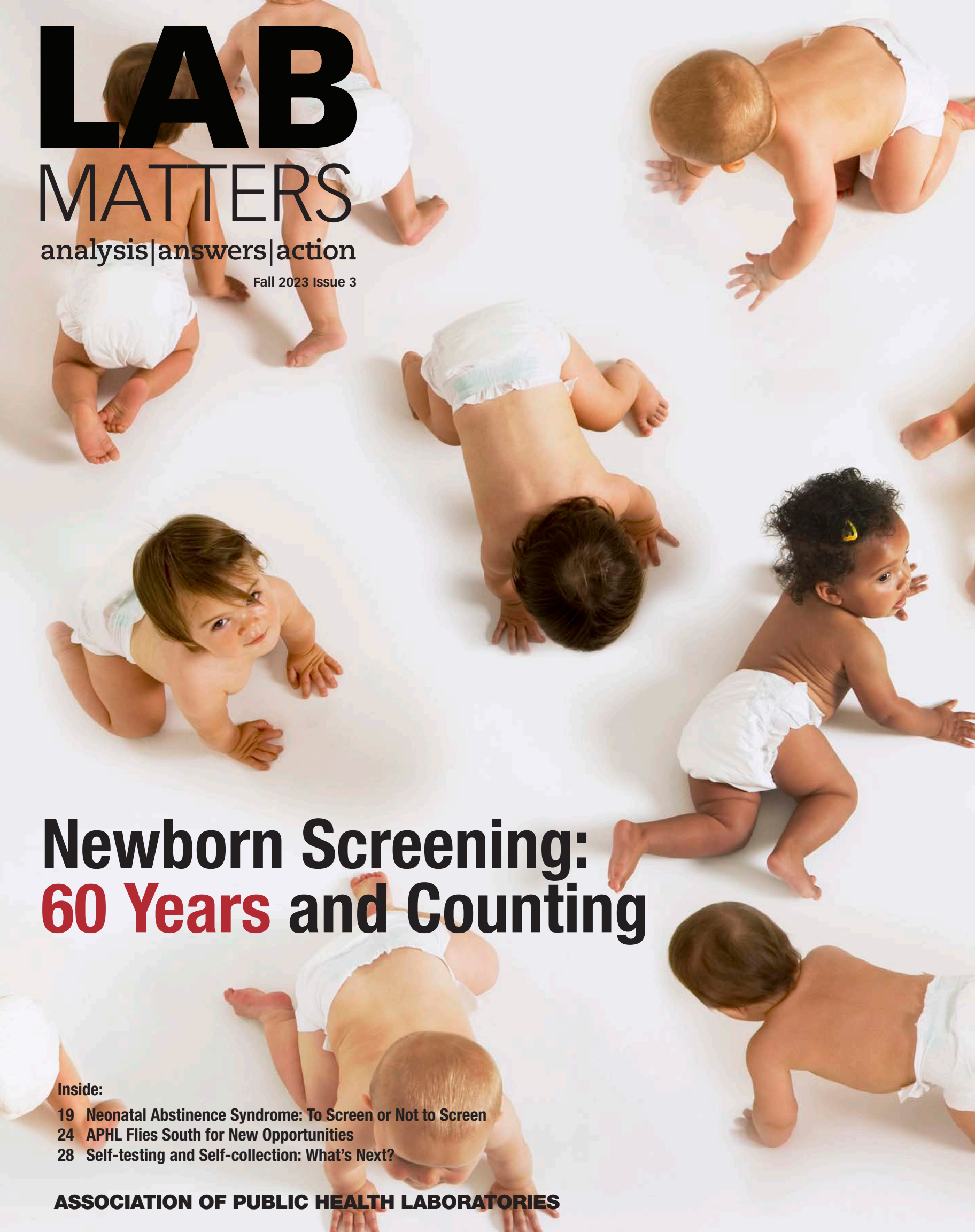




LAB MATTERS

analysis|answers|action

Fall 2023 Issue 3

Newborn Screening: **60 Years** and Counting

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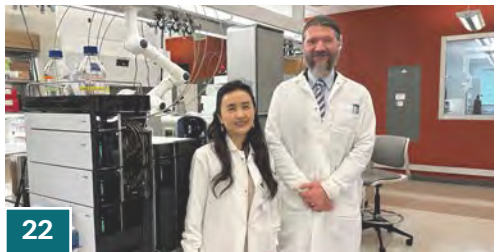
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The Association of Public Health Laboratories (APHL) works to strengthen laboratory systems serving the public's health in the US and globally. APHL's member laboratories protect the public's health by monitoring and detecting infectious and foodborne diseases, environmental contaminants, terrorist agents, genetic disorders in newborns and other diverse health threats.

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Public Health on the Frontier



Timothy Southern, PhD
President, APHL

When I graduated high school, kiddos like me who had an interest in science were told we could go into teaching or medicine. As a high school student, I always enjoyed science, and I developed a very strong interest in research and medicine during my first years in college. It wasn't until I went to the National Institutes of Health for a postdoctoral fellowship 13 years later that I finally realized the exquisite intersection of science, research and medicine was just screaming "public health" at me. I think it is poignant when I look back and reflect on how little discussion there was about public health careers in high school and college, and how long it took me to find my true career path. We as a community have a lot of work to do to ensure we recruit and nurture the next generations of public health laboratory scientists and leaders. I am thankful for those of you who have taken up this banner and are working with academic institutions to reinforce existing training programs, implement new training programs and to open the horizons of students searching for a rewarding career, all while promoting inclusion and diversity within our ranks.

I think one of the most profound changes I experienced in South Dakota, even more than the impact of rapid innovation in science and technology during the pandemic, is the visibility of public health and public health laboratories. Before the pandemic, folks didn't know much about public health laboratories. We did our jobs, and because we were good at our jobs, we tended to stay out-of-sight and out-of-mind. The pandemic changed that. Public health laboratories were thrust

into the spotlight and became profoundly valued members of the public health community.

Unfortunately, our community is approaching a time when public health laboratories and the services we provide risk losing visibility and the critical support we need. We have already seen rescission of federal funding, a loss that has touched all jurisdictions. I am very thankful to APHL and our members who continue to advocate for the resources we all need to not just maintain our programs but to continue building so that we can address the threats of tomorrow. Please continue to tell your stories to all who will listen because these efforts will help define how we deliver critical public health laboratory services in the post-pandemic era.

I believe what defines a public health laboratory is fundamentally changing. For decades, public health laboratories were the teams that provided testing both as a part of routine duties and as a part of outbreak detection and response. During that time, I believe our role and impact as subject matter experts was largely understated. Throughout the pandemic, our scientists and leaders became the subject matter experts that reached into communities to better understand local public health needs, including the needs of populations at risk for severe disease outcomes. I believe the future will require public health laboratory scientists and leaders to be bridge-builders, partners and collaborators with the diverse populations we serve. I also believe that public health laboratories must lead continuous development of the broader laboratory systems within our jurisdictions. I look forward to working with each of you as we continue working toward strong laboratory systems capable of adapting to changing public health needs.

There are profound unmet needs among our member laboratories, especially those laboratories that serve predominantly rural and frontier populations. But as the pandemic proved, we are just as likely to see public health deserts in urban settings as we are in rural communities. A priority for my term is

to bring members together and to find ways to drive improvement of public health laboratory service delivery to our most vulnerable populations. I want to shine a bright light on what it means to be a laboratory in a predominantly rural and frontier state, what it means to serve rural, frontier and tribal populations who are culturally diverse and geographically separated from critical public health infrastructure and explore ways to improve public health laboratory service delivery to populations with profound vulnerabilities.

In the coming months, there will be an opportunity for you to support an initiative that seeks to understand rural, frontier and tribal community needs and then explore solutions to better meet those needs. I will ask you to help me better understand what it means to overcome the geographic and financial isolation of tribal, rural and frontier communities, and to explore solutions that will make the services that we provide more accessible and more affordable. As a part of this initiative, we will establish what it means to be a trusted voice to culturally diverse populations, how to engage those populations to better understand community needs and how to improve the flow of resources in severely resource-limited communities.

Even though I think I stumbled into public health, the reality is that an amazing public health career found me. I am fortunate to have had so many dedicated colleagues, mentors and friends in our community—wonderful people who helped me navigate my first decade of public health laboratory service. I truly appreciate being a member of a community committed to diversity, equity, inclusion and accessibility, and I am thankful to be surrounded by a community of professionals who know we still have important work to do to ensure public health for our diverse communities. Thank you for this opportunity to "pay it forward." I am looking forward to having these deep conversations with all of you as the year progresses. ■

Managing Expectations...and Creating Opportunities



Scott Becker, MS
Chief Executive Officer, APHL

“... APHL will always take every opportunity to advocate for its members, and the resources they need to do their work.”

Part of APHL's role as a member-driven association is to listen to our members and their experiences. Even though it may not seem like it for folks, we have emerged on the other side of a pandemic. For me, I feel and look different than in the “before” times. A few extra pounds, a few more wrinkles and more mature professionally. But one thing hasn't changed for us: the desire to learn from our experiences and prepare for the next surge. The lessons we learned as laboratorians have become ingrained in our laboratories and our work. But they haven't been inculcated into broader public health systems yet. Our wish may be to be more proactive in our approaches, but under newly manifested political pressures and challenges, it may seem like we are fighting an uphill battle. And we are.

In the past, we have always found innovative ways to do more with less. We always respond, no matter what. Yet, at the end of the day, everything we do as public health laboratories comes down to money—money that translates into reagents, equipment, technology, supplies and staff. We have just finished three plus years of pressure to deliver results and data, even faster than we had before.

And now we face an unprecedented funding recession. Not only are we going to have to pivot on how the work is done, but also managing the expectations of our partners and collaborators. It will be difficult for us to say “we are unable to perform this test because of X, Y and Z.” But if we continue to be expected to do more with less, we will harm ourselves and our communities. Please know that APHL will always take every opportunity to advocate for its members, and the resources they need to do their work.

We are also working to make sure that the lessons learned during the COVID-19 pandemic are not forgotten. Early in the pandemic, we realized that there was no widely accepted understanding of the national diagnostic system and how the different laboratory sectors work together in an emergency. Leaning on relationships we formed during the pandemic, we consulted with partners inside and outside government, academia, commercial laboratories, diagnostic manufacturers, regulators, CDC and others, to create a national diagnostic system “playbook.” Working with The Pandemic Center at the Brown University School of Public Health and with funding support from the Peterson Foundation, we are developing a series of very practical documents that describe roles, responsibilities and best practices before and during a biological emergency. These documents build on the wealth of knowledge that was gained by public health professionals and their partners during the US pandemic response. Our hope is that it will be utilized by the new Pandemic Office within the Executive Branch and, even though we hope it will not need to be used, all our hard-won knowledge and experience will not just disappear into history.

As an association, we have also just completed the process of reviewing and updating our **strategic map**. Through a more concentrated process of review and reflection, I believe we have further distilled our objectives into true, actionable items that will support the work of our members—providing them

with all the resources and guidance that they need to navigate their way over the cliff and into a more prepared landscape. You will note that the latest map is a two-year plan, which carries on some of the work of the past map. We will continue to engage members and find ways to allow the map to “come alive,” and support your important work. I had an “aha” moment when the Board met to refine this plan. It was this: what WERE we thinking when we created a plan in the fall of 2020? We had high hopes that the pandemic would soon wane, and we could get on with our work. But here we are, years later, picking up not where we left off, but in some ways in a better place because we are now able to see more clearly about what really matters to the members and the Association. ■

A Winding Road to a Newborn Screening Fellowship

By Rudolph Nowak, MPH, senior specialist, Marketing & Communications

As far as **Public Health Laboratory Fellowship Program** cohorts go, newborn screening is small. Eleven individuals make up the cohort. Daniel Fang, **Alabama Department of Public Health Bureau of Clinical Laboratories** and Cullan Kirwan, **Colorado Department of Public Health and Environment**, both had an early interest in the science field, although they had different paths into the public health laboratory landscape.

"I really like the aspect of learning about characteristics and features of the natural world that we can't see, and coming to understand how those operate," Fang said. "I didn't really consider pursuing it as a facet of my career until maybe halfway through my undergraduate degree." Public health was not a consideration for Fang until his senior year, when Willamette University announced an Introduction to Public Health class for the first time.



Daniel Fang, Alabama Department of Public Health Bureau of Clinical Laboratories

"My degree was already completed, but I wanted to explore other fields that I was particularly interested in," Fang said. "I recalled that public health class and thought 'Let's see if I can learn more about that,' and through a few Google searches I found the APHL Fellowship [program], and here we are."

For Kirwan the initial public health path was less direct.



Cullan Kirwan, Colorado Department of Public Health and Environment

"I didn't originally start as a [science] major. In high school I was pretty good at biology," Kirwan said. "I went to college and started off in architectural engineering but then changed my major back to biology just because I [had an] interest in DNA and microbiology."

Kirwan ended up graduating from the University of Wyoming with an associate of science as a Medical Laboratory Technician with a Phlebotomy certificate and bachelor's degrees in molecular biology, microbiology and medical laboratory science. A rotation in the public health laboratory doing microbiology whole genome sequencing allowed Kirwan to see laboratory science outside of a hospital setting, which led him to his fellowship.

"My professor sent me the [APHL] Fellowship link because he knew I didn't really want to stay in the hospital," Kirwan said. "On a whim I applied for this fellowship, and it has changed the direction of my career."

Lasting Impacts

Fang has used his fellowship to learn more about the different aspects of public health and the public health community.

"I think that the most impactful parts of this fellowship have been the conferences because of how many amazing people I've been able to meet and not only learn

the job that they do, but also be inspired by just how knowledgeable they are and how motivated they all seem to be," he said. "I think the biggest takeaway is just how expansive and robust the newborn screening program is."

"It really does put a face on what you're doing in the laboratory," Kirwan agreed. "In our laboratory, we never actually see patients. We're doing all the testing, but we never put a face on what we do. The fellowship experience really gives you the opportunity to put the face to the test that you're doing." He saw that impact by getting to meet patients and parents' advocates at newborn screening conferences.

"They're there because the test that you do has (had) an impact in their lives. What I mostly got out of it is that you understand what impact a test has and what tests benefit public health."

So where do their pathways lead from here? Fang is currently applying to medical schools.

"I have really come to enjoy the idea of being able to do research and clinical care and influence policy work all under one umbrella specialty," he said. "I want to pursue clinical genetics, which was influenced by the fellowship."

Kirwan has accepted a position at the Colorado Department of Public Health and Environment, but he is also going back to school.

