resistance gene targets; blaKPC, blaOXA-24/40, and blaOXA-48, blaVIM, blaIMP-1, blaNDM; collected from two wastewater treatment plants (WWTP) that together service the entirety of Milwaukee. Fecal markers HF183, and lachno3, and host organisms Escherichia. coli, Klebsiella pneumoniae, and Acinetobacter baumannii were quantified in addition to ARGs.

ARG targets were quantified using PCR-based methods and further analyzed for genetic variation and community abundance through sequencing-based methods. All targets were quantified in both WWTPs at high abundances, with levels as high as 5E+06 copy number (cn)/L for blaKPC. Most ARG targets showed moderate to low correlations to fecal anaerobe human microbiome markers, rather, blaKPC, blaOXA-48, and blaNDM showed stronger correlations to K. pneumoniae or E. coli, and blaOXA-24/40 targets to A. baumannii. The blaVIM gene was the only target to express a strong positive correlation to human markers across both WWTPS. The amplicon produced by each assay was sequenced. We were able to confirm our assays were picking up expected organisms and found 1-2 dominant sequence types that matched exactly to reported clinical strains. Only a few minor sequences (<1.0%) appeared as non-specific amplification.

Both treatment plants showed their own dynamics, suggesting local characteristics are the primary driver of concentrations. Levels varied across seasons as did their presumptive bacterial hosts, suggesting growth dynamics within the sewer conveyance system can influence concentrations. Through analysis of the microbial community using 16S rRNA gene sequencing, we confirmed there are sewer dominant and environmental dominant organisms that enrich in the community from

upstream to downstream in the sewer conveyance system, while human fecal marker organisms remain the same, or decrease in relative abundance. These findings suggest that environmental reservoirs may be influencing the detection of AMGs via WWS. Understanding the ecological dynamics of bacteria harboring ARGs will be important for understanding reservoirs and interpreting wastewater surveillance data.

Presenter: Melissa Schussman, University of Wisconsin - Milwaukee, School of Freshwater Sciences, schussm2@uwm.edu

Poster 180

Detection of Multiple Respiratory Pathogens in Air Samples Collected from K-12 Schools Using Standard Biotools Respiratory Pathogen Assay Panel

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Environmental sampling has become an efficient way to detect pathogen genetic material from congregate spaces without requiring cumbersome and costly testing of individuals. Air can be sampled from smaller congregate spaces, and the pathogen genetic material detected complements that are obtained by pathogen detection from wastewater from larger communities. We have sampled air from K-12 school settings for SARS-CoV-2 and Influenza A virus since 2023. We are now using multi-pathogen detection assays to expand the number of pathogens whose genetic material is identified. Here, we describe the application of the novel Standard Biotools Respiratory Pathogen Assay Panel (RPP assay) to nucleic acids isolated from air samples. Air samples were collected twice weekly from eleven Wisconsin K-12 schools beginning in September of 2024 up to the present day. RNA was isolated, and samples were tested in duplicate with the RPP assay. This assay detects 18 targets corresponding to viruses commonly observed during respiratory virus season. SARS-CoV-2 and Adenovirus were detected at nearly steady frequencies for the entire duration of the monitoring period. In November and December of 2024, an increase in the frequency of detection of other respiratory viruses was observed, including Human Coronaviruses NL63 and HKU1 (HCoV NL63 and HCoV HKU1), as well as sporadic detection of influenza A and Respiratory Syncytial Virus (RSV). These data suggest that the Standard Biotools RPP assay can be used to detect respiratory pathogens from air cartridge samples. This study is ongoing, and we plan to compare results across the entire 2024/2025 school year from the RPP assay to other multiplex PCR assays utilized in our laboratory.

Presenter: Amy Ellis, University of Wisconsin Madison, ellis2@wisc.edu

Poster 181

Ensuring Public Health Laboratories reach their Medical Community, Brownsville, 2024–2025

P. Hernandez, UT Health Houston School of Public Health, Brownsville campus, M. Morris, UT Health Houston School of Public Health, C. Romero, City of Brownsville Health, Wellness and Animal Services, A. Rodriguez, City of Brownsville Health, Wellness and Animal Services, S. Fisher-Hoch, UT Health Houston School of Public Health, J. McCormick, UT Health Houston School of Public Health

Wastewater surveillance is an important emerging tool for understanding the epidemiology of pathogens at local, state, and national levels and is increasingly replacing surveillance of individuals. Utilizing wastewater surveillance provides a great advantage because of its broad reach across communities. Collaborations between wastewater facilities, public health laboratories and authorities generates data that is crucial to the maintenance of community health and safety. The Cameron County wastewater surveillance conducted at the UTHealth Houston Brownsville campus addresses the challenge of timely communication of circulating pathogens to the local medical community. In a joint effort between our laboratory, city authorities, and clinics we created a standard tool designed to communicate this information weekly to busy emergency rooms, pediatric urgent cares, and other

clinics across Cameron County to assist in appropriate diagnosis and treatment.
We created an eye-catching, simple and concise dashboard, detailing key information from wastewater surveillance by our research laboratory and information extracted from the Texas Epidemic Public Health Institute. Additionally, we included any vector-borne disease findings from the One Health Sciences Laboratory in Brownsville. Using various communication channels, we implemented our program through local health authorities, hospitals, and clinics to share this data with sites providing acute medical care. We first implemented this in a pilot program with partners then evaluated the impact so that we were able to modify the design and delivery to best meet their needs.
We then studied the impact of the program by reviewing comments and reactions and used data from clinics, including antibiotic usage, to determine the practical effects of the program. The feedback provided was collected using the QuestionPro platform.
We have shown that wastewater surveillance can be effectively conducted locally. Sharing the data in a timely fashion with those who need to know is an additional task. Our study has shown that the public health impact of local wastewater surveillance is both appreciated and of immediate benefit to authorities, clinics, and our communities.

Presenter: Paola Hernandez, UT Health Houston School of Public Health, Brownsville campus, paola.a.hernandez@uth.tmc.edu

Poster 182
H5N1 Preparedness and Continuity of Operations for the Public Health Lab of East Texas: Lessons for All in Response
M. Crum, UT Tyler School of Medicine and Public Health Lab of East Texas, M. Crum, UT Tyler Health Science Center

The Public Health Lab of East Texas (PHLET) has a small staff and is responsible for infectious disease testing for a 35-county region of East Texas. This work will describe how we utilized lessons from COVID-19 and other responses to formulate an early continuity of operations plan for our lab. The PHLET was the first to respond to the SARS CoV-2 outbreak in 2020. The months that followed gave us insight into the various gaps in our testing and resulting workflow that prevented the most effective response in that situation. The objective of this work is to share our response gaps from the past and determine best utilization of our laboratory staff, available supplies and reagents, and funding to have the ability to continue operations during the next pandemic. This analysis will focus on readiness during the early stages of an outbreak before a pandemic officially begins spreading via human-to-human transmission. Our lab meets on a regular basis to review and test our response plans. We also reviewed our staffing and their various roles and responsibilities, with a focus on cross-training. A plan was developed based on our staffing and testing capabilities to decide when to shift to a larger testing platform, and what that trigger point would be. The team decided to order reagents and supplies, including PPE in case there is a supply shortage. Lastly, we designed ways to relieve stress and tension related to work responsibilities, and to maximize communication and cooperation during a pandemic response.

Conclusion: The design of our continuity of operations plan is a shared resource that we hope can assist other public health laboratories in their planning and response before the next pandemic. The goal is assisting public health laboratories in surge capacity and preparedness efforts against emerging infectious disease threats, including but not limited to those pathogens that cause pandemics.

Presenter: Michelle Crum, UT Tyler School of Medicine and Public Health Lab of East Texas, michelle.crum@uttyler.edu

Poster 183
Detection of Carbapenemase Resistant Pseudomonas aeruginosa via Wastewater Surveillance
B. Seitter, Utah Public Health Lab, G. Pinas, Utah Public Health Laboratory, G. Pinas, Utah Public Health Lab, B. Troutman, Utah Public Health Lab

Carbapenem-resistant Pseudomonas aeruginosa (CRPAs) is one of the most concerning and difficult

to treat antimicrobial resistant organisms. It has been shown to contaminate medical devices and products, surfaces, water sources, thereby causing important outbreaks in healthcare settings. Resistance to multiple classes of antibiotics and high pathogenicity make CRPA an important target of infection prevention and surveillance. Wastewater surveillance has proven to be a promising way to detect pathogens of interest on a community level. Here we validated and optimized a culture method for the detection of CRPAs in wastewater.

A selective chromogenic medium for P. aeruginosa (RAPID P. aeruginosa Agar for Water Testing, Biorad) was evaluated. Presumptive P. aeruginosa colonies are blue-green on the RAPID P. aeruginosa Agar. Meropenem was added at 4 µg/mL to the medium to select for CRPA and incubations were performed at 37°C. The strains PA AR0054 (carbapenem-resistant) and PA 27853 (carbapenem-susceptible) were used for medium QC. Organism concentration prior to plating was achieved by centrifugation. Bacterial species identification was performed by MALDI-TOF mass spectrometry (Bruker). Carbapenemase production was detected through the phenotypic mCIM test. mCIM positive isolates were screened for the expression of KPC, NDM, VIM, IMP and OXA-48-like carbapenemases via the CARBA 5 lateral flow chromatography device (Hardy Diagnostics) and molecularly characterized by whole genome sequencing (WGS) (Illumina). Raw influent wastewater was collected as a 24h composite sample from 34 wastewater treatment plants (WWTP) in Utah.