"This fellowship changed my aspirations in terms of what I want to do. I am currently in an MPH program in Epidemiology and I'm trying to understand more public health perspectives on additional concepts." ■

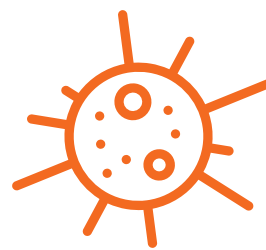
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Clinical Chemistry

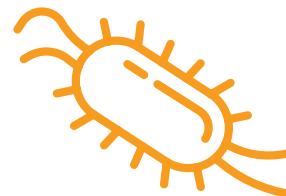


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APHL 2023, In Their Own Words

By Rudolph Nowak, MPH, senior specialist, Marketing & Communications

Fellowship flowed and friendships formed during **APHL 2023**. One hundred and twenty Public Health Laboratory Fellowship Program participants gathered in Sacramento, CA for five days of professional and personal development. For some fellows, the conference was the biggest event of their fellowship, for others it was a steppingstone for their future endeavors or another experience in their public health careers. They took advantage of opportunities to meet, network and celebrate their accomplishments with their cohort and others in the public health community. They might have had a little fun, too.

What did you gain from the Fellowship preconference gathering?



Faith Weber, Food Safety Fellow, **Virginia Division of Consolidated Laboratory Services**

"This was the first opportunity I got to meet people from different programs, see what they did and learn about other projects."

"Food safety and newborn screening are pretty small programs. I didn't know how few of us there were. So, we were small enough to all introduce ourselves easily."



Whitney Heard, Infectious Disease Fellow, **Alabama Department of Health, Bureau of Clinical Laboratories**

What was your experience with your cohort before the conference?



Amna Elbadawi, Infectious Disease Fellow, **Public Health Laboratory of East Texas**

"We have a fellows' GroupMe, so throughout the year if things came up or if the Fellowship Team released information, we were able to help each other out by passing that (information) along. But it was cool to meet people in person because obviously you're just kind of all numbers on paper until you see how many (fellows) there are."

"I think it was great seeing everybody—even people in your own cohort. I'm an infectious disease fellow and it's one of the biggest cohorts, but it was interesting just discussing everyone's scientific point of view. Especially the PhD- and master's-level fellows who acted as mentors at times."

Faith Weber

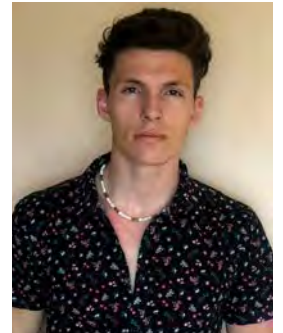
What was the highlight from the preconference presentations?

"[Marisa Hattab, Douglas County, Nebraska DEI Officer] talked about how we should grow our social and professional circles for people who we can count on [for support]. That's something I never really thought about."

Amna Elbadawi

Were you surprised by anything at or during the conference?

"I did not meet a fellow I didn't like, and I think that it is very uncommon where you meet a large group of colleagues, and you get along with all of them. It was incredibly fun, very friendly, a wonderful cohort."



James Zumhagen, Biorisk Management Fellow, **Missouri State Public Health Laboratory**

"The sessions were able to address less-than-fun topics in an engaging way, and I was surprised by that. But that did make it more fun."

Whitney Heard

"Outside of the conference, it was fun getting to network off the grid. I met up with the director of our laboratory and a couple other directors. I wasn't expecting to do that with a bunch of directors as a fellow. But it was cool to talk to them and they asked how I became a fellow, my background, and then also about playing college softball."

Faith Weber



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- **Eligible candidates:** Current student enrolled at an accredited institution and in pursuit of a relevant certificate, associate's, bachelor's, master's or doctorate* degree
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- **Hours and program length:** Flexible hours with an average of 20 hours a week, length varies

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* Genomic data
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APHL - CDC Public Health Laboratory Fellowship Program

Fellowships support a wide spectrum of public health laboratory program areas, such as: bioinformatics, biorisk management (biosafety), environmental health, infectious disease, informatics, food safety, newborn screening and quality management. Each fellow will be trained in alignment with established public health laboratory core competencies and functions and will work on unique program-specific projects and other initiatives in host laboratories.

- **Eligible candidates:** Bachelor's, master's or doctorate degrees
- **Applications:** Accepted year-round
- **Flexible start and end dates**
- **Hours and program length:** Approximately 40 hours a week, one to two years



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Planning for the Future in Arizona

By Kathryn Wangsness, assistant bureau chief, Arizona State Public Health Laboratory and Pranav Chhaliyil, laboratory operations intern, Arizona State Public Health Laboratory



Pranav Chhaliyil interviews Kathryn Wangsness for Arizona's Knowledge Retention Project. Photo: Arizona State PHL

In government, staff previously served most of their careers in the same organization. During their long tenures, they acquired skills and developed strategies for their positions, then passed along their knowledge to staff who were hired to replace them. But even before the COVID-19 pandemic, the nature of careers in public health laboratories began to change—becoming more technical and requiring more knowledge. And when someone left the laboratory or retired, their knowledge went with them.

In 2015, the APHL Knowledge Management Committee developed a **toolkit** to capture details relating to a position, especially the different aspects of the job. It was curated to cover various positions from Laboratory Scientist to Laboratory Director. Building off the toolkit, the Knowledge Retention (KR) Project was developed to preserve the resources and strategies acquired by staff before retirement or organizational changes to create a resource that will be useful to successors and allow them to benefit from the knowledge of predecessors.

In September 2022, then-Office Chief of Quality Assurance and Improvement and now Assistant Bureau Chief of the

Arizona State Public Health Laboratory Kathryn Wangsness introduced KR to her laboratory after the Arizona Management System identified staff who were critical to the agency and/or were retiring. Over the span of nine months, Wangsness and Laboratory Operations Intern Pranav Chhaliyil conducted in-depth interviews with more than 40 employees.

Casual Yet Professional

Multiple 30–60-minute interview sessions were held either in person or on Google Meet with staff, and were audio recorded with the permission of the interviewee. The emphasis was placed on helping the employees see the value of helping future generations in the agency by sharing their stories, experiences and tips.

“I would tell them the project is like writing a biography to encourage them to share their journey,” Chhaliyil reflected. And the response was overwhelmingly positive. Interviews were filled with laughter and stories from decades of experience in the agency. Some even took five sessions to complete the toolkit due to the level of detail and amount of information staff passionately shared. Questions were not provided prior to the

interview in order to obtain authentic responses.

Original pre-made questionnaires based on the original Knowledge Management Committee toolkit were used for specific positions. Post-interview surveys were used to collect links to frequently accessed documents, SOPs, contacts, journals and more. And a feedback survey was used to collect data on the project itself, as well as the interview experience. Alongside the great experiences, there were some challenging situations that helped staff understand more about how the project can be more approachable in its introduction and where some employees were coming from (e.g., worried about their job standing). There were turning points in the project, leading to a meeting between Wangsness and Chhaliyil, where they walked through an interviewee's journey from the email interview request to the questions asked, finding places where information could be misinterpreted, giving rise to a change in communication style to a feedback survey asking what the interviewee felt about the project and how it can be improved.

The Knowledge Retention Project is a unique approach to preserving and transferring employee wisdom across generations with holistic questions addressing various aspects of a position from management style to roles in budget development.

“This has been a great opportunity in helping retain information on tips and strategies developed by staff as well as identifying patterns in employee experiences that can be considered as feedback for the laboratory,” Wangsness said. The team thanks Lorelei Kurimski and the APHL Quality Systems & Analytics team for introducing KR to them and encourages other agencies to implement the project to record the knowledge and experiences of their valued employees. ■

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APHL 2023: Where Laboratory Science and Public Health Meet

Over 1,500 public health scientists and administrators attended **APHL 2023** in Sacramento, CA and virtually May 22–25. APHL welcomed over 120 participants from the Public Health Laboratory Fellowship Program: an APHL-CDC Initiative. Zubin Damania, MD, brought his comedic wit, rapping skills and videos to the Dr. Katherine Kelley Session. And APHL presented its annual awards for outstanding achievements in laboratory science, creative approaches to solving today's public health challenges and exemplary support of laboratories serving the public's health.

The following awards were presented:

Lifetime Achievement Award. Recognizes individuals who have established a history of distinguished service to APHL, made significant contributions to the advancement of public health laboratory science or practice, exhibited leadership in the field of public health and/or positively influenced public health policy on a national or global level. This is not a retirement award, but a true Lifetime Achievement Award.

- **Ralph Timperi**, MPH, former director, Massachusetts State Public Health Laboratory; retired, APHL
- **Eric Blank**, DrPH, former director, Missouri State Public Health Laboratory; retired, APHL

Gold Standard Award. Given to an APHL member who makes or has made significant contributions to the technical advancement of public health laboratory science and/or practice.

- **Amy Miles**, director, Forensic Toxicology Section, Wisconsin State Laboratory of Hygiene

Silver Award. Honors a laboratorian with 10-15 years of service in a Governmental public health laboratory (either public health or environmental/agricultural laboratory). The honoree will be recognized as a leader both within their home laboratory as well as external to their laboratory.

- **Elizabeth Nazarian**, research scientist, Bacteriology Laboratory, Wadsworth Center, New York State Department of Health

On the Front Line Award. Honors an individual or laboratory outside of the APHL membership who makes significant contributions to the advancement of public health laboratory science and/or practice.

- **Nathan Grubaugh**, PhD, MS, associate professor, Department of Epidemiology of Microbial Diseases, Yale School of Public Health; affiliated faculty, Yale Institute for Global Health

Emerging Leader Award. Honors an individual whose leadership has been instrumental in one or more advances in laboratory science, practice, management, policy or education early in their career.

- **Emily McCutchen**, MS, manager, Nebraska Public Health Laboratory
- **Michael Perry**, MS, MEd, associate director, Biodefense Laboratory, Wadsworth Center, New York State Department of Health

Leadership in Biosafety and Biosecurity Award. Honors a laboratorian with over 10 years of related service in the field of biosafety and biosecurity in a state and/or local public health laboratory. The honoree will be recognized as a leader both within their home laboratory as well as external to their laboratory (for example, by serving in a leadership role in committees/taskforces at the national level).

- **Leah Gillis**, PhD, adjunct professor, DrPH Public Health and Clinical Laboratory Science and Practice Program, College of Public Health, University of South Florida

LEAD Award. Established in 2021, this award recognizes the legacy of Eva J. Perlman, APHL's first chief learning officer, who over three decades helped shape the public health laboratory workforce and represented the attributes



2023 APHL Awards Ceremony winners. Photo: Wale Ariztos

of ‘serving those who have served.’ This award honors an individual who exhibits the attributes of a leader, encourager, advocate and developer, and who has 10 or more years of service in a state, local or territorial public health laboratory, or other public health laboratory partner.

- **Kristina McCallum**, manager, Biochemistry Laboratory, Division of Laboratory Services, Colorado Department of Agriculture
- **Kelly Winter**, PhD, MPH, branch chief, Training and Workforce Development Branch, Division of Laboratory Systems, US Centers for Disease Control and Prevention

Champion of the Public Health

Laboratory Award. Recognizes federal, state and local elected officials or executive branch employees who have recognized the importance of state and local governmental laboratories that perform testing of public health

significance either through support of legislation or federal agency decisions.

- **Demetre Daskalakis**, MD, MPH, director, Division of HIV Prevention, US Centers for Disease Control and Prevention

Presidential Award. The APHL Presidential Award was selected by Dr. Daphne Ware during her Presidential year (2022–2023) for the significant contributions that were made to the association’s work to promote policies that strengthen public health laboratories.

- **Judith Monroe**, MD, president and chief executive officer, CDC Foundation

Healthiest Laboratory Award. Given to an APHL member laboratory that is committed to safety, environmental process, environmental policy and employee health and wellness.

- **Winner:** **Nebraska Public Health Laboratory**
- **Runner-up:** **San José-Santa Clara Regional Wastewater Facility Laboratory**

Thomas E. Maxson Education, Training and Workforce Development Award.

Established in 1998 in memory of Dr. Maxson, this award honors an APHL member who is a public health or clinical laboratory practitioner, trainer or educator who has made significant contributions to public health laboratory practice by creating, delivering or developing continuing education opportunities, programs, policies or practices for the laboratory community.

- **Crystal Fortune**, laboratory biosafety coordinator and newborn screening short-term follow-up coordinator, Montana State Public Health Laboratory ■

APHL Presents New Member Values

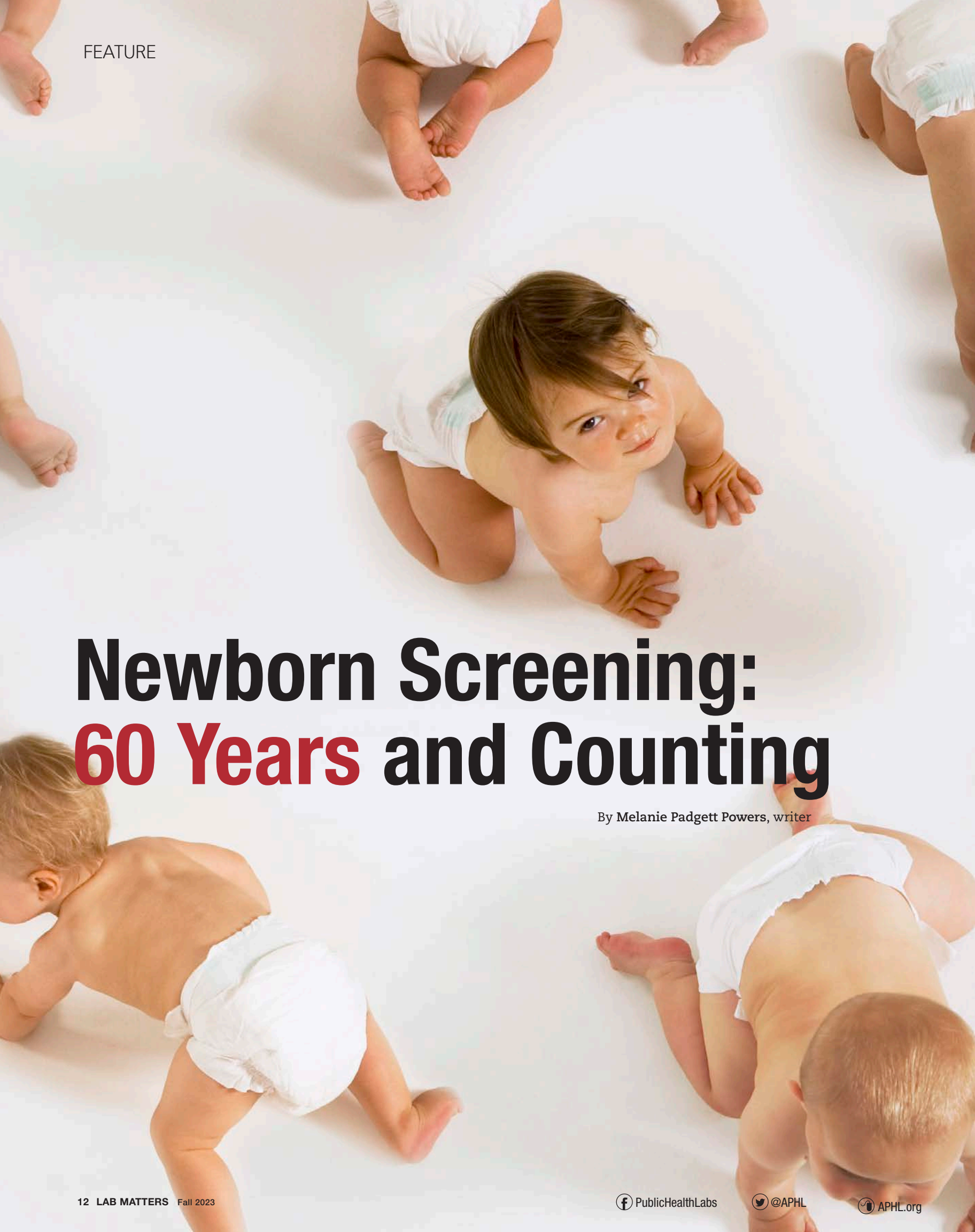
After the changes wrought by the COVID-19 pandemic, APHL leadership began seeking member input about putting APHL’s values into words. As a result, APHL announced four core values that would be added to its membership statements and serve as touchpoints for APHL members going forward.