An initial spike-in experiment showed that the growth of P. aeruginosa is supported in all WWTP influent samples. Good organism recovery (up to 100%) in all samples tested indicates that the RAPID P. aeruginosa Agar is suitable for the isolation of CRPA from wastewater. All the green colonies that grew in the spiking experiment were identified as P. aeruginosa, confirming the stated specificity of the medium. White and grey colonies encompassed a range of other Pseudomonas species and Stenotrophomonas maltophila. A second experiment was performed to screen all Utah WWTP samples for the presence of CRPA. 24 of the 34 WWTP samples analyzed yielded green colonies. Only isolates from 15 individual WWTPs were identified as P. aeruginosa. Green colonies from the remaining nine sites were speciated as: Stenotrophomonas maltophila, Aeromonas hydrophila, Aeromonas caviae and Pseudomonas otiditis. A total of 22 P. aeruginosa isolates were tested for carbapenemase production via mCIM. Three isolates, each from a different WWTP, were mCIM positive and were further characterized using the CARBA-5 assay and WGS. Two isolates did not harbor any known carbapenemase, while one of them expressed of a VIM-2 carbapenemase. WGS analysis indicated that the VIM-2 wastewater isolate is not related to any available clinical isolate from the State of Utah, yet belongs to a sequence type (ST235) commonly found in CRPA human infections.

This pilot experiment showed that CRPAs can be cultured from wastewater samples through this method, which we will employ for community level prospective surveillance. Further genomic characterization of CRPA wastewater isolates identified through this approach will allow us to establish correlation with clinical surveillance data and inform the molecular epidemiology of this important pathogen.

Presenter: Brooke Seitter, Utah Public Health Lab, uphlfellowbs1@utah.gov

Poster 184

Bayes inference of SARS-CoV-2 Evolution: Comparative Analysis of Genome-wide and Protein Specific Molecular Clocks

J. Linares-Perdomo, PhD, K. Oakeson, PhD, Erin Young, PhD, Utah Public Health Laboratory (Unified State Laboratory)

Materials and methods:
Data collection: SARS-CoV-2 genome sequences were downloaded from GISAID database, ensuring the inclusion of metadata such as collection dates for tip dating. Sequences were filtered to maintain high quality, retaining complete genomes with minimal ambiguities. The data sed was then partitioned by genome regions corresponding to individual structural and not-structural proteins and the full genome.
Bayesian phylogenetic analysis: Phylogenetic inference was conducted using BEAST v2.7.7.

Sequences were aligned, and tip dates were calibrated using sample collection dates. For site model selection, the HKY substitution model was used in conjunction with a gamma-distributed rate heterogeneity model to account for variations in substitution rates. Molecular clock models: The evolutionary rate was estimated using a random local model, allowing for rate variation across lineages. This approach was chosen to account for the potentially heterogeneous evolutionary dynamic across-
different proteins and the entire genome.
Prior distribution and demographic model: A coalescent Bayesian Skyline model was applied as the demographic prior
to capture changes in effective population size over time. For the rate changes analysis, a poison distribution using the
RRateChanges parameter was used.
Analysis implementation: Markov Chain Monte Carlo (MCMC) chains were run for 20M generations, sampling every
10000 generations. Convergence was assessing using Tracer v1.7.2, with effective sample size (ESS) values > 200
indicating robust parameter estimates. Maximum clade credibility (MCC) trees were generated using TreeAnnotator and
visualized using Figtree V1.4.4.
Results:
These findings reveal distinct mutation rates and evolutionary pa]erns between genome-wide and protein-specific
molecular clocks, reflecting the heterogeneous dynamics of SARS-CoV-2 evolution. Notably, certain proteins exhibited
accelerated rates, suggesting adaptive pressures and functional constraints shaping viral evolution. These results
highlight the importance of integra\ng protein-specific analyses with genome-wide studies to comprehensively
understand the molecular evolution of the virus.
Discussion:
The evolutionary dynamics of SARS-CoV-2 is critical for tracing its spread and adaptation. In this study, we applied Bayesian
inference analysis methods to investigate muta\on rates and molecular clocks across genome-wide and protein-specific
regions.
Conclusion:
This comparative approach provides insights into the evolutionary mechanisms driving SARS-CoV-2 diversification,
offering a framework for future studies on viral adaptability and phylogenetic reconstruction.
Keywords: Bayesian Inference analysis; BEAST, BEAUTI, Poison distribution, Coalescent model, Markov Chain Monte Carlo.

Presenter: Kelly Oakeson, Utah Public Health Laboratory (Unified State Laboratory), koakeson@utah.gov

Poster 185

Key Performance Indicators for Evaluation of Novel Automated Wastewater Methodology

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Wastewater surveillance is an emerging frontier in the public health sphere. Methods in sample concentration, extraction, and analysis are constantly evolving as more organizations adapt methods for their programs. When developing a new program, determining which methodology to implement is a

key decision. We have identified four key performance indicators (KPI) to consider when evaluating: accuracy, method complexity, hands-on time, and total nucleic acid (TNA) quality. Here, we describe the evaluation of a novel automated concentration method using these KPIs.
Influent wastewater was collected from thirty-five wastewater facilities and processed twice per week at Utah Public Health Laboratory using the Promega Maxwell RSC Enviro TNA Kit in a manual direct capture (DC) method. Briefly, samples go through protease treatment, centrifugal sedimentation of solids, and column filtration. In terms of hands-on time, the DC method required two analysts working for 3.5 hours (7 hours combined hands-on time). This is the standard to which we evaluated an alternative concentration method over four collection dates. Twenty-two sites were additionally tested using a novel automated approach, a Promega kit on the Kingfisher Flex instrument. The selected samples for the rapid capture (RC) method were chemically inactivated prior to a bead-based concentration on the Kingfisher. The RC method required one technician 45 minutes of hands-on time with an additional hour on the Kingfisher instrument.
For both methods, TNA extraction was performed on the Maxwell RSC automated instrument. All samples were suspended into a final elution volume of 80µL and stored at -80°C prior to analysis. Eluted samples from both methods were analyzed using the Bio-Rad QX600 ddPCR system and QX Manager software. Data from five respiratory targets (Influenza A, Influenza B, Respiratory syncytial virus, SARS-CoV-2, and bovine respiratory syncytial virus) were compared using two-way ANOVA in the statistical programming language, R. Analysis showed the Kingfisher RC method produced fewer copies/well in most samples and ANOVA revealed statistically significant differences in measured concentrations across all targets.
We sequenced the SARS-CoV-2 genome using the Illumina COVIDSeq protocol. The analyzed Illumina sequencing data went through data quality control, in which the reads with an average Phred quality score below 30 and length shorter than 50 bp were discarded. The trimmed and human-filtered reads were mapped to the reference SARS-CoV-2 genome using bowtie. Samples with less than 40% genome coverage at 10X depth were considered to have low genome coverage and were excluded from further variant analysis.
Using the outlined KPIs we compared the two methods. While the novel RC method was less complex and required significantly less hands-on time than the DC method, the TNA quality it produced failed to meet our needs for downstream analysis. However, it is important to note that nucleic acids used for sequencing were processed without any prior cleanup-step. Future workflow improvements will include dilution of the extracted nucleic acid or a cleanup step prior to sequencing. Overall, the new method shows promise for implementation in the lab, provided TNA quality can be improved. This method may be particularly valuable when analyzing wastewater solids for specific targets that may be enriched in this matrix.

Presenter: Christopher Fajardo, Utah Public Health Laboratory, cfajardo@utah.gov

Poster 186
An Expanded Analysis of Echinocandin Resistance in *Candida auris*: Correlating FKS1 Mutations with Antifungal Susceptibility Testing Minimum Inhibitory Concentration Breakpoints
T. Iverson, Utah Public Health Laboratory

Due to the multidrug-resistant profile of Candida auris, echinocandins—agents that inhibit β-(1,3)-D-glucan synthesis in the fungal cell wall—are frequently employed as a first-line treatment. However, the emergence of echinocandin resistance in certain C. auris strains highlights the need for rapid identification of resistant isolates to optimize treatment strategies and prevent further spread of resistance.

The primary mechanism of echinocandin resistance in C. auris involves mutations in the FKS1 gene, specifically within the conserved hotspot regions HS1 and HS2. These mutations alter the glucan synthase complex, thereby reducing the binding affinity and efficacy of echinocandins. This study aims to identify FKS1 mutations linked to echinocandin resistance in C. auris by correlating these mutations with antifungal susceptibility testing (AST) minimum inhibitory concentration (MIC) breakpoints, informed by guidelines for related Candida species.

Whole-genome sequencing (WGS) of 3,351 C. auris isolates collected between December 2021 and December 2024 identified 48 isolates (~1.43%) harboring FKS1 mutations. Among these, p.Ser639Phe (Clade I) and p.Ser639Tyr (Clade III) were significantly associated with echinocandin resistance. Thirty-one of the mutant isolates belonged to Clade I (2.13% of all Clade I isolates), whereas 17 were from Clade III (0.98% of all Clade III isolates). Notably, nine of the resistant Clade I isolates were in the FKS1 HS1 region, while Clade III had only one resistant isolate in this hotspot. Collectively, these results underscore the extended timeframe required to collect a sufficient number of mutation-positive isolates for robust analysis.

Rapid molecular detection of FKS1 mutations can facilitate timely clinical decisions and support the selection of appropriate antifungal therapy before phenotypic resistance is confirmed. Understanding the prevalence and distribution of these mutations is critical for surveillance and the management of C. auris outbreaks.

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Poster 187
Software Containers for Public Health Bioinformatics
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To make bioinformatic analyses scalable, portable, and reproducible, many bioinformaticians have turned to containers to run their bioinformatic tools. Software containers are isolated computational environments that resolve dependency conflicts and provide static digital space for software to be run. Just as a kit streamlines laboratory workflows by providing a consistent set of equipment and reagents, containers offer a standardized and replicable environment for running bioinformatics tools.

Bioinformaticians in public health are in a unique subfield of bioinformatics with goals focused on actionable surveillance and response for public health interests. This smaller scope needed container support for specific subsets and options for bioinformatic tools not available in larger community projects. To address this challenge, the State Public Health Bioinformatics consortium (StaPH-B) maintains two repositories for Docker images at Docker Hub (https://hub.docker.com/u/staphb) and quay.io (https://quay.io/organization/staphb). Both Docker and Apptainer (Singularity) can pull these images for use in Nextflow, WDL, and Snakemake workflows as well as standalone analyses with a container manager or the StaPH-B toolkit (https://github.com/StaPH-B/staphb_toolkit). The open-source nature of the project allows anyone to contribute to the images hosted in these repositories by submitting a Dockerfile to a GitHub repository (https://github.com/StaPH-B/docker-builds).

The 180+ images hosted by StaPH-B have been pulled over 16 million times. The most popular bioinformatic tools include pangolin, samtools, iVar, VADR, FastQC, and Freyja.

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

Poster 188
Powassan Virus Surveillance in Vermont: Utilizing an RNA-extraction Control to Optimize qPCR Screening of *Ixodes scapularis* Ticks
C. VanWinkle Orzell, Vermont Agency of Agriculture

Deer Tick Virus, or Powassan virus (Lineage II, DTV), is a single-stranded RNA virus of the Flavivirus genus transmitted to humans by the blacklegged tick (Ixodes scapularis). It is the causative agent of Powassan virus disease, a rare but serious illness presenting as meningitis or encephalitis that can lead to permanent neurological symptoms and/or death; currently there is no treatment for infection. Blacklegged ticks are the most abundant ticks in Vermont, and Powassan virus disease was reported in the state in 1999, 2022, and 2023. The Vermont Agency of Agriculture, Food and Markets' Tick Surveillance Program tracks tick infection rates over five-year cycles, during which ticks are collected

from every county. Ticks collected from 2015–2019 showed a 0.4% prevalence of Powassan virus infection, with subsequent years' screens resulting in less than 0.1%. These data from the Vermont Agriculture and Environmental Laboratory were generated using a singleplex TaqMan reverse transcriptase (RT) qPCR assay targeting the ns5 gene of the POWV lineage II virus (DTV). While an in-well (per sample) nucleic acid extraction control was utilized (Ixodes scapularis actin gene target), the extraction control was unable to differentiate between RNA and genomic DNA. To control for possible false-negative results, it is imperative to detect the presence of RNA and control for failure of RNA extraction or degradation. To address this shortcoming, we pursued the development of a custom assay target spanning an exon-exon junction, as the DNA sequence containing the intron would not be amplified. Initial experiments using our Ixodes scapularis actin-based custom assay showed diminished but low-copy amplification of DNA. Later collaboration with the University of Massachusetts, Amhurst (Rich et al., 2024), resulted in our adoption of an Ixodes tick calreticulin-gene–based assay exploiting the exon-exon junction design and capable of controlling for RNA extraction and quality, thus ensuring against false negative results and improving the quality and integrity of Vermont's Powassan virus surveillance data. Shown in this poster are our methods of tick processing and nucleic acid extraction followed by thermocycling conditions and amplification plots of both the actin- and calreticulin-based RNA control assays with Ixodes scapularis total nucleic acids.

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Poster 189
Collaborative Restructuring in a Public Health Lab Microbiology Unit
K. Seiler, Vermont Department of Health Laboratory, J. Warrington, Vermont Department of Health Laboratory, L. Desautels, Vermont Department of Health Laboratory

The Vermont Department of Health Laboratory (VDHL) Microbiology Unit underwent a restructuring in 2024 following the hire of a new Microbiology Program Chief. In alignment with public health infrastructure objectives of Healthy People 2030, this restructuring was in part motivated by recognition of the need for a strong, engaged public health workforce. It also served to proactively address potential workforce retention issues at VDHL by addressing imbalances and gaps in the pre-existing structure of the Microbiology unit. The approach to restructuring of the Microbiology Program was done collaboratively with participation of all members of the unit participating in a "Blue Sky" exercise. In this approach, each microbiologist was given an opportunity to provide their vision and model of a restructure of the entire Microbiology unit, given the parameter that the model had to be test-centered rather than employee-centered. The Program Chief took all models, found commonalities among them and created multiple integrated draft models of possible restructuring. These draft models were assessed and modified through a collaborative, iterative process with the laboratory directors. A final model was generated and presented to the Microbiology Unit for feedback. Feedback was used to finalize the restructure model, create the staffing plan for the restructure, and presented to partners. The Microbiology Unit was successfully restructured in summer 2024. As a result, a new subunit of Microbiology was created, increases in training to ensure greater bench depth was planned, and staff were more evenly and equitably distributed across units. The collaborative and iterative approach created transparency in the restructure process and illustrates the power of inclusivity and collaboration to create positive change in laboratory operations and structure.

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Poster 190
Can We Build It? Yes, We Can! Overdose Biosurveillance in Vermont
S. Stanton, Vermont Department of Health Laboratory, J. Warrington, Vermont Department of Health Laboratory, J. Eisenhauer, Vermont Department of Health Laboratory, N. Plocharczyk, Vermont Department of Health Laboratory

In 2022, Vermont recorded the second most overdose deaths per 100,000 in New England and ranked 8th highest in drug overdose fatalities per capita nationwide. In Vermont, non-fatal overdose drug use

patterns have not been uniformly investigated or tracked. This information can quickly inform targeted intervention policies. In support of overdose prevention efforts and in conjunction with Vermont hospitals, the Vermont Department of Health Laboratory (VDHL) has utilized funding from the CDC's Overdose Data to Action grant to implement an overdose biosurveillance program for determination of the substances present in the urine samples of non-fatal overdose patients reporting to Vermont emergency departments . This work is intended to inform overdose crisis response efforts by connecting specific usage trends to demographic data in efforts to direct prevention resources where they are needed.

To synchronize all facets of overdose biosurveillance development and implementation, the Vermont laboratory appointed a Biomonitoring Coordinator to assume responsibility for both procedural and analytical aspects of the program. Collaboration was key to the efficacious launch of overdose biosurveillance in Vermont – collaborations both within the State of Vermont government and externally with various regional- and national-level organizations. Intra-state collaborative partners have included the laboratory's Safety Compliance Team and the Organic Toxicology Team, the Division of Substance Use Programs, the Division of Health Statistics and Informatics Infectious Disease Epidemiology, and the Human Services Division of the Vermont Attorney General's Office. External partners include APHL and members of APHL collaborative communities (the Opioid Community of Practice and the OD2A Technical Workgroup), the Vermont branch of the Drug Enforcement Agency, Vermont hospitals, the South Carolina Department of Health Laboratory, the Rhode Island Health Department Laboratory (via funding from APHL's Peer-to-Peer Regional Consortia) and Agilent Technologies.

Several innovations have arisen from work to develop an overdose biosurveillance program in Vermont. These include the establishment of a Biomonitoring Coordinator, a novel methodology for sample procurement, creation of a user guide for partnering hospitals, formation of a statistically acceptable plan for sample de-identification in regions with smaller populations, and work towards codifying required participation in overdose biosurveillance through Vermont's statutes regarding regulated drugs rules.

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Poster 191
Harnessing Chemical Response Networks in Environmental Emergency Scenarios
A. Woods, Virginia Division of Consolidated Laboratory Services

During July of 2024, a warehouse containing pesticides, herbicides, fertilizers, and organic compounds located in South Central Virginia, caught fire after a car drove into two propane tanks within close proximity to the building. As part of the response, runoff samples from the water used to extinguish the fire were collected and submitted to the Chemical Terrorism Preparedness and Response laboratory at the Department of General Services (DGS) Division of Consolidated Laboratory Services (DCLS) for chemical emergency response testing.

The Chemical Terrorism Preparedness and Response laboratory includes staff members funded by federal grants as part of the Laboratory Response Network – Chemical (LRN-C) and Food Emergency Response Networks (FERN). Utilizing training and instrumentation provided through the LRN-C and FERN, staff members were able to perform mass spectrometry screening to identify and confirm various pesticides, herbicides, and organic chemicals present in the runoff.

This emergency response chemical testing was a first line of defense in the assessment of the environmental impact of the warehouse fire. Four environmental samples were tested by gas chromatography mass spectrometry and liquid chromatography high resolution mass spectrometry by the Chemical Terrorism Preparedness and Response laboratory and subsequently transitioned to other environmental testing areas at DCLS once the response shifted from emergency testing to routine surveillance. Ongoing support from federal partners including training and funding for emergency response chemical testing greatly increases the capability of DCLS to respond to environmental

emergencies.

Presenter: Amanda Woods, Virginia Division of Consolidated Laboratory Services, amanda.woods@dgs.virginia.gov

Poster 192
Optimization and Evaluation of Cell Lysis and DNA Purification Methods for the Enumeration of *Legionella* Species in Environmental Water
Y. Farias Valdovinos, Wadsworth Center, N. Biswas, Wadsworth Center, T. Hattenrath, Wadsworth Center

Legionella species are the pathogens responsible for Legionnaire's disease, a serious respiratory illness transmitted via aerosolization of environmental water sources. Despite significant advances in waterborne disease monitoring, conventional culture-based techniques for the identification Legionella spp. remain laborious, often requiring as much as 15 days to produce results. In contrast, molecular methods that target specific DNA sequences offer a faster and more efficient approach for detecting these pathogens in environmental water samples that often contain diverse background flora. In this study, we focused on optimizing cell lysis and DNA purification techniques to enhance the sensitivity and accuracy of RT-PCR assays for Legionella detection.
We evaluated two primer/probe sets designed for: 1) broad Legionella spp. detection, targeting the 23S rRNA gene (Li et al., 2023), and 2), Legionella pneumophila serogroup 1 by targeting the lipopolysaccharide (LPS) biosynthesis gene cluster (Merault et al., 2011; Donohue et al., 2014). Water samples with known concentrations of L. pneumophila were used to optimize primer and probe concentrations, with RT-PCR efficiency assessed using a standard curve of known concentrations of synthetic DNA standards. DNA extraction was performed using the boiling method, which involves bacterial lysis with Tris-EDTA buffer and heat treatment, followed by centrifugation and storage of the supernatant. The optimized RT-PCR assays for the 23S rRNA genes detected all 22 Legionella spp. tested, while the assay for LPS gene identified only L. pneumophila serogroup 1. RT-PCR assays achieved an average efficiency of 97%, with an r^2 value of 0.9996, indicating a high degree of precision. These findings underscore the importance of optimizing DNA extraction and lysis protocols to enhance the efficiency of molecular diagnostics in the identification of Legionella spp. Future research will evaluate the efficacy of various cell lysis buffers and different DNA extraction kits, focusing on DNA concentration, purification efficiency, and PCR amplification performance. Furthermore, the optimized techniques can be applied to downstream applications, such as digital-PCR, offering a promising next step for further enhancing sensitivity and specificity in Legionella detection within complex water samples.