Community: We actively foster meaningful connections and belonging among members and partners to promote collaboration, growth and diversity of thought that maximizes our contributions to advance public health.

Diversity, Equity, Inclusion and Accessibility: We embrace, promote and model a culture of diversity, equity, inclusion and accessibility and recognize it as essential to the work we perform and the communities we serve.

Innovation: We meet the diverse and ever-changing public health landscape through forward-thinking and adaptive leadership.

Scientific and Professional Integrity: We are a trusted leader in public health, striving for continuous improvement and the highest level of ethics and honesty in laboratory science and practice.



FEATURE

Newborn Screening: **60 Years** and Counting

By Melanie Padgett Powers, writer



Four-year-old Fitz Kettler pulls his stepstool up to the kitchen counter, eager to help his mom stir the cupcake batter.

“He really likes to help with the baking because he knows there will be a blueberry muffin or a cupcake that he can eat afterward,” mom Christina Kettler said.

One day she came into the kitchen to find her sweet boy using his cutting board and toddler-safe knife to cut up watermelon into tiny cubes for his baby sister, Zoe. Fitz is a healthy and happy child, but these family scenes were not a given four years ago.

“I want to say it changed our life, but it’s actually the very first thing that gave Fitz his life. ... I’m just forever grateful to everyone involved over the decades to have newborn screening.”

Christina Kettler, mother of Fitz Kettler

When Fitz was born in June 2019 via donor egg in vitro fertilization (IVF), his newborn screening (NBS) results were indicative of an immune deficiency, which would make him highly vulnerable to infection and illness. He was placed in a sterile room at Rady Children’s Hospital in San Diego, and whole genome sequencing confirmed he had a form of severe combined immunodeficiency (SCID), a rare disease more commonly known as “bubble boy disease.” Specifically, Fitz had **Artemis SCID**, a severe form of primary immunodeficiency caused by mutations in the DCLRE1C gene. The Kettlers were told their baby likely wouldn’t live to see his first birthday.

Then Fitz was able to get into a gene therapy trial at University of California, San Francisco, that was accepting kids with Artemis SCID. His stem cells were harvested, and a healthy copy of the DCLRE1C gene was introduced. In the laboratory, his stem cells began making healthy new cells. At two months old, Fitz was given the healthy cells via an infusion into his port in just 15 minutes. It worked. Fitz is now a regular preschooler with a strong immune system. He’s been sick a few times, including with asymptomatic COVID-19, but he recovered like any healthy kid. He’s up to date on his vaccines, including live vaccines for chickenpox, COVID-19, and measles, mumps and rubella.

The Start of NBS

The United States NBS program—which has improved and saved countless lives—celebrates its 60th anniversary in 2023. All 50 US states, as well as Puerto Rico and Washington, DC, have NBS programs.

“Newborn screening has impacted hundreds of thousands of families in the developed world,” said Dianne Webster, PhD, vice president of the International Society for Newborn Screening. “It’s saving money and time and grief and giving people better lives. There’s absolutely no question about that.”

In the US, NBS starts in the hospital with a simple heel prick to gather a few

drops of blood when a baby is one to two days old. (A hearing test and heart screening are also conducted.) Public health laboratory professionals are typically the next step in this critical public health program, screening almost 4 million babies in the US each year to uncover rare but serious, treatable disorders.

“I want to say it changed our life,” Kettler said, “but it’s actually the very first thing that gave Fitz his life. ... I’m just forever grateful to everyone involved over the decades to have newborn screening.”

NBS was created in 1963, after Robert Guthrie, MD, PhD, developed a blood test to screen for **phenylketonuria** (PKU) in New York. His successful pilot studies led other states to begin screening newborns. By the mid-1960s, almost every state was screening for PKU. Screening soon followed for congenital hypothyroidism, other inborn errors of metabolism, and hemoglobinopathies, including sickle cell disease. Now, each US NBS program screens for at least 31 disorders, according to the **APHL Newborn Screening Technical assistance and Evaluation Program** (NewSTEPS). The federal **Recommended Uniform Screening Panel** (RUSP) recommends that all newborns be screened for 37 core disorders and 26 secondary disorders. APHL plays an important role in the nationwide program, hosting both **hands-on trainings in the laboratory and didactic webinars and conference sessions**. APHL hosts a Newborn Screening Symposium every fall, provides resources online, monitors state and federal legislation, and supports global initiatives to expand NBS.

Over the past 60 years, the technology behind NBS has advanced tremendously. Public health laboratory professionals and NBS experts point to several milestones that launched the program forward. But,

among all the successes, there have been challenges—many of which remain today.

NBS Leaps Forward

Bradford L. Therrell, MS, PhD, MD (hon), remembers screening for only PKU in the mid-1970s and keeping manual records. Therrell is director of the **US National Newborn Screening and Global Resource Center** and professor (ret.) at the University of Texas Health Science Center at San Antonio. He credits the invention of the punch index machine for improving NBS efficiency in the 1970s. He vividly remembers inventor Robert Phillips demonstrating his punch index machine at a conference in 1975. The machine could punch four holes—with the right size and number needed for consistent, quality testing—in the filter paper used in the screening test.

Before that, for each test, laboratory workers had “to do the same thing over and over and over with a simple hand punch, like what’s used to punch holes in notebook paper, so preparing samples for testing was very expensive and time consuming,” Therrell said. But the punch machine allowed “you to punch four samples, or multiples of four, for the same amount of energy that you needed to prepare a single sample,” he explained. “That was a very important accomplishment in newborn screening, and it really moved things along.”

As for the data itself, microcomputers became available at about the same time. It can be easy today to underestimate the massive advancement and



ubiquitousness of computers. Before the late-1970s, laboratories were forced to collect data by hand. Then, thanks to powerful microprocessors that shrunk the size of computers, microcomputers became available. Before that, computers were huge, expensive machines that few could afford.

“We were able to utilize microcomputerization, and computerization in general, to handle the data in the newborn screening laboratory program and to handle the follow-up information,” Therrell said. “So, we went from hand-recording all of this information to computer-recording it, and it changed everything, especially in the larger screening programs.”

The next leap forward came with the invention of tandem mass spectrometry for chemical analysis. “It was a major shift in the science and technology of newborn screening because it allowed a laboratory to screen for an entire group of disorders from a single specimen punch,” said Guisou Zarbalian, MS, MPH, manager of Newborn Screening and Genetics at APHL.

Tandem mass spectrometry “revolutionized” NBS, said Susan Berry, MD, professor of genetics and metabolism in the Department of Pediatrics at University of Minnesota. “That was a big, big change, and newborn screening went from many states doing five or six tests to most states, with time, doing ... in the 20s to 30s, or sometimes even more testing.”

After tandem mass spectrometry allowed for more efficient screening, there was a call for a uniform screening panel. “Prior to the initiation of the RUSP, it was a terrible Wild, Wild West,” Berry said. “Some states did lots of screening and some did little. The general sense of the Recommended Uniform Screening Panel was that we wanted to try to be as fair as we could for everybody so that all kids could be screened uniformly.”

In 2002, the federal [Health Resources and Services Administration’s \(HRSA\)](#) [Maternal and Child Health Bureau](#) asked the [American College of Medical Genetics \(ACMG\)](#) to develop guidelines for newborn screening. According to NIH, ACMG considered 81 conditions, ultimately

placing 29 of them in a core screening panel. This became the RUSP. In 2003, the [Advisory Committee on Heritable Disorders in Newborns and Children](#) was formed to advise the secretary of the US Department of Health and Human Services (HHS). In 2005, that committee recommended a panel of conditions to the HHS secretary as the nation’s RUSP. It was adopted in May 2010 with the addition of SCID, according to NIH.

Ongoing NBS Challenges

Diseases and conditions are added to the RUSP based on evidence that supports the potential net benefit of screening, the ability of states to screen for the disorder and the availability of effective treatments, according to HRSA. That third part—the availability of effective treatments—has become one of the challenges of NBS, leading to passionate arguments and both philosophical and real-world debates among parents, disease advocacy groups, researchers and public health professionals.

Berry points to the example of Krabbe disease, a rare genetic condition with no direct treatment and no cure. Babies diagnosed with Krabbe disease progressively get worse and often die before age 2. However, some states have included Krabbe disease as part of their NBS panel. Usually, the argument for adding it to a panel, Berry said, is that you can do a bone marrow transplant, if you start that transplant very early in life.

“The problem is it’s very difficult to accomplish that,” she said. “And there’s very poor evidence that it really makes a big difference other than prolonging life. So, it’s very, very controversial among the newborn screening community.”

At its February 2023 meeting, the Advisory Committee on Heritable Disorders in Newborns and Children decided not to recommend adding Krabbe disease to the RUSP. It was a tie vote, and the committee requested

a scientific review of the evidence and more discussion with the nominating group, Hunter’s Hope Foundation, which aims to support research and increase awareness of Krabbe disease and related leukodystrophies.

As a part of these debates, Berry said the community needs to ask, and think back, to the purpose of NBS. Is the purpose to detect all disorders in children? “That’s very different than the original objective of newborn screening, which was to do early detection so you could change outcomes because of early treatment,” she said.

Berry said some argue that NBS is the best—and perhaps only time—to find out this genetic information so we should screen for more diseases, which can help the scientific community learn more about these rare diseases. Others worry that that will dilute the original purpose of NBS, which could make parents reluctant to screen their baby.

“There’s a lot of philosophic debate about where NBS should go, and there are reasonable arguments on both sides,” Berry said.

How different programs count tests also invites reflection and scrutiny. When screening for hemoglobinopathies, for example, one state might count hemoglobinopathies as a single test, while another might count possible detection of each individual hemoglobin variant as a test, which leads to a



“The newborn screening community must be vigilant in continuing to educate providers and new parents about the value of this important public health screening program. This education includes challenging discussions about newborn screening research that incorporates ethical principles while remaining equitable and representative of the entire state’s population.”

Richard Olney, MD, MPH, Genetic Disease Screening Program division chief for the California Department of Public Health

larger number depending on how the analysis is run. A third state might count hemoglobinopathies as three tests because the RUSP lists three hemoglobin conditions or the state determines there to be only three clinically significant sickle hemoglobinopathies, Therrell explained.

The lack of standardization among how the states count the tests in their panel affects public perception, he said. States already have discrepancies in the number of actual tests included in their panels, but the counting issue confuses the matter even more. Both the general public and legislators “perceive that because you’ve got a larger number out there, you’re doing better than everybody else,” he said. An APHL task force is studying this issue.

A Look at Outcomes and Privacy

A gap in the NBS system is the lack of data about long-term outcomes, Berry said. In short, does NBS work? “We know bits and pieces of it, and we know a lot of anecdotes, but no one has ever gone back for almost any disorder ... to study long-term outcomes.”

Her state of Minnesota has been using tandem mass spectrometry since 2001, which means there are young adults who were diagnosed with a rare genetic disease because of NBS. “No one’s systematically going back to those adults and saying, ‘how you doing?’ I think that’s a shame. The promise of newborn screening was that we’d have improved outcomes and people would live better lives, and we honestly don’t know if they do.”

A large ongoing discussion is about privacy. Some states store dried blood spots for years, using them for quality control, research and implementation of new screening tests. However, lawsuits have challenged the storage and use of dried blood spots, primarily because DNA can be extracted from them. In one New Jersey case, law enforcement used an

infant’s dried blood spots to identify a criminal suspect related to that child. In a Michigan lawsuit, lawyers argued that the existing consent form—which is required from parents before their baby’s blood can be used for research—was vague and that parents were not sure what they actually signed.

“There’s a tug of war between advocates who have interest in a specific disorder and advocates who are pushing for genetic privacy and have concerns about security of data and sometimes even how newborn screening is conducted,” Zarbalian said. “Some families feel really uncomfortable with the state obtaining a child’s blood and storing it and using it for different processes that are necessary to operate a newborn screening program.”

Furthermore, there has been increased public scrutiny of public health programs in recent years. “Building the public’s trust and support for newborn screening is key to the success of the program,” said Richard Olney, MD, MPH, Genetic Disease Screening Program division chief for the **California Department of Public Health**. “The newborn screening community must be vigilant in continuing to educate providers and new parents about the value of this important public health screening program. This education includes challenging discussions about newborn screening research that incorporates ethical principles while remaining equitable and representative of the entire state’s population.”

Improving Education

Education of NBS in general is an ongoing issue. Do parents really know what the heel prick is for and what the results mean?

Although most parents won’t think twice about it, for those 12,000 families who get a phone call informing them their baby needs follow-up testing, it can be a confusing process. Parents who took part in the Parent/Patient Panel during APHL’s 2021 Newborn Screening Symposium said it didn’t help that it was often a stranger calling them, not a physician they had an existing relationship with.

Natalie and Eric Lamb received a phone call in September 2020 informing them their daughter, Etta, tested positive for spinal muscular atrophy. The Lambs are both nurses, but they didn’t know the neuromuscular doctor on the phone and didn’t understand what she was talking about. “She basically just started spitting a bunch of genetic information at us, and all we heard was complete gibberish,” Eric said during the conference session.

Other families shared similar stories: When Diane and John Pytel received a call in 2006 that their newborn, Mia, had PKU, John thought it was the “state” calling. He assumed it was a Pennsylvania bureaucratic mistake because it was just some stranger on the phone. The parents on the panel all agreed that better education is needed, perhaps during pre-pregnancy planning—and it shouldn’t be just a pamphlet handed to them by a nurse. Because of lack of





“ [Newborn screening is] still one of the most important things we’ve ever done. It’s a little-recognized, tremendous public health advance. ... I just hope that by continuing to help people understand it better, they’ll realize how much public health has done to improve the lives of children through newborn screening.”

Susan Berry, MD, professor of genetics and metabolism in the Department of Pediatrics at University of Minnesota

proper communication and education about the importance of NBS, the parents said they’ve heard of resistance from other parents. Reasons given include thinking the government is going to have their baby’s DNA stored in a database, that there are no genetic diseases in their family so it’s unnecessary, and for home birth families, that it’ll disrupt their bonding and they’ll “do it later.”