Presenter: Yadira Farias Valdovinos, Wadsworth Center, yadira.farias@health.ny.gov

Poster 193
Highly Multiplex Amplicon Sequencing of *Salmonella* in New York State
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Introduction: Salmonella species are a leading cause of foodborne infection worldwide. Rapid identification of Salmonella species serotypes is crucial for surveillance purposes, to guide outbreak/cluster detection and epidemiological investigations. Traditionally, isolates have been submitted to the Wadsworth Center (WC) for confirmation and serotyping by whole genome sequencing (WGS). However, with increased use of culture independent diagnostic testing (CIDTs) at the clinical and diagnostic level, bacterial isolation of foodborne pathogens has decreased, requiring a re-evaluation of current methods. Highly multiplexed amplicon sequencing (HMAS) is a new tool that has the potential to increase speed and effectiveness of the surveillance process and streamline new public health technology with traditional clinical microbiology practices. In 2023, the Centers for Disease Control and Prevention (CDC) piloted HMAS at 10 public health laboratories (PHLs) in the

United States, including the WC.

Methods: HMAS is a specimen-based metagenomic approach that uses an integrated fluidic circuit (IFC) to amplify up to 48 samples. HMAS uses three pieces of sequenced data from each sample, utilizing both the primary sample and isolate. Stools are enriched in Tetrathionate (TET) broth and plated onto xylose-lysine-tergitol (XLT4) agar. Colony morphologies suspicious for Salmonella species are picked from XLT4 agar to brain heart infusion (BHI) and triple sugar iron (TSI) agars and sent to matrix assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS) for identification of Salmonella spp. If an isolate is confirmed as Salmonella species it is sent to WGS and DNA is extracted from both the primary stool enriched in TET broth and the recovered isolate. HMAS library preparation is performed using the X9 high-throughput genomics system, sequenced on the Illumina Miseq and analyzed by the Step-Mothur pipeline. WGS sequence files are analyzed and uploaded to both the PulseNet national database and the National Center for Biotechnology Information Sequence Read Archive (NCBI SRA).

Results: To date, thirty-eight Salmonella isolates have been recovered from ninety-eight CIDT-positive stool samples. Thirty-two out of thirty-eight are included in the core dataset. Twelve out of thirty-eight were recovered using TET broth enrichment. Eighty-three percent of enriched stools passed quality control (QC) compared to thirteen percent of unenriched stools. Seventy-eight percent of all isolates passed QC.

Conclusion: Results show that TET enriched stools perform better than raw stool specimens, therefore since November 2024, only enriched stools are being assessed for this study. In the future, we will continue to evaluate the effectiveness of the HMAS protocol, making necessary improvements for optimal sequencing outcomes to develop metagenomic culture-independent testing workflows. The CDC hopes to implement a national CIDT workplan in support of PHLs.

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Poster 194

Determining Mercury Species in Whole Blood by Isocratic Liquid Chromatography Coupled to Vapor Generation Inductively Coupled Plasma Tandem Mass Spectrometry

P. Parsons, Wadsworth Center - NYSDOH, C. Palmer, Wadsworth Center, New York State Dept of Health, P. Parsons, Wadsworth Center, New York State Dept of Health

Speciation methods provide a more detailed picture regarding human exposure to toxic metals/metalloids and their effects on human health. The toxicity of methylmercury (MeHg) differs considerably from inorganic mercury (iHg), such that their separation and quantification in whole blood is helpful in identifying sources and possible pathways of exposure. Liquid chromatography (LC) has advantages over gas chromatographic (GC) methods for separating iHg from MeHg species due to the former's compatibility with uptake rates of common nebulizers used with ICP-MS and the latter's requirement for derivatization for an effective separation. Here we report an improved speciation method that was developed to separate and quantify MeHg and iHg in whole blood using isocratic LC elution with determination by ICP-MS/MS. Chromatographic separation of MeHg and iHg is achieved in ~4 minutes using a C8 reversed phase column. In those rare cases where exposure to ethyl mercury (EtHg) is expected, or where a CRM is known to contain EtHg (e.g., NIST SRM 955c), EtHg can be captured by extending the LC elution time to 8 minutes. Adding a vapor generation (VG) step post column, enhances the signal to noise ratio, and achieves even lower limits of detection (LODs). With optimized sample preparation, the LC-VG-ICP-MS/MS method LOD for both iHg and MeHg is 0.2 µg/L. Method validation was conducted using NIST SRM 955c Toxic Metals in Caprine Blood and NIST SRM 955d Toxic Elements and Metabolites in Frozen Human Blood. Additional validation data were collected using archived blood materials from multiple Proficiency Testing programs and External Quality Assessment schemes materials, and with blood-based quality control blood materials provided by the US CDC, previously analyzed for Hg species using isotope dilution with GC coupled to ICP-MS.

Presenter: Patrick J. Parsons, Wadsworth Center - NYSDOH, patrick.parsons@health.ny.gov

Poster 195
Identifying Inhibitors of *Vibrio* Species Quorum Sensing Transcriptional Regulators
A. Chang, Wadsworth Center, New York State Department of Health, B. Liang, University at Albany, J. Paczkowski, Wadsworth Center

Vibrio species are a Gram-negative, comma-shaped bacteria found in aquatic environments. They often infect aquatic organisms and can cause various illnesses in humans, such as foodborne illness, gastroenteritis, or septicemia. Increasing coastal water temperatures in the United States are predicted to lead to an increase in Vibrio infections in coming years, specifically in the eastern portion of the country. Antibiotic resistance among these bacteria makes infections difficult to treat, posing a significant public health issue. A potential alternative treatment may include targeting regulators of the quorum sensing pathway. This pathway is required for pathogenesis but not bacterial growth. Thus, it is important to investigate the structure and function of these targets. Vibrio species bacteria use quorum sensing to communicate and regulate group behaviors, such as those responsible for virulence gene expression. Two quorum sensing transcriptional regulators of interest are SmcR from Vibrio vulnificus and OpaR from Vibrio parahaemolyticus. Investigating the structural basis of ligand binding in SmcR and OpaR can reveal potential inhibitors of these transcriptional regulators. A computational method of virtual screening was used with the molecular docking program Autodock Vina to screen libraries of compounds for predicted binding affinity to SmcR and OpaR. Docking results guide predictions for how an inhibitor may bind to the target protein in vitro. These predictions can then be tested in vitro using isothermal titration calorimetry (ITC), a method that calculates binding kinetics between a protein and ligand. The small molecule thiophenesulfonamide PTSP (3-phenyl-1-(thiophen-2-ylsulfonyl)-1H-pyrazole) known to bind to SmcR was predicted to bind comparatively worse in OpaR. ITC was performed to measure the binding affinity of PTSP in SmcR, OpaR, and mutants SmcR R179C and OpaR F180T. Another small molecule identified through virtual screening, (4-(benzo[d][1,3]dioxol-5-ylmethyl)piperazin-1-yl)(5-((4-chlorophenoxy)methyl)furan-2-yl)methanone, showed stronger predicted binding in OpaR F180T as compared to wild-type. The predicted binding patterns of this small molecule in each target protein will be tested with ITC. Computationally identifying and conducting in vitro assays for small molecule compounds of SmcR and OpaR will allow a greater understanding of ligand binding and thus inform potential inhibition of these target proteins for future in vivo assessment and drug efficacy in animal models.

Presenter: Annmarie Chang, Wadsworth Center, New York State Department of Health, annmarie.chang@health.ny.gov

Poster 196
Molecular Detection of Antimicrobial Resistance in Pathogenic *Neisseria* Species
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The Centers for Disease Control and Prevention has reported a rise in drug-resistant Neisseria gonorrhoeae (NG) and Neisseria meningitidis (NM) in recent years. Targeted next-generation sequencing (tNGS) assays are a powerful molecular tool allowing for rapid characterization of bacterial isolates and primary specimens. The Wadsworth Center Bacteriology Laboratory (WCBL) has developed a tNGS assay for detecting antimicrobial resistance (AR) in NG and NM.
The assay targets genes known to confer resistance to antibiotics used in the treatment of NG and NM infections, including the penA gene (penicillins and cephalosporins), gyrA and parC genes (ciprofloxacin), and the 23S rRNA gene (azithromycin). It utilizes a multiplex PCR amplification approach coupled with long-read Nanopore sequencing to generate high-coverage sequence datasets. The reads are quality-filtered, grouped into gene-specific bins, and high-quality consensus sequences are reconstructed de novo for each gene. These consensus sequences are then compared to reference databases (NG-STAR, NCBI Bacterial Antimicrobial Resistance Reference Gene Database)

to identify mutations associated with drug resistance.
Comparisons of tNGS results with antimicrobial susceptibility tests using gradient strips (ETEST®) has demonstrated a strong concordance between predicted and observed AR for penicillin (13/13 low level AR NM isolates with mosaic penA gene), cefixime (9/9 AR NG isolates with mosaic penA gene), and ciprofloxacin (23 AR NG isolates, 23 with gyrA gene mutations, 19 with parC gene mutations; 2/2 AR NM isolates with gyrA gene mutations). For azithromycin, 5/8 NG isolates had mutations in the 23S rRNA gene. The remaining three isolates are being assessed by whole genome sequencing to determine alternative AR mechanisms.
Additional testing for clinical validation is underway for both isolates and clinical specimens includes determination of specificity, sensitivity, reproducibility, and accuracy according to the New York State (NYS) Clinical Laboratory Evaluation Program (CLEP) requirements. Implementation of this tNGS assay will allow for rapid and improved surveillance of AR NG and NM in NYS and could potentially impact patient treatment and infection control measures.