“Many parents are not aware that their newborn is going to get screened shortly after birth,” Olney said. “Ideally, parents should be informed about newborn screening while they are pregnant, and getting this message out through the cooperation of prenatal care providers is an ongoing challenge.”

Incorporating Next-generation Sequencing

An area that fits into both the success and challenge columns is next-generation sequencing, which includes whole exome sequencing and whole genome sequencing (WGS). Laboratories now have the ability to sequence large amounts of a person’s DNA in a much shorter time, although it is not a routine part of NBS. WGS can be a life-saver when used to discover the cause of a child’s severe illness. But should it be done routinely on seemingly healthy babies? And what to do with that information?

“You’re going to find a lot of stuff you can’t do anything about if you do that,” Berry said. “Is that right, to be done as part of newborn screening? That’s a big challenge and a big area of controversy.”

A genome sequence is a lot of data and it’s “still pretty mysterious,” Berry said. “Even when we get a gene sequence back using just the coding sequence for the proteins, we don’t always know if the genetic error that we see is something that causes a problem, that is it’s pathogenic, or if it’s just something that’s a variation from person to person that is benign.”

Webster quoted her colleague, who said: “We need to define what we mean by early detection.” As Webster explains, “There are patients who presented in adulthood who think it would be good if they’d have known about their disease earlier. But if you were to ask a number of families if they want to know if their newborn baby was going to develop late-onset Pompe disease when they were in their 30s or 40s, the family may not want to know.” Furthermore, one must consider the autonomy of the child—should they be told about a disease early in their childhood that may develop when they are an adult?

Like her older brother, Zoe Kettler, now 16 months old, was born via donor egg

IVF. She was the Kettler’s “Hail Mary baby” as their last remaining embryo to transfer. The couple knew Zoe would be an Artemis SCID carrier but not have the disease. Nevertheless, the Kettlers nervously awaited the NBS results after Zoe was born. “It was a much different experience waiting for that newborn screening test than the first time when I didn’t think there was anything to be concerned about,” Christina Kettler said.

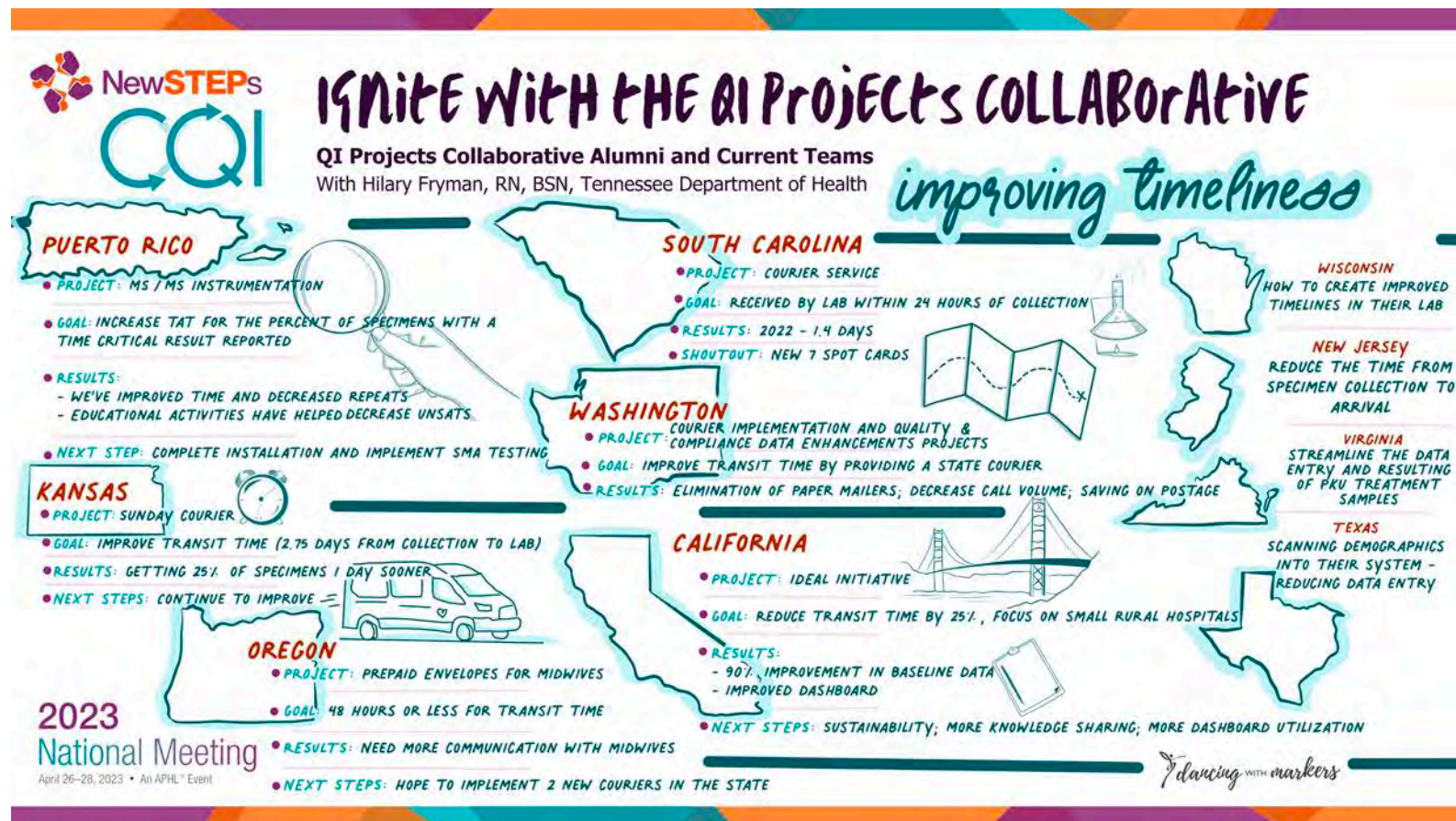
She choked up as she reflected back on that day: “I remember when the doctor came in and told me that it was fine, it was negative. I was crying and I felt so relieved.”

Despite the challenges, complexities and ongoing questions, newborn screening is one of the leading public health success stories.

“It’s still one of the most important things we’ve ever done. It’s a little-recognized, tremendous public health advance,” Berry said. “I just hope that by continuing to help people understand it better, they’ll realize how much public health has done to improve the lives of children through newborn screening.” ■

Transforming Newborn Screening through Continuous Quality Improvement

By Ashley Comer, senior specialist, Newborn Screening & Genetics



Illustrations of state QI project that were generated at the NewSTEPs CQI National Meeting.

Newborn screening (NBS) has been a cornerstone of public health for decades, ensuring early detection and intervention for infants with potential health conditions. As advancements continue to shape the field of NBS, the need for continuous quality improvement (CQI) becomes increasingly important. The APHL Newborn Screening Technical assistance and Evaluation Program (NewSTEPs) led a successful CQI initiative, empowering NBS programs throughout the United States and Puerto Rico to enhance their processes and ultimately improve outcomes for newborns.

60 Years of Newborn Screening

This year marks 60 years of newborn screening. What began as one screen for Phenylketonuria (PKU), now has evolved to include screenings for

an expanding panel of conditions, advanced technologies and complex systems. However, with growth and innovation comes the need for continual improvement. Recognizing this, NewSTEPs embarked on a journey to foster CQI culture within NBS programs.

Newborn Screening Quality Improvement Projects Collaborative

Thanks to funding from the Health Resources and Services Administration (HRSA), NewSTEPs initiated a CQI program that supported the Quality Improvement (QI) Projects Collaborative. Starting in 2019, this collaborative brought together 34 NBS QI project teams to participate in several CQI training opportunities over a five-year period. Each team identified individual project

aims and metrics and applied CQI methods. Through webinars, discussion groups, coaching calls and national NewSTEPs CQI meetings, the QI Projects Collaborative encouraged NBS QI project teams to enhance their understanding and application of CQI science. By adopting CQI methodologies, these teams were better equipped to identify and address areas for improvement within their NBS processes and systems.

Plan, Do, Study, Act

The application of CQI led to impressive improvements. NBS programs participating in the QI Projects Collaborative reported enhanced efficiencies, reduced turnaround times and increased accuracy in screening test results. During the 2023 NewSTEPs CQI National Meeting, the QI Projects

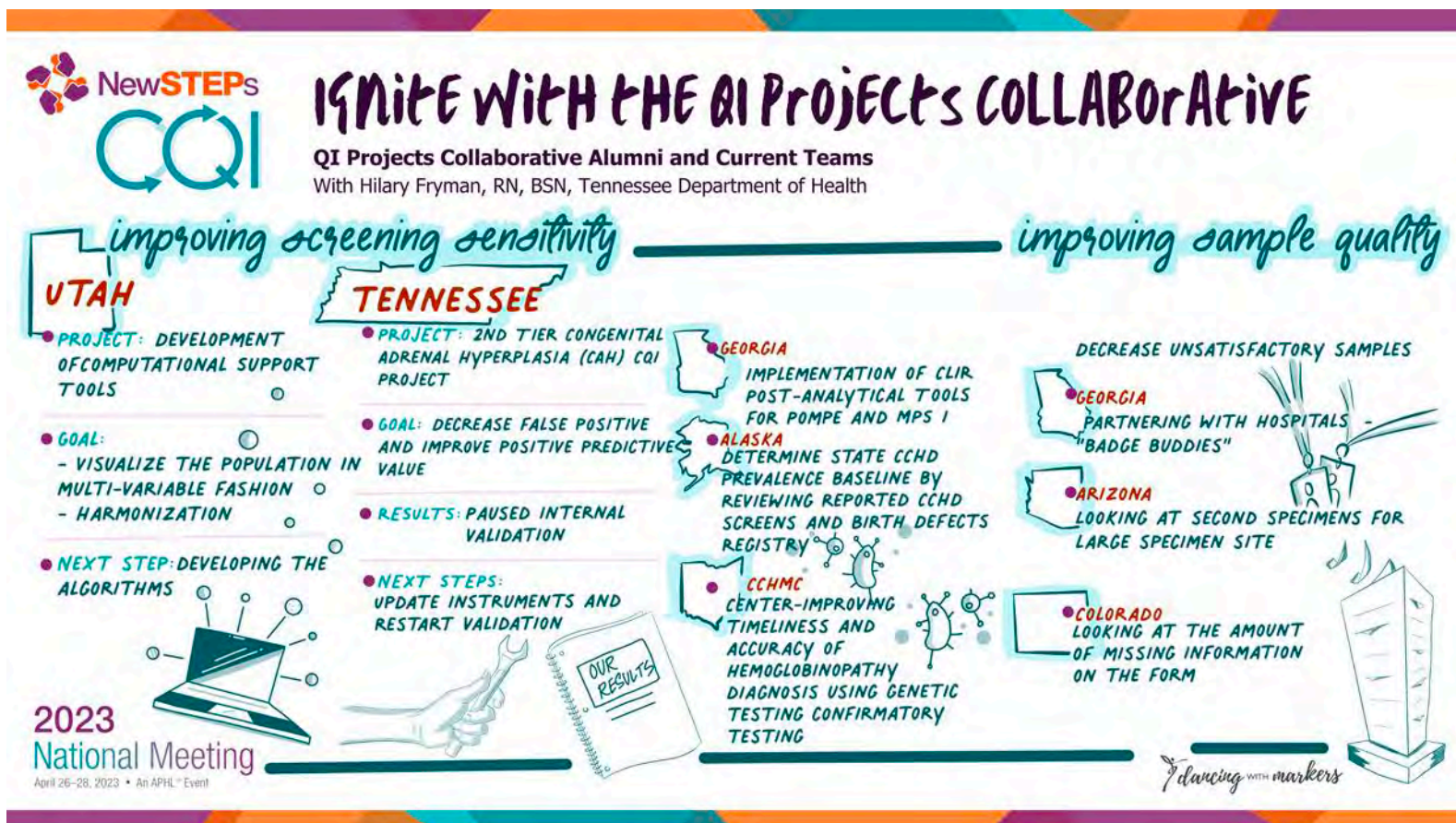


Illustration of state QI projects that were generated at the NewSTEPs CQI National Meeting.

Collaborative teams engaged in ignite-style presentations that were visually captured by a live graphic note-taker to share the improvements. Through the NewSTEPs QI Projects Collaborative, participating NBS programs were able to identify and address gaps in their screening processes and share this experience across programs. Moreover, the collaboration between NBS programs fostered the sharing of practices and lessons learned, further enhancing the overall quality of newborn screening.

The success of this CQI initiative has demonstrated the power of collaboration, knowledge enhancement and data-driven decision making. By embracing CQI principles and methodologies, NBS programs can elevate their screening processes, strengthen their systems, and ultimately improve outcomes for newborns. As NBS continues to evolve, the importance of CQI cannot be overstated. Through ongoing commitment to continuous improvement, we can ensure a brighter, healthier future for all newborns. ■



Neonatal Abstinence Syndrome: To Screen or Not to Screen

By Kelsey Granger, MHS, specialist, Environmental Health

As public health continues to combat the opioid overdose epidemic, the impact on vulnerable populations must be considered. While Neonatal Abstinence Syndrome (NAS) symptoms are non-specific, they are caused by *in utero* exposure to opioids or other substances known to result in withdrawal. According to 2020 data from the Healthcare Cost and Utilization Project (HCUP) managed by the US Agency for Healthcare Research and Quality (AHRQ), one newborn with NAS is diagnosed every 24 minutes—about 59 diagnoses a day. While NAS impacts neonates across all demographic groups, a 2021 HCUP report found that neonates with NAS are significantly more likely to be non-Hispanic White, Medicaid-billed, residing in ZIP codes in the lowest quartile of median income and living in nonmetropolitan counties.

Raising the Alarm

The estimated rate of NAS increased 82% between 2010 and 2017. In response to these shocking data, and to address the role of laboratory data in NAS surveillance, APHL's Overdose Biosurveillance Task Force (OBTF) convened a panel of experts across related disciplines including neonatology, obstetrics and gynecology, analytical chemistry, epidemiology and clinical and public health laboratory science. Lengthy debate among the experts

around universal screening and the use of newborn screening dried blood spots (NBS DBS) in NAS surveillance resulted in a **2022 report** which concluded that laboratory data has the potential to enhance NAS surveillance and provided the following recommendations:

1. Laboratory testing is recommended for symptomatic neonates, not for screening of all newborns.
2. Residual newborn dried blood spot specimen collected for the detection of diseases on the Recommended Uniform Screening Panel should not be used for NAS surveillance.
3. Population-based research studies are recommended to determine the prevalence of *in utero* opioid exposure.

To track cases more easily, universal screening for NAS utilizing existing screening systems may seem appealing but ultimately may not be suitable. Immunoassay screening methods are accessible and possess rapid turnaround time that can quickly inform treatment decisions but come with challenges: only a recent drug exposure is detectable, sporadic drug use cannot be reliably detected, and this method is prone to false positive and false negative results, which can lead to inaccurate inflation

or underestimation of NAS cases. As the opioid epidemic evolves, novel substances including fentanyl analogs have emerged, and may not be detectable using immunoassay. For definitive (confirmatory) results, toxicology testing using HPLC-MS/MS or HRMS is indicated.

Finding a Way Forward

NBS DBS tested according to the **Recommended Uniform Screening Panel (RUSP)** are a common feature of newborn screening programs across the US and may also appear an appealing specimen for universal NAS screening. However, APHL does not recommend the use of NBS DBS collected for the detection of diseases on the RUSP for NAS surveillance. Neonates present withdrawal symptoms 24–48 hours after birth, while conditions included in the RUSP otherwise go unrecognized without laboratory testing. Results from toxicology testing of dried blood spots may not be available for 5–7 days after birth and would not inform treatment in a timely manner. Inclusion of drug screening in NBS DBS could pose challenges to obtaining informed consent for critical NBS testing or could even deter birthing individuals who use drugs from choosing to give birth in a hospital.

While NAS occurs across racial groups, it is disproportionately concentrated among at-risk low-income, rural populations. Downstream impacts of universal screening methods are not without consequence and, if instituted without proper precaution or protection of the individual, can significantly negatively impact birthing individual and infant bonding through involvement of child protective services and subsequent legal challenges that can extend to future births without indication of drug use. In some states, these impacts can result in criminal prosecution of the birthing individual. The OBTF recommends that testing of symptomatic neonates be prioritized to best utilize laboratory and public health resources and minimize potential social and ethical implications of neonatal drug testing. ■



Neonatal abstinence syndrome is characterized by a combination of variable symptoms, including convulsions, tight muscle tone, fussiness, excessive crying, poor feeding, slow weight gain, breathing problems, fever, sweating and blotchy skin.

CALL FOR ARTICLES:

From The Bench

a regular feature of *Lab Matters*



Lab Matters, APHL's flagship publication, is seeking submissions from laboratorians at all levels of practice for "From the Bench," a member-driven section of its quarterly magazine.

We welcome both technical and non-technical articles covering topics across public health laboratory science, administration, careers and management. Articles may be 600-1200 words in length.

We're looking for a few key components in "From the Bench" articles –

- 1. A compelling story.** Tell us about an initiative that streamlined an administrative process or fostered relationships with external communities. Describe how your lab has worked with other public health partners to stop an outbreak or respond to an environmental health threat. Or tell us how you dealt with an initiative that failed.
- 2. A complete story.** Give us an article with a beginning, middle and end. The end should provide some resolution of the narrative. You don't need to have fully implemented the new algorithm, analyzed all the research data or completed the electronic laboratory reporting system discussed in your article.
- 3. An insider's view.** We want articles told from the unique perspective of a laboratory scientist that will be interesting and informative for APHL members and partners.

**Share your
story with
peers!**

We can help!

We will work with you to write the article, from connecting you to a writing consultant to providing editing assistance if you wish to write the article yourself.

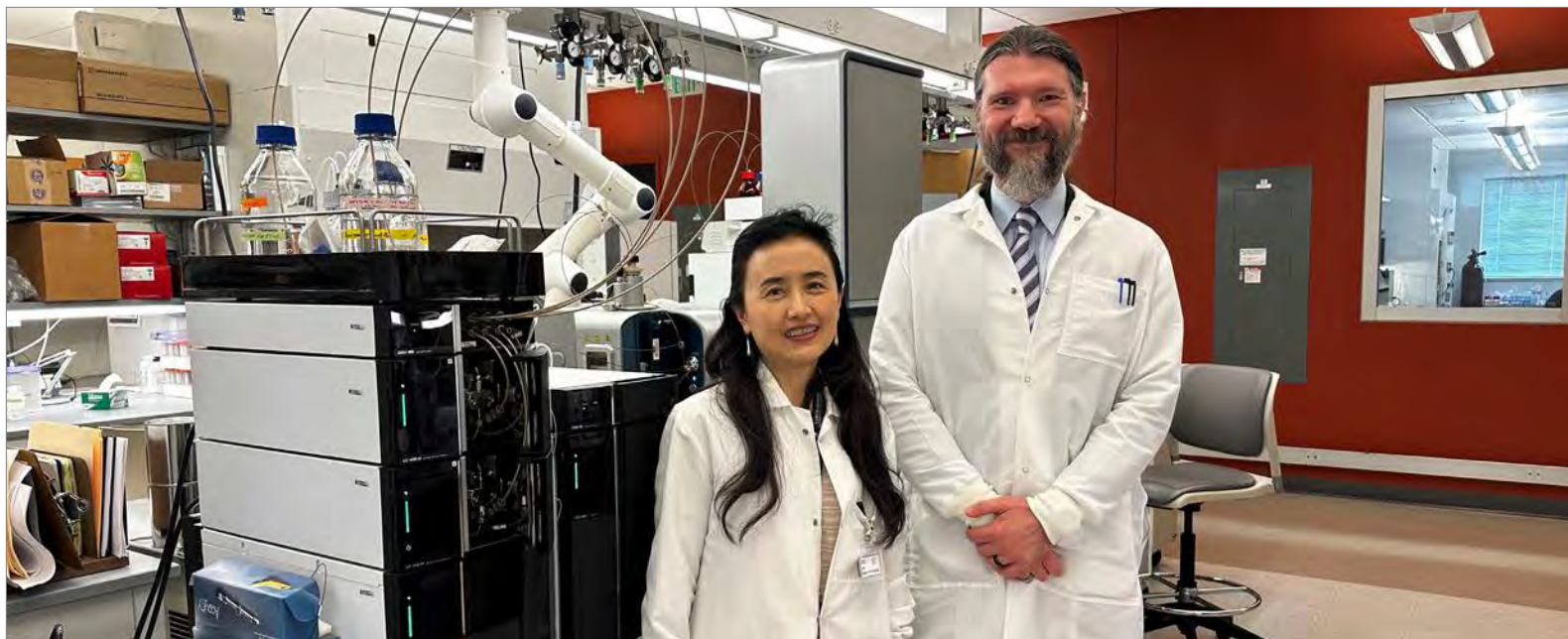
If you have **questions** or would like to **submit a story idea**, please contact:

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A \$50 Billion Reason to Examine US EPA's State Revolving Funds as a Laboratory Resource

By Erin Morin, MHS, specialist, Environmental Health



Maryland's public health laboratory was approved \$1.2 million to purchase a high-sensitivity mass spectrometer to quantify PFAS at parts-per-quadrillion levels, and a high-resolution mass spectrometer for unknown contaminant identification. As a Safe Drinking Water Act principal state laboratory, they also have secured a Memorandum of Understanding with their state program that will provide \$300,000 per year from the DWSRF for safe drinking water compliance testing. From left to right: Zhao Cao, who developed most of Maryland's PFAS and non-targeted methods, and Dr. Sinisa Urban, chief of environmental sciences, who directs the projects and obtained the funding. Photo: Blair Knouse

Signed in November 2021, the Bipartisan Infrastructure Law provides a \$50 billion public health investment over the course of four years to strengthen the nation's water systems. Much of this funding will be available to states through the [US Environmental Protection Agency's \(US EPA's\)](#) state revolving fund (SRF) programs. Depending upon your state's eligibility, SRF programs may be an untapped funding resource for your laboratory for a variety of uses to support water quality testing.

US EPA's Drinking Water and Clean Water State Revolving Funds

US EPA's SRFs two sister funds, the [Clean Water State Revolving Fund \(CWSRF\)](#) and the [Drinking Water State Revolving Fund \(DWSRF\)](#) have operated since 1987 and 1996, respectively. SRFs are considered partnerships between US EPA and all 50 states and Puerto Rico, operating like banks by providing subsidized loans for water quality and public health projects from congressionally appropriated funds.

CWSRF eligibility is based on project type and primarily funds wastewater infrastructure and water quality projects whereas DWSRF eligibility is limited to public- or privately owned community water systems or non-profit non-community water systems (e.g., schools). Set-aside funds from this loan may be used to support laboratories.

Bipartisan Infrastructure Law

The Bipartisan Infrastructure Law has [considerably increased CWSRF and DWSRF dollar amounts](#), creating new general supplement and emerging contaminants funds for each SRF. The emerging contaminant funds, in particular, represent an unprecedented opportunity for laboratories to apply for instrumentation funding that will help them address contaminants that pose a new or re-emerging risk to public health and are known or expected in the environment. CWSRF and DWSRF emerging contaminants projects can focus on per- and polyfluoroalkyl substances (PFAS), or contaminants

that do not have Clean Water Act water quality criteria or are listed on [US EPA's Contaminant Candidate List \(CCL\)](#), respectively.

Emerging Contaminants in Small or Disadvantaged Communities Grant

Separate from SRFs, an additional \$5 billion of Infrastructure Law funding will be awarded through the [Emerging Contaminants in Small or Disadvantaged Communities grant](#). States are to use this funding to make grants to eligible emerging contaminant projects and/or activities in small or disadvantaged communities. Laboratories should collaborate with their environmental health partners to provide the needed data for these emerging contaminants projects. Eligible projects are outlined on the webpage and must also focus on PFAS or other contaminants listed on the CCL. The webpage and [implementation guide](#), specifically Appendix D, are good resources when writing the proposal. ■

Applying for Your State's SRF

The [Council of Infrastructure Financing Authorities](#), a non-profit that represents the state revolving funds, recommends the following steps when applying for your state's SRF:

1. Check your state's [SRF funding status](#), read your state's [intended use plan](#), and determine the contaminant(s) of emerging concern on which your state is focusing.
2. Determine if your laboratory might be able to get smaller amounts of sustained funding through the DWSRF program management, local assistance and/or SRF administration set-asides. A handful of laboratories have been able to establish annual funds through these set-asides from 1–60% of the set-asides, or for specific dollar amounts of \$50,000–300,000. Working with their state drinking water programs that receive DWSRF dollars, state laboratories have dedicated these funds to special projects, equipment, supplies, staff salary and other laboratory needs.
3. Reach out to the [clean water](#) and [drinking water](#) SRF managers in your state to determine how to apply and what your laboratory may be eligible for.
4. Determine the application's deadlines and requirements—it differs among states! You should be able to apply for this funding through 2026. Technically, you should be able to submit multiple projects in a funding cycle. Your SRF might also need to develop a pipeline of projects to fund in the future to ensure they spend the funds.
5. Take advantage of the current CWSRF pipeline for laboratory equipment—especially if it is dedicated to [emerging contaminants testing](#) (e.g., an instrument needed to bring on a new PFAS method). It's possible the CWSRF might be able to transfer funds over to the drinking water SRF.
6. Don't get discouraged if your state has never funded a laboratory project! Be clear about the need to protect your jurisdiction's public health. Be clear about the need that laboratory testing meets to protect your jurisdiction's public health. Please contact sarah.wright@aphl.org if you need assistance with any of this.

MEMBER EXCLUSIVE

The Public Health Pricing List

The Public Health Pricing List is a *members-only* listing of discounts and special offers that APHL negotiates with corporate partners.

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For more information about the public health pricing list discount offerings, please contact Camille Walker, Manager, Corporate Relations, camille.walker@aphl.org.

Strengthening and Enhancing Foodborne Outbreak Response Capabilities

By Kirsten Larson, manager, Food Safety

Food safety is an ongoing priority for public health that requires collaboration across multiple disciplines and all levels of government. Developed by APHL, the [Council to Improve Foodborne Outbreak Response \(CIFOR\) Food Safety Programs Reference Guide](#) provides a snapshot of this breadth in governmental food safety efforts. CIFOR has recently introduced some new tools and resources to assist public health laboratories in their efforts to detect and respond to foodborne outbreaks (FBOs) within their own jurisdictions.

Addressing FBO Collaboration Needs

Given the range of programs and activities outlined in the Reference Guide, it is important to regularly review goals and objectives to maximize relevance and efficacy. CIFOR recently updated its strategic plan to better align with current food safety efforts. Several APHL staff and member representatives from APHL's Foodborne Diseases Committee provided a critical laboratory voice in the strategic planning process and helped ensure that public health laboratory challenges are forefront in the new plan.

The CIFOR Council approved six new strategic goals along with corresponding objectives intended to address ongoing FBO detection and response challenges. Work groups comprised of CIFOR members and discipline-specific experts will be convened in the latter half of 2023 to begin work on strategic goals and objectives.

APHL members interested in participating in any of the work groups should contact Kirsten Larson at kirsten.larson@aphl.org.

Resources to Address FBO Challenges

As CIFOR looks to the future, it has continued to develop and update tools and products to evaluate program performance, enhance collaboration among stakeholders, improve outbreak surveillance and provide model practices in FBO investigations. Most CIFOR tools and resources are designed with public health laboratories in mind.

- CIFOR Guidelines Learning Modules – The CIFOR Learning Modules provide summaries of the [Third Edition CIFOR Guidelines](#), a comprehensive resource for local, state and federal agencies involved in FBO investigation and control. The modules cover topics such as legal preparedness, planning and preparation, surveillance and outbreak detection, control measures and multi-jurisdictional outbreaks. After completing these modules, the goal is for learners to seek additional information in the CIFOR Guidelines and partner with other professionals to apply these key points in practice. The modules are available on [APHL's Learning Center](#), and continuing education credits are available upon completion of the modules.
- Third Edition CIFOR Toolkit – The [Third Edition CIFOR Toolkit](#) serves as a companion piece to the CIFOR Guidelines and provides hands on tools to help agencies identify which recommendations work best within their jurisdictions. APHL recommends that PHLs meet with epidemiologists and environmental

CIFOR Strategic Goals

Founded in 2006, CIFOR is a multidisciplinary collaboration among local, state and federal agencies, and multiple public health associations that develops guidelines, tools and products to facilitate effective responses to foodborne outbreaks (FBOs).

Goal 1: Increase the capacity of public health systems to identify and eliminate contributing factors and environmental antecedents associated with foodborne disease outbreaks.

Goal 2: Increase communication and collaboration among the network of public health professionals responding to foodborne disease outbreaks.

Goal 3: Improve the ability of public health professionals who investigate foodborne disease outbreaks to implement effective recruitment and retention strategies.

Goal 4: Identify and promote strategies to reduce the time from specimen collection to completion of whole genome sequence analysis.

Goal 5: Identify and promote strategies to collect, integrate, analyze and act on data more rapidly and completely during foodborne disease outbreaks.

Goal 6: Develop and promote model practices and other tools using a cross-organizational, multidisciplinary approach to improve foodborne outbreak investigation.

health staff within their respective jurisdictions to work through sections of the toolkit. A coordinated, multidisciplinary approach ensures that improvements are made across the whole food safety system.

Public health laboratories are encouraged to utilize CIFOR tools and resources to assess, strengthen and enhance their FBO response capabilities, especially in collaboration with cross-disciplined professionals working within their jurisdiction.