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Poster 197
Implementing Dried Blood Spots for Confirmatory Hepatitis C Virus RNA Testing: Training and Collaboration Building with Other Public Health Labs
R. Hallack, Wadsworth Center/NYSDOH, L. Styer, Wadsworth Center/NYSDOH

Description of the Project: The Bloodborne Virus Laboratory (BVL) at the Wadsworth Center, New York State Department of Health, provides hepatitis C virus (HCV) RNA testing services to many community-based rapid testing sites including syringe exchange programs. Many clients have a history of injection drug use and are at high risk for HCV infection. Plasma is the preferred specimen for HCV RNA testing; however, venipuncture blood collection is often challenging to perform on people who inject drugs, and many will refuse the blood draw which means they will not receive the necessary diagnostic HCV testing recommended by the CDC. Dried blood spot (DBS) specimens collected by fingerstick are a viable alternative sample type that does not require venipuncture. We implemented DBS testing in 2020 using a modified Hologic Aptima HCV Quant Dx assay that was validated for DBS and approved for clinical use by the New York State Clinical Laboratory Evaluation Program. Currently, we perform qualitative HCV RNA testing on DBS collected at rapid testing sites from across New York State to confirm positive HCV antibody tests. In 2024, we received 806 DBS from 27 different sites and returned 237 HCV RNA detected, 455 HCV RNA not detected, and 24 invalid or indeterminate results. Due to DBS quality issues, 90 tests were canceled.

Specific problem: HCV RNA DBS testing is relatively simple to implement yet underused to reach difficult to test individuals. Having learned of our success, public health labs outside of New York have expressed interest in learning more about our DBS HCV RNA testing service. To assist these laboratories, we developed a comprehensive 8-hour in-person training program and invited public health laboratory scientists from a nearby state to participate.

Summary of Implementation: The training covered 4 main features of testing: validation design including examples for sensitivity, reproducibility, stability and accuracy studies; utilization of contrived and fingerstick collected DBS; collection, storage and shipping instructions for submitters; and quality control. Trainees observed the entire testing process and received copies of standard operating procedures to cover the entire testing process including forms, tracking sheets, lists of supplies needed including vendors and part numbers, DBS elution buffer formulation to make in-house, and continued support post training. The two public health scientists returned to their state laboratory with a clear plan for implementing DBS HCV RNA testing. Within one month of training, the scientists presented their validation study proposal to their Quality Assurance Team and gained approval to proceed.

Summary: Offering a concise, complete, in-person training program to implement DBS as a specimen type at laboratories using the Aptima HCV Quant Dx Assay will increase testing uptake for persons at high-risk for HCV infection when venipuncture blood cannot be collected. The expansion of HCV

testing supports the Viral Hepatitis National Strategic Plan to eliminate the public health threat of viral hepatitis by 2030. The BVL will continue to build partnerships with laboratories interested in this training program.

Presenter: Renee Hallack, Wadsworth Center/NYSDOH, renee.hallack@health.ny.gov

Poster 198
Accelerating Informatic Workflows: Google Cloud Platform with NextFlow Tower Integration
S. Bruce, Wadsworth Research Center, New York State Department of Health

The Bioinformatics Core is leading a major initiative to to migrate and optimize fourteen existing Next Generation Sequencing (NGS) pipelines to the Google Cloud Platform (GCP) using NextFlow Tower. The project aims to enhance parallel processing, scalability, and performance of pathogen data analysis, while empowering laboratories to run pipelines independently through an intuitive web-based interface. The integration of NextFlow Tower with GCP will lead to significant improvements in scalability, performance, and usability for informatic workflows. By transitioning to a self-service model, labs gain autonomy while the Bioinformatics Core can prioritize pipeline development and innovation.

Presenter: Spencer Bruce, Wadsworth Research Center, New York State Department of Health, spencer.bruce@health.ny.gov

Poster 199
Accelerating Data Analysis and Cutting Costs: Harnessing Business Intelligence (BI) and LIMS for Operational Efficiency
N. Ruehlen, Washington Department of Health, Public Health Laboratories, A. Terrenzio, WA Public Health Laboratories, A. Lathum, WA Public Health Laboratories, V. Aoki, Washington State Department of Health, Public Health Laboratories

Background
In today's fast-paced laboratory environment, efficient data analysis is essential. Our dated laboratory information management system (LIMS) posed challenges in streamlining data analysis tasks. To address this, we developed a business intelligence (BI) semantic model with dashboard creation capabilities for the LIMS data. This model automates time-consuming tasks, such as cross-referencing specimen submissions to identify past positive results, significantly enhancing laboratory operations by allowing staff to swiftly determine if additional testing is needed.
Before implementing the LIMS BI model, all specimen submissions for syphilis Treponema pallidum particle agglutination assay (SYPH TP-PA) and chemiluminescent microparticle immunoassay (CMIA) were tested regardless of previous-positive status. The BI model introduced a customized dashboard, enabling rapid and efficient identification of patients with previous-positive results who do not require further SYPH TP-PA or CMIA testing. This innovation has led to significant cost savings, estimated at approximately $50,000 per year.

Methods
The Washington Public Health Laboratories (WAPHL) Quality Assurance (QA) and Sexually Transmitted Infection Laboratory (STI) teams developed a master semantic model of the LIMS data using Microsoft Power BI. This model serves as the backbone for subsequent customizations performed on a case-by-case basis following an established WAPHL BI dashboard development process.
Validation of the final dashboard is carried out and documented to ensure the accuracy and reliability of the data presented in the dashboards. This involves cross-verification with created data sets from direct queries of the LIMS SQL server and subject matter expert user testing.
Utilizing the BI automation capabilities, dashboard data are refreshed throughout the day at scheduled intervals from the LIMS SQL database.

Results
Creating the BI semantic model of the LIMS data has allowed the WAPHL STI team to realize

significant savings in laboratory staff time and materials by eliminating unnecessary testing, which was unachievable with traditional queries of our LIMS system. This is estimated to save the laboratory approximately $50,000 per year.

Conclusion
The integration of BI with our outdated LIMS has proven to be a transformative solution for enhancing laboratory operations. The dashboard allows laboratory staff to view accurate, current data without cumbersome queries or analysis steps. This modernization has enabled the automation of SYPH TP-PA and CMIA previous-positive data analysis capabilities, improved operational efficiency, and achieved notable cost savings.

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Poster 200
Laboratory Procurement Requests, Purchasing and Payments, Oh My: Enhancing Procurement and Business Workflow Visibility in the Washington Public Health Laboratories
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Background: Efficient business and procurement processes are critical for the effective operation of the Public Health Laboratory. Delays in any stage of the procurement cycle, from initial request to final payment, can impact research, diagnostic testing, and overall laboratory function. The current procurement process at the Washington State Public Health Laboratories faces various challenges such as under-shipments, delays in invoice processing, lack of procurement tracking, and funding expiration due to untimely invoice receipt and processing. Taken together these challenges have hindered the procurement workflow and caused significant payment delays. To address these challenges, a comprehensive solution is required to improve data visibility and streamline and optimize related business and procurement processes.

Methods: This project involves the development of a Power BI dashboard to track the entire procurement journey from the original request through procurement, purchasing, accounting, and settlement of payments. The dashboard links purchase orders (POs), quotes, invoices, and tracks check issuance, providing a holistic view of the procurement process.
The dashboard integrates updated daily data from the Office of Financial Services (OFS) - Purchase Orders Library on SharePoint.com. These data include PO numbers, vendor names, and dollar amounts.

Expected Outcomes: The implementation of the Power BI dashboard is expected to enhance visibility and efficiency of the procurement process. Each lab section will gain better visibility into its spending and budgets, leading to improved budget management and allocation. By tracking procurement data accurately, the dashboard aims to mitigate the risks related to funding expiration and optimize the overall procurement workflow. Ultimately, this will support more effective financial management and operational efficiency within the Washington State Public Health Laboratories. Additionally, the dashboard aims to improve communication and collaboration between business partners including requesters, procurement officers, accounting departments, and vendors.

Keywords: Procurement, Power BI, Dashboard, Public Health Laboratories, Washington State, Efficiency, Transparency, Budget Management, Data Visualization, SharePoint, Microsoft List

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Poster 201

60% Access Antibiotics: Where Are We in Washington State?

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Background:
Antimicrobial resistance (AMR) is a global health crisis. The overuse and misuse of antibiotics contribute significantly to the emergence and spread of drug-resistant bacteria. This threatens the effectiveness of treatments for various infections, leading to increased morbidity, mortality, and healthcare costs.
The World Health Organization (WHO) launched the AWaRe (Access, Watch, Reserve) classification as a key stewardship strategy to guide and optimize the use of antibiotics. This framework categorizes antibiotics into three groups: Access, Watch, and Reserve. Access antibiotics are recommended as the first or second choice for most common infections, as they have a lower potential for resistance development. Watch antibiotics are prioritized as key targets for stewardship programs and monitoring due to their increasing resistance potential. Reserve antibiotics are considered last-resort options for the treatment of confirmed or suspected multi-drug-resistant infections. The WHO recommends that at least 60% of all antibiotics prescribed should be from the Access group to mitigate the risk of antimicrobial resistance (AMR).
Understanding the current trends in Access antibiotic prescribing practices, is essential for effective AMR surveillance and intervention.

Methods:
This study will utilize publicly available Medicare Part D claims data for outpatient settings in Washington State over the ten-year period from 2013 to 2022.
- Data Extraction: Relevant data points will be extracted from the claims data, including:
a) Prescriber information (Location, specialty)
b) Prescription details (Drug name, dosage, days' supply, route of administration, total drug cost)
c) Date of prescription
- Antibiotic Classification: All prescribed antibiotics will be categorized according to the WHO AWaRe classification system based on their spectrum of activity, resistance potential, and clinical importance.
- Data Analysis:
a. Consumption Calculation: Antibiotic consumption will be measured as the total number of antibiotic days supplied.
b. Access Antibiotic Percentage: The percentage of Access antibiotics prescribed will be calculated annually and compared to the WHO's 60% threshold.
c. Trend Analysis: Time-series analysis will be conducted to identify trends in Access antibiotic prescription rates over the ten-year period.
d. Subgroup Analysis: Subgroup analyses will be performed to examine variations in Access antibiotic use by: Route of administration, prescribing institution type, and drug cost
- Data Visualization: A Power BI dashboard will be developed to visualize the data, including interactive maps, charts, and graphs. This dashboard will allow for dynamic exploration of the data and facilitate the identification of key findings.

Expected Outcomes:
• Determine the current percentage of Access antibiotic prescriptions in Washington State.
• Analyze trends in Access antibiotic consumption over the past decade.
• Identify variations in Access antibiotic use across different routes of administration, prescribing institutions, and counties.
• Generate insights to inform targeted interventions to optimize antibiotic prescribing practices and mitigate antimicrobial resistance.

Keywords: Antimicrobial resistance, Access antibiotics, Antibiotic stewardship, Medicaid Part D, Outpatient care, Washington State, Public health, Data analysis, Power BI.

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Poster 202
Determination of Glyphosate, Aminomethylphosphonic Acid (AMPA) and Glufosinate in Drinking Water Using Direct Analysis by LC-MS/MS
S. Oehrle, Waters Corporation, S. Adams, Waters Corporation, S. Hird, Waters Corporation

Reliable analytical methods are needed for detection, quantification, and identification of glyphosate, aminomethylphosphonic acid (AMPA), and glufosinate in drinking water. Accurate and reliable methods are required to monitor these widely used pesticides in drinking water from different sources. This work describes a simple direct analysis method, based on liquid chromatography coupled to tandem mass spectrometry (LC–MS/MS), which avoids the need for lengthy and laborious derivatization and solid-phase extraction (SPE). Samples of water were injected directly into the LC-MS/MS (ACQUITY™ Premier System with Xevo™ TQ Absolute Tandem Mass Spectrometer System) using the Anionic Polar Pesticide Column. The performance of the method was successfully evaluated in three different types of drinking water. The extremely high sensitivity of the Xevo TQ Absolute System was demonstrated with reliable detection for all three analytes at concentrations as low as 10 ng/L (0.01 µg/L). The performance of the method was evaluated in-house and by an inter-laboratory study using spiked water samples. Results from both studies showed that the trueness of the method was between 82 and 110%. Close agreement was observed with the repeatability, within laboratory reproducibility, and between laboratory reproducibility, all being < 16% RSD. The method is considered sensitive, specific, accurate, and suitable for the determination of these challenging analytes in a range of different types of drinking water, for checking compliance with regulatory limits, and to support studies focusing on human exposure.

Presenter: Stuart Oehrle, Waters Corporation, stuart_oehrle@waters.com

Poster 203
Harnessing the Power of Mass Spectrometry and Automation to Reduce Sample Size, Sample Preparation Time and Increase Laboratory Efficiency
S. Oehrle, Waters Corporation, K. Organtini, Waters Corporation, I. Wan, Promochrom Technologies

US EPA Method 1633 has become the foundational method for analysis of PFAS in non-potable water matrices, soils, biosolids and tissues in the United States. The method consists of sample preparation using weak anion exchange (WAX) solid phase extraction (SPE) with graphitized carbon black (GCB) clean up. This EPA method is a performance-based method allowing for changes to be made as long as data quality equivalency is demonstrated. With this method becoming very much in demand, laboratory throughput and efficiency must be considered for maximum laboratory success. Automation and instrument sensitivity are investigated as avenues to decreasing required sample size and therefore increasing laboratory efficiency.
A highly sensitive LC-MS/MS system was utilized for this analysis, allowing for reduction in sample volume size while still achieving equivalent results and method detection limits to the method. Sample volumes tested ranged from 500 mL (the recommended volume in EPA 1633) down to 50 mL using an automated solid phase extraction (SPE) system for sample preparation of water samples to eliminate the need for human interaction during the sample preparation process. To make the sample preparation process fully automated, a stacked dual layer cartridge containing both WAX and GCB chemistries was utilized instead of having to perform the GCB step manually.
Equivalency was established by demonstrating that all quality control guidelines were achieved including retention time stability, ion ratio stability, ongoing calibration verification, and recovery. Equivalency of the automated SPE system and stacked dual layer cartridge is also demonstrated as a complete workflow.
A method detection limit study will demonstrate equal or lower MDLs using the automated, reduced sample volume approach. Additionally, a wastewater certified reference material was processed in an equivalent reduced sample volume and was within all certified levels for the 40 PFAS covered by EPA

1633.

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Poster 204
Identifying COVID Surges in Wastewater from Limited Sampling
T. Driscoll, West Virginia University, J. Lambert, West Virginia University, A. Jones, West Virginia University, T. Driscoll, West Virginia University

Wastewater testing is a valuable tool for following trends in the abundance of SARS-CoV-2 and other infectious disease pathogens among communities. It has been widely adopted in the United States since early in the COVID-19 pandemic, with 49 states and territories contributing regularly to the CDC's National Wastewater Surveillance System (NWSS). A common method for generating data involves collecting a 24-hour composite sample from a treatment facility, concentrating viral particles, extracting nucleic acids, and quantifying targets using digital PCR. It is not clear how often we need to collect in order to identify trends in COVID levels accurately and quickly; a single sample is not sufficient to identify a sustained trend due to the inherent noise in the data, a consequence of the physicochemical variables that influence the wastewater system. This information is critical to the utility of wastewater in informing effective downstream public health responses. The purpose this study was to determine how collection frequency affects the accurate identification of sustained COVID increases (surges) in wastewater. Using a long-term wastewater data set that included daily collections from a treatment facility over four consecutive years (Nov 2020 - Nov 2024), we developed a model for identifying COVID surges using wastewater data. We compared our model results to an observed pattern of surges in this data set. We show that the start of a COVID surge in wastewater can be identified with reasonable accuracy within 7 days of surge inception using a model built from the full data set. Models using less frequent collections (1 and 2 days per week) were not as accurate; however, these models can be tuned to still provide useful information and facilitate timely downstream public health responses. In conclusion, high-frequency wastewater sampling is the most accurate and fastest way to identify COVID surges; however, less-frequent sampling can perform reasonably well if used appropriately.

Presenter: Timothy Driscoll, West Virginia University, timothy.driscoll@mail.wvu.edu

Poster 205
Wastewater Sequencing of SARS-CoV-2 from Small Sewersheds
T. Driscoll, West Virginia University, A. Jones, West Virginia University, G. Chimino, West Virginia University, T. Driscoll, West Virginia University

Wastewater testing is a valuable tool for following trends in the abundance of SARS-CoV-2 and other infectious disease pathogens among communities. In addition, genomic sequencing of RNA extracted from wastewater can be used to estimate the proportion of circulating COVID variants, reveal patterns in viral distribution and evolution, indicate the severity of disease, and even drive treatment choices. In this study, we used the Oxford Nanopore MinION platform to sequence SARS-CoV-2 from 17 community wastewater treatment facilities between August 2022 and January 2024 and estimated the proportion of COVID variants using freyja. Briefly, 10 ml influent samples were concentrated using Ceres Nanoptraps Microbiome A beads followed by total RNA extraction. Extracted samples were further reverse transcribed and Sars-CoV-2 amplified with tiling amplicon primer sets. Sequencing was performed using either the Oxford Nanopore MinION or PromethION platforms. The resulting long reads were analyzed for the presence of SARS-CoV-2 mutations and their corresponding variants within individual sewersheds over time using the freyja software package. Results were published on the West Virginia Wastewater dashboard (https://wvuvectors.shinyapps.io/WaTCH-WV/) and raw sequencing data submitted to the NCBI SRA. We found that the appearance of variants at the national level generally preceded their appearance in our test sewersheds. To determine the number of required long-reads to sufficiently sequence a wastewater sample and identify the maximum number of variants present, we performed a rarefaction analysis by sub-sampling from 200,000 mapped reads down to 10,000 mapped reads per sample and analyzing each sub-sample with freyja. Our results

indicate that 50,000 mapped reads are sufficient to identify all variants in a sample, and that threshold is largely independent of sewershed size, date of collection, or total number of variants in the sample. Our study suggests that long-read sequencing is useful for detailed variant analysis within small sewersheds in resource-limited settings.

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Poster 206
Comparison of the Kingfisher Apex and Kingfisher Flex Extraction Instruments for Influenza, SARS-CoV-2 and Measles PCR
M. Carlson, Wisconsin State Laboratory of Hygiene, E. Hanson, Wisconsin State Laboratory of Hygiene, R. Griesser, Wisconsin State Laboratory of Hygiene, A. Bateman, Wisconsin State Laboratory of Hygiene

Background:
High throughput extraction platforms are key to laboratory efficiency, especially during outbreaks and seasonal response. The Wisconsin State Laboratory of Hygiene (WSLH) currently uses the Kingfisher Flex for influenza and SARS-CoV-2 (SC2) extractions. The Kingfisher Apex demonstrates similar methods and workflow efficiency to the Flex. WSLH also has no 96-well extraction platforms for measles. Hence, a validation of the Apex would allow for efficient measles detection while also updating instrumentation for influenza and SC2.