For more information on APHL's Food Safety program, please contact foodsafety@aphl.org. ■

APHL Flies South for New Opportunities

By **Matthew McCarroll**, lead specialist, Global Health, **Reshma Kakkar**, informatics manager, Global Health and **Noah Hull**, technical manager, Global Health



APHL CEO Scott Becker presents at the CDC CFA conference in Chile

In Spring 2023, APHL received additional funding from the **US Centers for Disease Control and Prevention (CDC)** for the **Global Public Health Data Innovation (GPHDI) program**, the global version of the Data Modernization Initiative (DMI). The requirement was to expand the countries APHL works in for GPHDI from four to eight. Three of these countries are in the Central and South America region—Honduras, Colombia and Paraguay. At the same time, CDC's newly formed Center for Forecasting and Outbreak Analytics (CFA) and the CDC South America (SAM) regional office initiated a conference in April 2023 to begin work in the South America region, and invited APHL as a key partner.

Data Analytics and Forecasting for Public Health

The conference provided a platform for public health officials in South America and the United States to share their experiences performing data collection, analytics and utilization of these data for forecasting outbreaks and events of public health significance. Speakers included representatives from CDC (US and in-country offices), the **Pan American Health Organization (PAHO)**, country Ministry of Health officials and academic and non-profit partners. Four APHL staff traveled to Chile to attend this forum and

share innovative laboratory data solutions that have been developed within the US and globally.

Laboratory data play a critical role in global disease forecasting and analysis. Data are needed by multiple decision makers both internal and external to the Ministry of Health, including public health agency staff, epidemiologists and healthcare providers. For laboratories to share data effectively, they need the capability to electronically exchange data with systems and stakeholders. When laboratory data can be quickly transferred from the laboratory to the decision makers then outbreak mitigation efforts have an improved impact on the severity of the outbreak. During his presentation on the role of the public health laboratory, APHL CEO Scott Becker issued a call to action to *include* the laboratory, *inform* the public health decision makers, *invest* in data modernization and to do this using *innovative* solutions.

Looking Ahead

APHL has begun efforts to strengthen laboratory capacity throughout the South American region. The association recently completed a training on metagenomics in Paraguay. APHL is also working with the Paraguay Division General of Surveillance to develop a solution for

capturing laboratory data from public hospitals and reference laboratories into a national laboratory data warehouse to enable electronic laboratory reporting with surveillance systems. This work is being completed using proof-of-concept solutions that have proven successful in countries within the Africa region supported by APHL.

In Colombia, APHL performed an evaluation on their bioinformatic and information systems. A digital roadmap is being developed that includes bioinformatic training of trainers, data standardization, centralization and visualization. Additionally, APHL is working with the CDC SAM office to develop a country mentorship program for the region that promotes laboratory accreditation and certification. ■

The Role of Laboratory Leadership and Governance in Quality Management System Sustainability

By **Jully Okonji**, senior laboratory specialist, APHL Kenya, **Edwin Ochieng**, country director, APHL Kenya and **Reshma Kakkar**, informatics manager, Global Health

Leadership and governance are emerging concepts in healthcare; however, there is limited consensus on the definition, measure and implementation approaches within laboratories. The **World Health Organization (WHO)** defines leadership and governance in healthcare as setting priorities for the maintenance and improvement of the population's health, assessing progress toward the attainment of those priorities, and ensuring that all relevant actors are held properly to account for their actions. These priorities are necessary to ensure sustainable development in healthcare. Applying this definition to laboratories is key for ensuring quality systems and building sustainable laboratory capacity. Laboratory leaders must clearly understand their roles, responsibilities and the expectations of the people they serve.

APHL Kenya partnered with two public health laboratories in Kenya to demonstrate these concepts of leadership and governance. The two laboratories had exhibited no improvement in their quality management systems (QMS) for several years. Donors were wary of investing resources due to lack of progress, poor commitment from laboratory leadership, and absence of cooperation between the laboratory and the hospital with which it was associated.

APHL Kenya adopted a detailed and carefully considered approach, which targeted county (equivalent to a state) laboratory leadership, hospital management and facility-level management that emphasized:

- Laboratory managers understanding the expectations of their roles.
- Building a strong laboratory team through effective communication.
- Developing solid communication strategies.
- Identifying strengths, weaknesses, opportunities and threats.

The strategy involved recognizing challenges and successes at the outset and determining solutions collectively. The county laboratory leaders, with APHL's assistance, reached a consensus on a standard approach to operations, which included: 1) a situational analysis to identify strengths and weaknesses of the laboratories, opportunities and

external threats; 2) provision of a work environment conducive to effective laboratory operations; and 3) establishment of a strong foundation guided by situational analysis to better understand the hurdles to sustainable development (Figure 1).

County laboratory leaders were guided by APHL to identify opportunities for sustainable development under three themes: human resources, financial resources and environmental/infrastructural resources (Figure 2). An overriding requirement was the allocation and commitment of resources to enable the laboratories to function optimally without cessation of essential services. County laboratory leaders also identified the possibility of collaboration between hospital and laboratory leaders to leverage existing capacity and improve the quality of laboratory services.

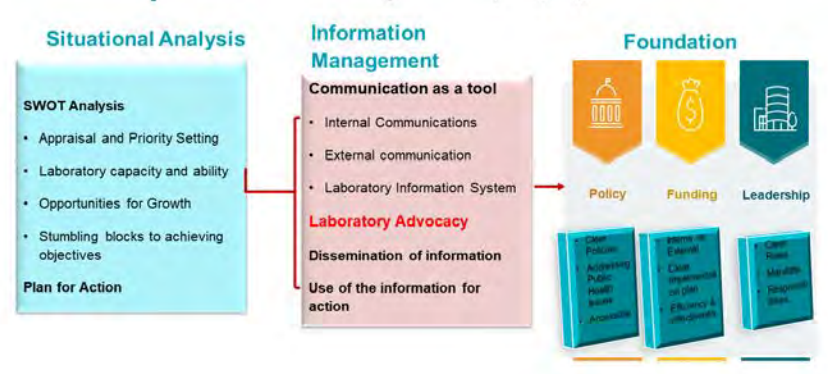


Figure 1. Situational analysis using participatory approaches.

Human Resource	Financial Resource	Environmental Resource
• Skills and Specialties • Acknowledge efforts and celebrate achievements • CMEs • Power of Delegation • Development of Cascade in leadership	• Internal/External • Review of test cost • Initiate a new test menu • Improve quality and efficiency • Laboratory leadership wisdom- Clinical/Lab interface	• Repair worn-out infrastructure i.e. lights, water, sinks, etc • Staff amenities • Space for equipment • Biosafety biosecurity

Figure 2. Sustainability opportunities were identified during the work sessions.

APHL worked with facility-level laboratory managers to outline priority needs that leveraged available laboratory capacity. These included:

- The role of a robust workforce.
- Timely reagent inventory management.
- Appropriate equipment management through selection and procurement of equipment based on needs and capacities needed.
- Employing control measures and checks using internal quality controls and enrollment in quality assurance programs.
- Ensuring both input and outcome processes were governed by accountability, audits, and determination of outcomes in management forums.

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The Next Generation Sequencing Quality Initiative: Creation of Tools to Improve Sequencing Quality Management

By Shannon Mahoney, MS, senior specialist, Advanced Molecular Detection

As sequencing platforms and capabilities have largely advanced over the past decade, public health laboratories have been faced with the hurdle of adapting quality management systems to meet the unique challenges for next generation sequencing (NGS) workflows.

The US Centers for Disease Control and Prevention's (CDC) Office of Advanced Molecular Detection (OAMD) funded APHL and its Division of Laboratory Systems to launch the **Next Generation Sequencing (NGS) Quality Initiative** in 2019. It endeavors to develop tools and resources to aid public health laboratories in creating a robust quality management system (QMS) in their sequencing and bioinformatics workflows. To ensure the initiative created a comprehensive set of resources for quality management, it utilized the **12 quality system essentials** (QSEs) established by the Clinical Laboratory Systems Institute (CLSI).

This year, the NGS Quality Initiative is celebrating a major milestone in its drive to supply tools and products focused at improving the quality of NGS workflows. With over 100 published products, it is celebrating the successful development of resources that address all 12 QSEs. Customizable, ready-to-use resources that encompass the entire sequencing workflow from the wet lab protocols through bioinformatics analysis and internal assessments are now available for all public health laboratories. These resources are created for a variety of different audiences, including bench laboratorians, bioinformaticians, quality managers and laboratory managers, since they are all involved in NGS quality management and have unique needs.

By providing tools and templates that can easily be redesigned and reimagined to fit the needs of individual laboratories, the QMS can be useful for laboratories just beginning sequencing as well as for those with well-established sequencing programs. Recognizing the diversity and expansiveness of laboratory's sequencing programs, the initiative also has resources for all of the major sequencing

EXAMPLES OF POPULAR PRODUCTS

QMS Assessment Tool: The interactive assessment tool serves as a guidance document to assist laboratories in establishing and curating their own internal quality management system with sequencing activities in mind.

Identifying and Monitoring NGS Key Performance Indicators SOP: This guidance document assists laboratories in identifying and applying quality metrics to maintain high-quality NGS testing.

NGS Method Validation Plan: This template document provides laboratories new to sequencing with the insight to generate their own method validation plan by providing near step-by-step guidance for identifying assay type, determining acceptance criteria, permitting sample requirements and more.

Bioinformatics Training SOP: This guidance document helps direct laboratories in the development and implementation of training courses using a "Tell, Show, Do, Apply" learning methodology, aimed at developing the skills and knowledge necessary to perform bioinformatic analysis.

instruments, with over **60 published instrument-specific documents**, ranging from pre-installation checklists to maintenance logs to instrument SOPs.

Of the over 100 products hosted on the QMS, two of the most downloaded products include the **QMS Assessment Tool** and **NGS Method Validation Plan**, which serve critical roles in allowing laboratories to both onboard new NGS platforms and assays, as well as ensure high quality testing is being maintained through the facilitation of their own internal QMS. The products provide users with a fundamental, yet comprehensive, process for building out sequencing operations through interactive, step-by-step templates.

As sequencing operations in public health laboratories grow alongside the NGS Quality Initiative, APHL looks to ensure that resources continue to evolve with the advent of new sequencing and

bioinformatics tools. With products focused on all 12 CLSI QSEs, laboratories can expect resources to support almost every facet of testing, regardless of their sequencing experience. The NGS Quality Initiative hopes to continue to develop and revise the resources to ensure their products stay relevant and up to date with the current sequencing workflows. Looking ahead, the Initiative hopes to continue to expand the development of its resources to best serve the public health community, including more customized tools to aid in clinical accreditation standards for sequencing and greater resources for quality management in bioinformatics analysis. If you have any questions about this work or suggestions for resources, please contact ngsquality@cdc.gov. ■

Self-testing and Self-collection: What's Next?

By Sarah Buss, PhD, D(ABMM), manager, HIV, Hepatitis, STI and TB



Self-collection and self-testing are not new concepts, as in-home self-monitoring of blood glucose has been the standard of care for patients since the 1980s. Although the OraQuick In-Home HIV Test was **US Food and Drug Administration (FDA)** approved in 2012, widespread home-based testing for infectious diseases was not commonplace in the United States until COVID-19 upended the world. The pandemic showed that in-home collection and testing models have the potential to increase access to and uptake of testing services, likely because of the convenience, privacy and anonymity afforded to patients.

When self-testing or self-collection programs are properly implemented, the resulting increase in testing could lead to earlier detection, linkage to care and, ultimately, reduced transmission and disease incidence. To address how best

to implement and address obstacles in this space, Megan Crumpler, PhD, director of the **Orange County Public Health Laboratory** and APHL president-elect, moderated a roundtable at APHL 2023 entitled, “Is Self-testing the Future or Will We Adhere to the Past?” This roundtable engaged members in discussing the utilization of different approaches and highlighted the benefits and challenges laboratories face as public demand for access to these models surges.

Finding the Best of Both Worlds

Jessica Zamora, MPH, from the National Coalition of STD Directors (NCSD), discussed NCSD’s **Check Yourself** initiative, a collaboration with **LetsGetChecked**. This model pairs self-collection with laboratory-based testing for several different sexually transmitted infections (STI) in home-collected urine and fingerstick whole blood specimens.

STI programs and health departments contract with NCSD to participate, and a nursing line is available for follow up and referral. Notification systems trigger alerts (e.g., no sample received within a week of sending kit) and reports are delivered electronically with automated reportable disease notifications sent to health departments. Zamora explained that Check Yourself uses prepaid shipping and temperature monitoring devices to help assure quality preanalytical processes. When asked about specimen return rates, Zamora cited NCSD data that demonstrated that specimen return rates were higher when collection kits were picked up at a clinic (~90%) compared to those picked up in the community or by mail (~30%).