Initial Extraction Instrument Comparison:
During initial testing, twenty positive specimens for each virus (influenza A, influenza B, SC2, and measles) were extracted on the Apex and Flex (in parallel) using a MagMAX Viral/Pathogen II Nucleic Acid Isolation Kit (MVP II). The measles samples were also extracted on the BioMérieux EMAG, the current validated instrument for measles PCR. A checkerboard extraction was performed for SC2 and measles to assess carry-over. Real-time reverse transcriptase polymerase chain reaction was performed on the Applied Biosystems 7500 Fast Dx Real-Time PCR Instrument (ABI 7500). The performance between the Apex and Flex for influenza, SC2, and measles was similar. All qualitative results matched. The average Ct values fluctuated by < 1 for each virus. The EMAG demonstrated a better measles extraction than both the Apex and Flex with Ct values roughly 2.3 cycles lower. Checkerboard accuracy was identical between instruments, with neither showing splashing nor carryover. This initial comparison of the Kingfisher Apex and Flex demonstrated a similar performance, warranting additional comparative testing.

Future Extraction Instrument Comparison:
An additional 10 positive specimens for each virus (influenza A, influenza B, and SC2) will be extracted on the Apex and Flex (in parallel) using the MagMAX Viral/Pathogen II Nucleic Acid Isolation Kit DX. The DX kit will be more widely supported with recent changes in FDA regulations when compared to the MVP II kit. A checkerboard extraction will also be performed for SC2 to assess carry-over. Real-time reverse transcriptase polymerase chain reaction will be performed on the CFX Opus Real-Time PCR System since the ABI 7500 is no longer supported. It is expected that the Kingfisher Apex and Flex will demonstrate similar performance using the MVP II DX Kit. If results demonstrate minimal deviations, we plan to fully validate the Kingfisher Apex for these assays.

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Poster 207
Indecision 2025: A Comparison of the Thermo Fisher QuantStudio 5 Dx and the Bio-Rad CFX Opus 96 Dx Real-time PCR Instruments
M. Carlson, Wisconsin State Laboratory of Hygiene, E. Hanson, Wisconsin State Laboratory of Hygiene, A. Bateman, Wisconsin State Laboratory of Hygiene

The Thermo Fisher ABI 7500 Fast Dx real-time PCR instrument is a staple in most public health laboratories but will be phased out in the near future. As a result, many public health labs are currently

deciding which real-time PCR platform(s) to purchase to meet their future molecular testing needs. In this poster, we will compare two popular RT-PCR instruments that laboratories are considering as replacements for the ABI 7500 Fast Dx: the Thermo Fisher QuantStudio 5 Dx (QS5) and the Bio-Rad CFX Opus 96 Dx (Opus). The Wisconsin State Laboratory of Hygiene (WSLH) has multiple of each instrument and is currently evaluating them for many assays, including diagnostic and surveillance virology testing. The QS5 is a modernized version of the beloved ABI 7500 Fast, with a white LED lamp shone over excitation filters and a CCD camera recording fluorescence from behind emission filters. The QS5 has two software options: the desktop-based "QuantStudio 5 Dx Native" software, and the cloud-based Diomni software. The "QS5 Native" software is more similar to the ABI 7500 Fast Dx software, but with additional template locking features for IVD use. The other QS5 software option, Diomni, aims to be a comprehensive laboratory workflow solution and allows the user to access the software remotely from any computer. In comparison, the Bio-Rad Opus utilizes multi-colored LEDs mounted on an optical shuttle for fluorescence excitation and photodiodes for detection. This updated design minimizes well-to-well variation and reduces the amount of maintenance and calibration needed. The CFX Maestro software is not as flashy as Diomni but offers enhanced flexibility reminiscent of the ABI 7500 Fast Dx software. We are in the midst of a side-by-side comparison of these two RT-PCR instruments, performing the CDC Influenza SARS-CoV-2 Multiplex PCR assay and the CDC Influenza A H5 subtyping assay. This poster will describe our evaluation of each instrument including sensitivity, software usability, detection technology, cost, customer service, and overall experience.

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Poster 208

Wastewater Surveillance of Mpox: Method Development and Preliminary Surveillance Data

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Mpox, formerly known as monkeypox, is a zoonotic disease caused by Orthopoxvirus monkeypox, the monkeypox virus (MPXV), that can lead to severe illness in humans. It is most often spread through sexual contact, although transmission can occur through contaminated surfaces, infected animals, and close respiratory contact. Mpox is endemic in parts of West and Central Africa; however, in 2022 a global outbreak occurred with over 100 countries affected by clade II mpox outside of its endemic range (over 110,000 cases worldwide and 34,000 cases in the US). Currently, there is an uptick in the more severe and more transmissible type of mpox, clade I (clade 1b). Since January 2024 there have been over 55,000 cases and 1000 deaths in Central and Eastern Africa, multiple cases in Europe and at least one confirmed case of clade I in the USA. Given the concern regarding the spread of clade I and the documented efficacy and efficiency of wastewater surveillance in providing early-warning and tracking other pathogens, building wastewater monitoring for mpox clade I and II has become a priority for federal and state public health agencies across the United States.

Since detection of even a single mpox case in a community is of significant public health concern, optimization of wastewater workflow and molecular methods to maximize sensitivity and reproducibility is vital. The key components of the workflow that influence sensitivity are viral concentration and extraction, as well as PCR quantification. Here, we present a comparison of various concentration methods for MPXV including, use of Ceres Nanotrap particles, Promega Rapid Capture magnetic bead-based kit, and concentration from influent solids via centrifugation. Preliminary results suggest that Promega Rapid Capture and Ceres Nanotraps are effective in recovering mpox from wastewater. The selection of a sensitive and specific PCR assay is key to the success of MPXV wastewater monitoring. Some agencies have opted to use a broad PCR assay [Non-Variola Orthopoxvirus (NVO)], designed to detect all clades of mpox as well as its closest relatives, while others use assays targeting individual clades of MPXV. We compared multiple digital PCR assays, including the NVO assay, as well as clade I and II assays. Our data suggest that the NVO assay may not be optimal for mpox surveillance. In addition to the evaluation of different workflows, we also present preliminary results of

mpox wastewater surveillance in Wisconsin.

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Poster 209
Development of a Real-time PCR Assay for the Detection of *Mycobacterium gordonae* at the Wisconsin State Laboratory of Hygiene
N. Mack, Wisconsin State Laboratory of Hygiene, C. Seehafer, Wisconsin State Laboratory of Hygiene

Mycobacterium gordonae is described in the Manual of Clinical Microbiology as “one of the most commonly recovered mycobacteria in the laboratory” while also having “only rarely been associated with disease in humans”. At the Wisconsin State Laboratory of Hygiene (WSLH), M. gordonae is the second most isolated organism second only to Mycobacterium avium complex. Empirical data at the laboratory at WSLH has shown M. gordonae to be difficult to identify by MALDI-TOF, often leading to identification by 16S or rpoB DNA sequencing, which is both expensive and time consuming. We determined that developing a real-time assay to identify M. gordonae would reduce time spent by microbiologists to identify this non-pathogenic organism and get results to submitters more quickly. A validation was conducted for an assay similar to the Mycobacterium tuberculosis complex and Mycobacterium avium complex real-time PCRs already being performed at WSLH. The validation was done with previously identified M. gordonae isolates and well as other Mycobacterial species and non-Mycobacterial species to ensure the PCR primers and probe do not cross-react with other species. The validation showed that this PCR can detect M. gordonae, however, it does not distinguish between M. gordonae and M. paragordonae which are closely related. Implementation of this assay began in May of 2024 and has significantly reduced microbiologist time spent identifying M. gordonae.

Presenter: Nicholas Mack, Wisconsin State Laboratory of Hygiene, nicholas.mack@slh.wisc.edu

Poster 210
Validation of Multiplexed PCR Method for the Detection of β-Lactamase Genes in Carbapenem Resistant Gram-negative Bacteria
P. Ross, Wisconsin State Laboratory of Hygiene, E. Baldwin, Wisconsin State Laboratory of Hygiene, A. Valley, Wisconsin State Laboratory of Hygiene, A. Bateman, Wisconsin State Laboratory of Hygiene

Background: Carbapenem resistance is a growing concern among Gram-negative bacteria. As a Regional Laboratory for the Antimicrobial Resistance Laboratory Network, the Wisconsin State Laboratory of Hygiene (WSLH) performs a variety of tests to allow partners to identify, treat, and surveil carbapenem resistance Enterobacterales, Pseudomonas aeruginosa, and Acinetobacter baumannii. The WSLH uses Centers for Disease Control and Prevention’s (CDC) PCR assays to identify carbapenemases (KPC, NDM, VIM, IMP, OXA-48, OXA-23/24-40). While these PCR procedures have been the suggested method for identifying carbenepenem resistance genes in the past, they do not detect all gene variants for each gene and are labor intensive when performed in parallel for all Carbapenem Resistant Organisms (CROs). To streamline the process, WSLH validated the NG-TEST CARBA 5 assay for Enterobacterales and P. aeruginosa. However, A. baumanii is not approved for testing with the CARBA 5 assay nor does the CARBA 5 assay identify OXA genes other than OXA-48. We are validating the Streck β-Lactamase and OXA kits to streamline our current process for A. baumanii and increase the total detectable gene variants, since both kits contain multiplexed master mixes which test for a large variety of gene variants for each β-Lactamase gene.

Methods: Using the QuantStudio 5 (QS 5), we evaluated KPC, NDM, VIM, IMP, and OXA-48-like from the Streck β-Lactamase kit; and OXA-23, OXA-24/40, and OXA-48-like from the Streck OXA kit. 5 previous positive isolates of each gene were used to evaluate the accuracy of the Streck kits (n=35). According to the package insert, positive samples should have a Ct value between 10 and 26, and Streck’s technical note document suggests that a change in fluorescence (ΔRn) should be ≥1,000,000. Additional future testing will evaluate precision and reproducibility.