A challenge that laboratories may be struggling with is how to ensure that a self-collected approach meets the regulatory requirements that laboratories must follow. At the **Florida Bureau of Public Health Laboratories**, the method used for CT/GC testing was **approved** for use on vaginal specimens that were patient-collected in a clinical setting where support and counseling is available to explain procedures and precautions. Susanne Crowe, PhD, MHA, shared that her laboratory was able to complete a verification study prior to being able to offer testing for these specimens. Crowe explained that more self-collection options with valid regulatory claims are needed to further advance self-testing or self-collection initiatives. When self-collection is not covered by a test’s regulatory claim, standard CLIA verification and validation studies are not sufficient, and appropriate studies for

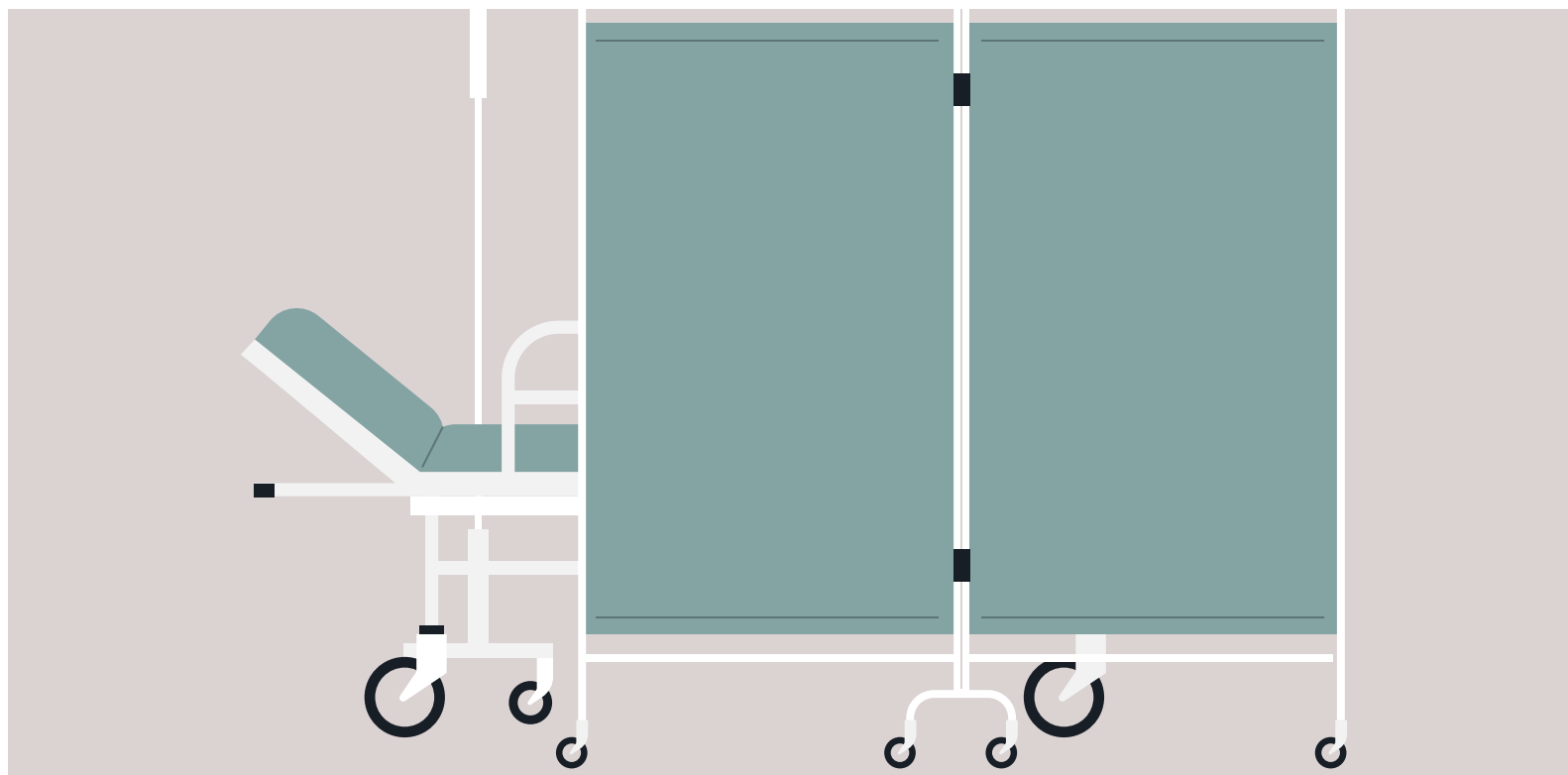
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There's an App for That: Determining Hospital HAI Prevalence in Developing Countries

By Paul Jankauskas, MS, MPA, manager, Informatics



Healthcare-associated infections (HAI) are a significant risk to patient safety, and the prevalence in hospital facilities is often unknown or under-characterized. Describing the burden of these infections is critical to decreasing risk and guiding infection prevention and control (IPC) initiatives in hospitals and other healthcare facilities. The **US Centers for Disease Control and Prevention's** (CDC's) Division of Healthcare Quality Promotion (DHQP) established the **Global Action in Healthcare Network-HAI** (GAIHN-HAI) module to detect, prevent and respond to HAIs in network facilities of four countries—Philippines, Indonesia, Brazil and Jordan. As part of this network, CDC is partnering with facilities to develop sustainable methods of measuring HAIs and using data for action in resource-limited healthcare settings.

HAI Point Prevalence Surveys

An HAI point prevalence survey (PPS) is a less resource-burdensome epidemiological method to measure the prevalence of HAIs in healthcare settings and provide actionable IPC data. Data are collected by IPC personnel using a

standardized protocol at a specific point in time to measure HAI rates among hospitalized patients during the survey period. CDC's GAIHN-HAI team funded partners to implement HAI PPS at network hospitals in the four countries and wanted to provide an efficient and intuitive tool to collect and analyze the data. They envisioned a responsive system in which users are guided through the complex HAI PPS process by following a series of simple step-by-step instructions. APHL was funded to work in cooperation with CDC to identify or develop a minimum viable product (MVP) for this purpose.

Project Progression & Identification of Informatics Solution

The project proceeded in four steps:

1. Discovery.
2. Define data requirements and develop project solutions roadmap.
3. Perform solutions landscape analysis and propose design and architecture.
4. Development and deployment of the MVP.

The GAIHN-HAI.org Web Application in Action

Data collection requirements were documented and tested at hospitals in the Philippines and Jordan and the first iteration of development of the MVP was completed by Deloitte developers in June 2023. The PWA development, test and production environments were deployed on the APHL Informatics Messaging Service (AIMS) platform and user acceptance testing was completed in July 2023. The GAIHN-HAI.org application will go live and be available to GAIHN-HAI hospitals to facilitate HAI PPS data collection this fall. The actionable data will be readily accessible to the participating hospitals to direct IPC activities and improve patient safety in an impactful manner. ■

Expanding Electronic Laboratory Reporting Beyond COVID-19

By **Patina Zarcone**, MPH, director, Datapult; **Millie Malai**, MPH, technical project manager, Datapult; **Victor Lee**, MD, vice president, Clinical Informatics, Clinical Architecture; **Lois Harry**, MD, clinical content analyst, Clinical Architecture; and **Brian Garrett**, product manager, Datapult

Centralized ELR, **Datapult's** COVID-19 electronic laboratory reporting service, is built on the **AIMS platform** and was used throughout the pandemic to report millions of test results to public health. The network of connections established between AIMS and surveillance systems can continue to be used for ELR beyond COVID-19; however, this expansion is challenging. For the expanded service (called Expanded ELR) to achieve its goal of reducing reporting burden, it must account for each jurisdiction's ELR rules with a one-to-many connection. Datapult, together with **Clinical Architecture**, is undertaking the work of developing Expanded ELR for this rule-driven, tailored reporting.

Building an Expandable Logic

A modular framework was developed to “build” each jurisdiction's ELR rules. These modules allow for uniform “reportability factors” (e.g., antibody, antigen, nucleic acid) and “reportability flavors” (e.g., resulted, positive, negative) to be combined in any permutation to adhere to each jurisdiction's published rules. To do this, ELR criteria across all jurisdictions were reviewed and decomposed into these modular units of meaning.

Name
Chancroid - H. ducreyi - POS (Org.Cx)
Chlamydia - C. trachomatis - POS (NA)
COVID-19 - SARS-CoV-2 - RES (NA)
Hepatitis A - Hep A Virus - POS (Ab.M)
Hepatitis B - Hep B Virus - RES (Ag.s)
HIV Infection - HIV - RES (VL)
Mpox - Mpox Virus - RES (NA)
Syphilis - T. pallidum - POS (Ab)
Syphilis - T. pallidum - POS (Org.MO)

Figure 1. Snippet of ELR Modules

Question	Resolution
How can one algorithm be built to use for variable jurisdictional reporting criteria?	Decompose the requirements into modules that can be combined for each jurisdiction's rules—in one algorithm
How can a data format be applied such that it can be consumed by any jurisdiction?	Create a uniform HL7 v.2.5.1 output that reflects all non-negotiables from each jurisdiction
If ELR information was not very detailed, could the rules be interpreted uniformly?	Utilize assumptions and reconcile directly with each jurisdiction

The Challenge to Expanding and Centralized ELR: Variability

Variability was the challenge to creating a system that housed every jurisdiction's ELR rules. Each jurisdiction documents differently their criteria, the detail in that documentation, and the types of test/results that are reportable. For a successful ELR service, questions needed to be resolved.

A Case for Centralizing ELR Criteria

A typical use case may be that a laboratory operates in one locale. There is only one set of reporting rules to understand and one connection that needs to be made. However, if the laboratory is a reference laboratory or national network of sub-laboratories, then multiple different connections would need to be made, with a parallel number of different rule sets and data requirements. The management of this variability posed our greatest challenge.

There are two straight-forward changes that can help significantly decrease the variability to improve timely and high-quality public health reporting data.

1. **A common ELR criteria template** – jurisdictional reporting criteria for ELR had to be inferred from a list of conditions. Harmonization of

laboratory-specific reporting rules could further distinguish between case reporting and laboratory reporting requirements.

2. **Making the rules easier to find** – it was a challenge to find the right documentation. ELR rules for all jurisdictions in one place and in one uniform template would simplify requirement gathering.

Public Health Filling Public Health Gaps

The distances between “non-traditional” public health data partners, public health laboratories, public health agencies and healthcare providers is narrowing, and as it does, the disconnects and misalignment are thrust into everyone's view.

Expanded ELR directly serves public health agencies' infectious disease surveillance programs. It singularly demonstrates the power of public health to build solutions and fill gaps that have existed in the shadows. It links the data pathways out of testing laboratories into epidemiologists. It highlights the wide variation in published reporting requirements across the country. It seeks to reconcile the variability of acceptable data standards. It is a much-needed step, and in the right direction. ■

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National Preparedness Month: Celebrating the Essential Work of Public Health Laboratories

By **Jill Sutton**, specialist, Emergency Preparedness and Response and **Rana Rahmat**, MPH, specialist, Laboratory Response Network

In recent years, outbreaks of emerging infectious diseases, natural disasters, environmental and industrial catastrophes and other public health emergencies have been stark reminders of why investment into the nation's public health infrastructure is critical in preparing for the public health threats of today—and tomorrow. Public health laboratories are a vital asset to our nation's preparedness and are essential for the early detection and response to public health threats. APHL collaborates across the association and works with key partners to support public health laboratories as they prepare for, respond to and recover from public health crises.

Every September, APHL, along with public health agencies and organizations from across federal, state, local, tribal and territorial levels, honors the importance of maintaining readiness by recognizing National Preparedness Month. This year, we asked two of our esteemed laboratory directors about their laboratories' approach to preparedness: Andrew Cannons, PhD, HCLD/CC (ABB), laboratory director at the **Florida Bureau of Public Health Laboratories—Tampa** (BPHL-Tampa), and Anthony Tran, DrPH, MPH, D(ABMM), laboratory director for the **California Department of Public Health's** (CDPH's) **Center for Laboratory Sciences**.

What does preparedness mean to your public health laboratory?

Cannons: Basically, preparedness is a state of readiness to try and mitigate the curve balls that are thrown at us every now and then. In Florida, hurricanes are one of our biggest threats and something we prepare for every year. Here at the BPHL-Tampa, every section has a hurricane plan for how to prepare their section should a storm threaten. We ensure our generators are fully functional and diesel tanks are full, and we test our notification system to ensure all staff can be notified and updated on the situation at all times. We also ensure that all staff are reminded to have a plan at home and be ready to evacuate if necessary. Staff are



Andrew Cannons, PhD, HCLD/CC (ABB), laboratory director at the **Florida Bureau of Public Health Laboratories—Tampa** (BPHL-Tampa)



Anthony Tran, DrPH, MPH, D(ABMM), laboratory director for the **California Department of Public Health's Center for Laboratory Sciences**

most important in the laboratory, and we want everyone to be as safe as possible.

Tran: The California Department of Public Health's Center for Laboratory Sciences is dedicated to an all-hazards approach for laboratory preparedness. We have the only Tier 1 advanced LRN-B and Level 1 LRN-C laboratories in the state of

California. Preparedness is an everyday activity at CDPH; planning ahead with drills, arranging schedules and ensuring laboratory staff, supplies and equipment are ready for operation at any given time.

How does your lab celebrate preparedness month every September?

Cannons: It kind of depends on what is happening. We have a monthly staff meeting and make preparedness a major part of that. This year, we are looking into doing some other events and tying them into public health. Of course, if we are dealing with a September storm or major outbreak, all cards might be off the table!

Tran: We have traditionally not celebrated National Preparedness Month, however, now is a wonderful time to start! We have an impromptu social committee and are in the midst of planning an event for September, so this year it will focus on preparedness and celebrate the hard work of our laboratorians who work every day to prepare for the next all hazards event.

How can APHL help your lab's preparedness needs?

Tran: It would be wonderful if additional resources were provided to the LRN laboratories and their staff. Ancillary activities such as preparations for wet laboratory trainings and outreach could use more resources and support from APHL. These could include templates and guidance/best practice documents on how to outreach and what is expected of specific tiers/levels of LRN laboratories.

How do you keep up staff readiness and morale at your laboratory?

Cannons: [We] keep them involved by providing staff with ways to offer input, and exercise without over exercising.



Attendees at the 2023 California Select Agent Partner Meeting gathered to network and exchange information. Photo: CA Center for Laboratory Sciences

Tran: Consistent communication among LRN laboratories and partners via regular meetings is key to staff readiness. As for morale, we have formed a committee devoted to planning events for the entire campus to ensure that there is time for both work and mental and physical rest, which is critical to maintaining good services. Additionally, we nurture a welcoming team atmosphere and enable collaborative work at the laboratory bench. We cross-train our staff on a multitude of laboratory tests to enable coverage for continued laboratory operations in the event of the unexpected. We create opportunities for teamwork and to instill a supportive atmosphere for cooperative laboratory operations. Most importantly, we respect each other and treat one another with dignity and expect everyone to maintain personal accountability. However, that being said, it is hard, and morale does suffer considering

that we and other preparedness programs are under-resourced and understaffed. National Preparedness Month offers an opportunity for this community to stand together to advocate for change and bring awareness and support to LRN laboratories across the nation. The more united we stand, the stronger we will be as a network!

What preparedness advice do you have for other public health laboratories?

Cannons: Definitely prepare for the worst and hope for the best! And while sometimes it looks like you can over prepare, that is way better than being underprepared.

Tran: Always be prepared for any threat and always expect the unexpected. The Friday afternoon calls from the FBI are real, thus ensuring that staff are trained and are mentally prepared for these types of calls. It is useful to exercise and

train to maintain competency, both tabletop and in the laboratory, as these assays can be difficult to perform and interpret. Preparedness is about cooperation, planning and being organized. The chance of success can be optimized with these three aspects in mind.

National Preparedness Month is a call to action for all sectors of the public health system and a reminder that public health threats can escalate quickly, cross jurisdictional lines, occur simultaneously or in quick succession. The inevitability of these events reinforces why maintaining preparedness is essential to mitigate the impact of future public health emergencies. ■

Increasing Diversity and Creating Inclusive Search Processes to Strengthen Student Recruitment

By Ladan Ghedi, MA, specialist, Academic Partnerships and Amra Handzic, MBA, specialist, Academic Partnerships



According to the *Journal of Public Health Management and Practice*, greater diversity of experiences and perspectives yields innovative public health approaches, stronger evidence and better training related to health equity to facilitate improvements in public health outcomes.

In alignment with APHL core values, it is important to ensure that potential academic organization partners prioritize diversity, equity, inclusion and accessibility (DEI) as a part of their institutional initiatives. This work involves engagement in outreach activities to academic institutions and other workforce-related organizations as well as professional associations.

From Theory...