Results: The Streck kit was 59.1% (13/22) accurate in the initial test run. The 9 samples that did not

have expected results on Streck all had false positives. One sample that was previously positive for IMP tested positive for IMP and VIM; 4 samples that were previously positive for KPC tested positive for KPC and NDM; and 4 samples that were previously positive for OXA-48 tested positive for OXA-48 and OXA-24/40 on the OXA kit. The 4 samples that had false positives on the OXA kit were tested in parallel for OXA-48 on the β-Lactamase kit. These 4 samples did not have any false positives using the β-Lactamase kit, which indicated that the β-Lactamase kit is more accurate than the OXA kit for detection of OXA-48. In all instances, the true positives were associated with HEX/VIC dye, and the false positives were associated with the TEX615/JUN/ROX dye. After repeating each sample, the false positives remained, but their fluorescence curves were lower (< 10,000 at peak) than those of true positives (~20,000-80,000 at peak). To investigate, we ran the samples in parallel on two PCR instruments (ABI 7500 and QS 5; the issue repeated on both instruments), discussed the issue with another AR Lab Network Regional Lab that runs these Streck assays, and reported the issue to the manufacturer. We are currently in the process of evaluating different extraction methods, different PCR cycling methods, and different dye calibrations to determine what could be the cause of the false positives and comparatively low ΔRn.

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Poster 211

Improving Performance of Methods for Pathogen Surveillance in Wastewater — A Proficiency Testing (PT) Program for SARS-CoV-2, Influenza and RSV

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Wastewater-based surveillance (WBS) for pathogens is increasingly recognized as a robust public health tool. WBS data can be a leading indicator of increased disease transmission, and WBS provides for more equitable community-monitoring, independent of healthcare seeking behaviors. However, implementation challenges remain. One obstacle is the lack of method standardization, with dozens of methods in use, and no effective way to assess and compare their performance. Here we describe the design and results from the first wastewater PT program in the US.

Laboratory performance was assessed using actual wastewater matrices, spiked with the target viral pathogens. Our overarching goal was to identify underperforming methods/labs and then work with those labs to improve outcomes. A composite influent sample from a Wisconsin water reclamation facility was collected in May'24 and spiked with inactivated SARS-CoV-2, influenza A/B and RSV A. Replicate samples were pulled from a churn and homogeneity verified. Three replicate samples were shipped to the participants overnight on ice and a sub-set was analyzed in-house for stability over a 2-week period. A large set of meta-data was also collected to capture method details and to allow independent verification of the reported results. In addition, data on internal spike control and fecal marker measurements were collected.

Thirty-one labs enrolled in the 1st PT round with 29 submitting results. 45% of participants used a solids removal method prior to viral concentration, while 55% processed whole influent. The most commonly applied concentration method was Ceres Nanotrap (69%), followed by InnovaPrep Concentrating Pipet (21%). The most popular extraction method was MagMax by ThermoFisher (55%) followed by Qiagen's AllPrep PowerViral (28%). All of the labs, except one, used a digital PCR platform for quantification. 11 of the 29 labs were contacted to troubleshoot discrepancies either in the metadata or in their reported virus levels – which after correction resulted in almost an order of magnitude improvement in the spread of the reported data. Overall, the SARS-CoV-2 data exhibited less variance, while the range of the influenza A/B, RSV and the fecal marker (PMMoV) data were almost an order of magnitude wider. Labs processing the influent's liquid phase showed decreased average recovery of SARS-CoV-2, RSV, as well as the spike-in process control and PMMoV. In contrast, recovery of

influenza A and B was overall similar between whole influent and the solid-depleted influent. Based on the viral recovery and precision, we ranked the performance of the different concentration/extraction methods. We also evaluated the use of spike-in recovery control and fecal marker concentrations as data normalization strategies.

Our PT program demonstrated that while the WBS field has made progress toward standardization of the sampling and laboratory methods, the need for inter-laboratory comparisons and common evaluation metrics is great. PT is an effective way of benchmarking performance and providing feedback to the laboratories for continued improvement. It is especially important for a growing field such as WBS to examine legacy and new methodology, as well as QAQC criteria, to improve data comparability and utility in the nationwide wastewater surveillance system (NWSS).

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Poster 212

Bactopia v4: Adapting to Nextflow's Future with Enhanced Efficiency and Compatibility

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Bactopia is a Nextflow pipeline for the complete analysis of bacterial genomes. Bactopia allows users on all types of systems, such as laptops, HPC, and cloud platforms, to quickly analyze bacterial genomes using hundreds of bioinformatic tools with Conda, Docker, and Singularity. Bactopia maintains extensive version control, data auditing, and testing to ensure reliability for its users. Bactopia can be rapidly scaled from a single sample to tens of thousands of samples, making it ideal for large-scale, ongoing genome surveillance projects. Here we introduce the latest major version release of Bactopia, version 4.

In response to major updates in Nextflow, a significant rewrite to Bactopia was necessary to remove its dependency on now-deprecated Nextflow features. This overhaul simplifies the codebase, streamlines development, and enhances compatibility with future releases of Nextflow. The v4 update includes key improvements such as the migration of standalone Python scripts to the bactopia-py package, promoting modularity and maintainability. Additionally, the introduction of in-job cleanup significantly reduces storage requirements, and enhanced reporting tools provide users with clearer, more actionable insights from their analyses. Together, these advancements ensure Bactopia remains a robust and scalable solution for genome surveillance while paving the way for future enhancements.

With these updates, Bactopia v4 positions itself to meet the growing demand for genomic tools that integrate seamlessly with evolving processes. Bactopia now has enhanced compatibility with the Seqera Platform and Nextflow ecosystem allowing users to take advantage of the latest developments in Nextflow. In addition, the improved modularity offers flexibility for extending Bactopia beyond bacterial genomics. Researchers can now explore applications in fungal and metagenomic analysis, further diversifying Bactopia's utility. The efficiency gains provided by in-job cleanup make Bactopia especially well-suited for resource-constrained environments, such as public health laboratories with limited storage infrastructure.

These improvements not only expand the pipeline's capabilities but also reinforce Bactopia's commitment to the global genomic research community. Enhanced training materials and outreach efforts are planned and will make the pipeline more accessible to new and existing users. By addressing current challenges and anticipating future needs, Bactopia is poised to continue supporting genome surveillance, public health research, and academic inquiry for

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Poster 213

Occupational and Behavioral Influences on Environmental Chemical Exposure in the US Population: Insights from NHANES on Perchlorate, Nitrate and Thiocyanate

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Abstract:
Background: Perchlorate, thiocyanate, and nitrate is common environmental chemicals, which are known as sodium/iodide symporter inhibitors (NIS). Accumulating evidence suggests their potential effect on human health, especially on thyroid function. However, these studies were constrained in estimating disparities in exposure and identifying sensitive populations.
Objective: This study aimed to assess the prevalence, trends, and disparities in the levels of perchlorate, nitrate, and thiocyanate exposure by occupation and lifestyle habits among U.S. populations, using data from the National Health and Nutrition Examination Survey (NHANES) from 2011 to 2018.
Methods: The study sample after excluding participants with missing data in the main and covariates consisted of 2973 people with a mean age were 42.83± 13.9; 1682 males and 1291 females. Four separate Generalized linear models were used to examine trends and disparities in exposure levels of log creatinine-adjusted perchlorate, nitrate, and thiocyanate by occupation groups and lifestyle habits, including smoking status, drinking Status, and exercise habits. Multinomial logistic regression was performed to assess the association between Thiocyanates tertiles and lifestyle habits adjusted for gender, age, race, and marital status.
Results: The Generalized linear models and Multinomial logistic regression results revealed Significant variations across occupation groups and lifestyle habits, with self-employed individuals have statistically significant increase in levels of perchlorate and nitrate as compared to employees of private companies ($\beta = 0.066$ [95% CI: 0.030 to 0.101]); ($\beta = 0.039$ [95% CI: 0.006, 0.072]) with $p < 0.001$. Non-Smokers and Non-Drinkers exhibited significantly lower levels of all three chemicals, particularly thiocyanates ($p < 0.001$, Wherein that smokers had significantly higher odds of being in higher thiocyanate tertiles (tertile 2: OR= 1.98 [95% CI: 1.00, 3.92], tertile 3: OR=4.70 [95% CI: 2.10, 10.60]). In contrast, individuals who engaged in regular exercise were associated with significantly lower exposure to thiocyanate (tertile 2: OR= 0.96 [95% CI: 0.55, 4.34], tertile 3: OR =0.19 [95% CI: 0.61, 4.37]).
Conclusion: In conclusion, this study reveals occupational settings and lifestyle factors significantly influence exposure to perchlorate, nitrates, and thiocyanates, with smoking individuals being more susceptible. Promoting physical activity could reduce exposure. Further research is needed to understand the mechanisms and potential sources of exposure. There is a need for public health policies to reduce exposure risks in vulnerable populations.

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Poster 214

A Comprehensive Workflow for Monitoring Pathogens and Antimicrobial Resistance in Wastewater: From Sample Collection to Data Analysis

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Wastewater surveillance has emerged as a critical tool in public health epidemiology, especially following the COVID-19 pandemic. Modern culture-independent sequencing techniques provide valuable insights into microbial dynamics, yet a lack of standardization across various stages of the process remains a significant challenge. In this study, we present a unified approach to wastewater surveillance, covering all stages from sample collection and preparation to target detection, data analysis, and report generation. To maximize sensitivity and accuracy, we applied both long-read sequencing on the PacBio Sequel II platform and short-read sequencing on the Illumina NextSeq 2000 platform. This dual approach enables concurrent detection of pathogens, antimicrobial resistance

(AMR) monitoring, and functional analysis within wastewater systems. Additionally, we employed ProxiMeta Metagenome, a proximity ligation (Hi-C) library-based sequencing platform, to detect interactions between DNA molecules originating from the same cell, aiming to enhance AMR detection by assigning AMR genes to their host for maximum utility. Raw wastewater samples from local WWTPs were processed using our in-house workflow. By integrating advanced sample preparation technologies from Zymo Research with PacBio long-read and Illumina short-read sequencing, our approach aims to deepen understanding of microbial behavior in wastewater environments.

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