Following a recent webinar, *Creating an Inclusive Search and Retention Process* hosted by the Eastern Association of Colleges and Employers, APHL staff gained additional relationship-building strategies and outreach techniques used to increase the diversity of the public health laboratory workforce through the promotion of career pathways in public health laboratory science. The objectives of this webinar were to learn how to attract and retain diverse talent, learning firsthand from DEI professionals the strategies and intentional recruiting techniques they use to diversify their workforce.

...to Practice...

To support workforce development in public health, the association's plan of action includes attendance at DEI-focused career fairs and conferences to promote career pathways in public health laboratory science to underrepresented students and alumni.

To ensure an inclusive search process for public health laboratory fellowship and internship programs, APHL utilizes diversity-focused job boards to ensure opportunities reach students and early career professionals across diverse backgrounds, geographical areas, disciplines and career stages. As recognized by the *US Centers for Disease Control and Prevention* (CDC) and the *Division of Scientific Education and Professional Development* (DSEPD), diversity and inclusion are essential for workforce development and improvement of public health.

APHL has partnered with organizations such as the *Tribal College Journal of American Indian Higher Education* and the *Historically Black Colleges and Universities* (HBCU) Career Hub to promote *Career Pathways in Public Health Laboratory Science: an APHL-CDC Initiative*. The HBCU Career Hub aims to educate around job search skills, workplace realities, employment equity and to support the career and professional goals of students, alumni and the broader HBCU community. The *Tribal College Journal* is a nonprofit media organization operating under the auspices of the *American Indian Higher Education Consortium* (AIHEC). Their website and e-newsletters are primarily focused on American Indian and Alaska Native higher education, which represents a suitable platform for sharing public health laboratory fellowship and internship opportunities.

...and Outreach

To further support DEI efforts related to the promotion of Career Pathways in Public Health Laboratory Science programs, APHL plans to participate in

recognition programs such as the *2023 National HBCU Week*. A White House initiative, National HBCU Week has been a notable opportunity to advance educational equity, excellence and economic opportunity through HBCUs, in partnership with HBCU leaders, representatives, students and alumni. This September, APHL will participate in the 5th Annual National HBCU Week Conference Career and Recruitment Fair for in-person job seekers in Arlington, VA.

In October, APHL will host five sessions to present on Career Pathways in Public Health Laboratory Science to 14 of the leading Hispanic-Serving Institutions (HSIs) in the country at the Fall 2023 HSI Virtual Career Expo. Additionally, we will focus on increased outreach to Tribal Colleges and Universities (TCUs) through various activities such as webinars and information sessions provided to community members.

Conclusion

The recruitment strategies and techniques adopted by the Academic Partnerships program will make a valuable impact on increasing the diversity of applicants into our public health laboratory science workforce. ■

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APHL Career Pathways Program Receives STEM Award

By Michelle Forman, manager, Media



APHL is proud to announce that the **Career Pathways in Public Health Laboratory Science: an APHL-CDC Initiative** received the 2023 Inspiring Programs in STEM Award from *INSIGHT Into Diversity* magazine, the largest and oldest diversity and inclusion publication in higher education. The Inspiring Programs in STEM Award honors college and university programs that encourage and assist students from underrepresented groups to enter the fields of science, technology, engineering and mathematics (STEM). Through the Career Pathways program, current students, early career scientists and established laboratorians participate in opportunities to explore or advance their career in public health laboratory science. This includes the recently launched

Public Health Laboratory Internship and Fellowship Programs.

The APHL Career Pathways program was selected by *INSIGHT Into Diversity* to receive this award because of the program's dedication to recruiting underrepresented students from STEM education disciplines and to building a larger highly trained public health laboratory workforce that represents the communities they serve. This commitment is in alignment with APHL's core value to embrace, promote and model a culture of diversity, equity, inclusion and accessibility (DEI).

"We are deeply grateful to be a recipient of this award that celebrates our engrained focus on diversity in the Career Pathways program's recruitment

efforts," said Christine Bean, PhD, MBA, MLS(ASCP), APHL Chief Learning Officer. "Our program has placed hundreds of fellows and interns in public health laboratories for hands-on experiences working closely with public health scientist mentors. We feel strongly that our focus on diversity is an investment in a more representative public health laboratory workforce in the future that will serve diverse communities across the nation. Thank you to *INSIGHT Into Diversity* for this honor."

Inspiring Programs in STEM Award winners were selected by the magazine based on efforts to inspire and encourage a new generation of young people to consider careers in STEM through

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GLOBAL HEALTH: The Role of Laboratory Leadership and Governance in Quality Management System Sustainability

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In the work conducted by APHL and the two laboratories, it became evident that leadership-supported governance structures resulted in laboratory leaders performing above average.

In the two years during which APHL worked with the laboratories to implement leadership and governance, both laboratories increased their testing capacities and experienced 100% staff

retention. County and facility laboratory leaders created leadership cascades that allow a systemic leadership approach rather than a personnel-centered leadership approach. This meant there was more willingness for the laboratory managers to delegate functions and authority to their deputies, and quality managers experienced a growth in their capacity to lead. Laboratory sections were managed by appointed section heads in contrast to the earlier structure where all responsibility and authority were concentrated within one leader.

Both laboratories achieved accreditation in 15 months and were able to fund their laboratory budgets, which included paying accreditation fees. Synergy between the hospital management and the laboratory leadership was established, enabling them to work together effectively for the good of the public's health. ■

INFECTIOUS DISEASES: Self-testing and Self-collection: What's Next?

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FDA approval or clearance are difficult for an individual laboratory to pursue.

Ryan Karsner, MD, FDA, joined the roundtable conversation to share his perspective. He indicated that the FDA was open to reviewing and approving self-tests and self-collected specimen collection devices if manufacturers

submit data to ensure safety and effectiveness. He discussed that risks still exist, which need to be mitigated, such as ensuring clear interpretations for self-tests, transport and disposal issues for home-based self-collection devices. He also mentioned that self-tests may not have performance characteristics equivalent to clinic-based collection and laboratory-based testing. Feedback from the audience to Karsner focused on the need for more guidance from FDA to get these tests and devices to market.

These topics continue to prove challenging as public health aims to increase access to testing, particularly for underserved populations, while also maintaining high-quality testing. As experts in diagnostic testing, the laboratory community should remain engaged in this discussion to help shape the development of self-testing and self-collection models and determine what role laboratories will play in the future. ■

TRAINING WORKFORCE AND DEVELOPMENT: APHL Career Pathways Program Receives INSIGHT Into Diversity Magazine's 2023 Inspiring Programs in STEM Award

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mentoring, teaching, research and successful programs and initiatives. The APHL Career Pathways program will be featured, along with 79 other recipients,

in the September 2023 issue of *INSIGHT Into Diversity* magazine.

"We know that many STEM programs are not always recognized for their success, dedication and mentorship for underrepresented students," says Lenore Pearlstein, owner and publisher of *INSIGHT Into Diversity* magazine. "We want to honor the schools and organizations that have created programs that inspire

and encourage young people who may currently be in or are interested in a future career in STEM. We are proud to honor these programs as role models to other institutions of higher education and beyond." ■

APHLTM 2023

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AGRICULTURAL, VETERINARY AND ENVIRONMENTAL TESTING TECHNOLOGIES AND PRACTICES

Anatoxin-a and Saxitoxin: Cyanotoxins of Increasing Frequency in Pennsylvania Freshwater Bloom Events

P. Higgins¹, A. Mickey¹, C. Sterling²; ¹Pennsylvania Department of Environmental Protection, ²Pennsylvania Department of Environmental Protection Bureau of Laboratories

The main harmful algal bloom (HAB) season in Pennsylvania (PA) typically lasts from May to October, and HAB events have been occurring in greater abundance and duration due to climate change. HAB events involving certain types of cyanobacteria can lead to the presence of different cyanotoxins in recreational waters. Anatoxin-a and saxitoxin are neurotoxins that are more potent in detrimental health effects than the other two common cyanotoxins of concern (microcystin and cylindrospermopsin). Many state and the federal recreational guidance documents only provide values related to the presence of microcystin and cylindrospermopsin and there is little consideration for the potential health effects when multiple toxin-producing cyanobacteria types are present in a single bloom event. During the 2022 HAB season in PA, anatoxin-a and saxitoxin were detected more frequently than in previous HAB seasons and multiple types of cyanobacteria that could produce two or more cyanotoxins were observed in individual HAB events. The prevalence of anatoxin-a and saxitoxin detection during HAB events, including multiple cyanotoxin events, suggests a need for additional focus on anatoxin-a, saxitoxin, and multiple cyanotoxin events in surveillance programs for freshwater bodies.

Presenter: Pamela Higgins, pahiggins@pa.gov

Comparing Methods Utilized to Test for PFAS in Aqueous Samples: EPA 537.1, EPA 533, ISO 21675

E. Walters; Biotage

Extraction protocols and analytical methods utilized for detecting per- and polyfluoroalkyl substances (PFAS) in aqueous samples have evolved rapidly over the past decade. Since the US Environmental Protection Agency (EPA) released method 537 in 2009, laboratories across the world have made significant progress in their ability to quantify these contaminants. From 2013–2015, EPA 537 was utilized for assessment monitoring as part of the Unregulated Contaminant Monitoring Rule (UCMR) 3 program and was updated to EPA method 537.1, shortly thereafter. In late 2019, EPA method 533 and International Organization for Standardization (ISO) Method 21675 were released, which in addition to EPA method 537.1, enable laboratories to quantify up to 37 PFAS analytes with exceptional precision and accuracy. In anticipation of the upcoming UCMR 5 program, it is beneficial to review these top methods utilized for evaluating PFAS in aqueous samples. This study focuses on comparing the extraction protocols and analytical requirements for each method. Results from these methods will be compared to assess method robustness, analyte detection limits, data accuracy and reproducibility. This evaluation demonstrates the value that can

be obtained from comparing protocols and analytical results from current methodology to make informed decisions about the future of PFAS monitoring.

Presenter: Evan Walters, evan.walters@biotage.com

ddPCR on Beach Water in San Diego County

S. Hallis, B. Costa, W. Jordan, J. Corrigan, W. Wooten; County of San Diego Health & Human Services Agency

Droplet digital polymerase chain reaction (ddPCR) was used for the detection of fecal indicator bacteria (FIB), *Enterococcus* spp., in San Diego County beach water samples collected as part of regulatory monitoring activities starting on May 5, 2022. The ddPCR method was approved by EPA for use only in San Diego County for the purpose of beach monitoring and notification. CDPH approved the County of San Diego's use of the ddPCR method at 1413 DNA copy number/100 mL threshold. Additionally, the County of San Diego Public Health Laboratory received approval from ELAP to add the alternative test procedure to the FOA certificate. ddPCR results are typically turned around within 10 hours compared to culture techniques which produce results in 24 hours. The faster turnaround time is more protective of public health as beaches can be posted earlier when numbers are in exceedance of the beach action threshold. Additionally, the beaches can be opened sooner as results can be provided the same day. Furthermore, PCR based water monitoring techniques are more sensitive than previously used culture techniques. Sampling of south county sites occurs daily, and other sites are sampled per AB-411 guidelines. At beaches in the south county, the community is disproportionately impacted by cross border sewage flows from Mexico and the Tijuana River Valley. Beach threshold exceedances have an impact on tourism dollars for the affected communities. South swell conditions were prominent during the spring and summer months resulting in many exceedances in south county. South swells may push sewage along the San Diego coastline. Peak tourism season generally runs between Memorial Day and Labor Day. This time period is the focus of data analyzed for this poster. In 2022, south county sites were in exceedance 60.2% of peak tourism season compared to 17.0% in non-south county sites. Imperial beach pier was in exceedance for 77.9% of peak tourism season in 2022 compared to 0% in 2021 (prior to ddPCR implementation); whereas Torrey Pines was in exceedance for 12.5% in 2022 compared to 0% in 2021. Imperial Beach Pier exceedances were observed with south swell conditions 44.6% of the time. Swell events appear to bring an influx of enterococcus which may persist for several days. In the absence of a swell event, copy number/100 mL decreases over time due to mixing and degradation. The impact of south swell on ddPCR results may indicate that Mexico serves as a point source of sewage leading to lengthy beach advisories for south county. Lastly, implementing this new test which is more sensitive and provides more accurate testing than previous methods, creates unique opportunities to manage the new data. One such creative solution initiated in San Diego is the creation of the warning tier to allow for proper public notification so that beach visitors can make an informed decision before entering the potentially impacted water.

Presenter: Samantha Hallis, samantha.hallis@sdcounty.ca.gov

Development and Implementation of a Canine Infectious Respiratory Disease Panel (CIRD)

T. Spikes, Y. Mosley, H. Naikare, L. Whittington, D. Baughman, R. Cox, M. Coarsey, A. Kalantari; Tifton Veterinary Diagnostic & Investigational Laboratory

Canines are the most popular companion animal in the world with nearly 50 million households having at least one dog in the United States alone. Due to their popularity, they are also one of the most often seen and treated animals at veterinary clinics. When these animals present with symptoms of an illness, the vet may order testing by collecting specimens from the animals that are then sent to a diagnostic lab. Tifton Veterinary Diagnostic and Investigational Lab intends to develop a diagnostic panel to test for the following canine infectious respiratory diseases: CAV, CHV, B. bronchiseptica, *Mycoplasma canis/cynos*, *Strep. zooepidemicus*, Influenza A, CRCoV, CDV and CPiV. This will be accomplished by employing stringent testing validation methods and utilizing Indical and Indimag nucleic acid extraction kits and real time PCR.

Presenter: Teddy Spikes, trs53790@uga.edu

Development of Novel Methods for Total Organic Fluorine Assessments in Environmental and Clinical Matrices

L. Duncan, M. Shafer; Wisconsin State Laboratory of Hygiene

Background. The PFAS (per- and polyfluoroalkyl substance) crisis facing the world is one of most pressing environmental chemical pollution emergencies in modern times. PFAS are now ubiquitous in the environment due to their persistence, mobility and extensive history of use, having been used in numerous industrial and consumer applications, with little regulation, since the 1950s. Many PFAS resist chemical and biological degradation, can bioaccumulate, and have been associated with a diverse array of adverse health outcomes. Preventing PFAS contamination of environmental reservoirs and subsequent human exposures is challenging because there are numerous potential sources, many of which are very poorly characterized. In addition, only a small number of the >5,000 known PFAS are typically quantified (20-40 at most), which limits efforts to identify PFAS compounds that are characteristic of specific sources. Further complicating our understanding of environmental cycling and bioaccumulation is the fact that many important classes of PFAS and precursors undergo transformation in the environment and human body to PFAS compounds of specific enhanced toxicity concern. **Approach.** On-going PFAS research efforts at the University of Wisconsin, State Laboratory of Hygiene, are directed at providing a more complete characterization of PFAS in environmental and clinical matrices through the application of new and advanced methods. In parallel with the development of new methods, we are collecting major environmental sources/vectors of PFAS and subjecting them to detailed PFAS characterization in an effort to define source specific PFAS chemical fingerprints. Lexi Duncan, our APHL fellow is integrated into this exciting and dynamic research endeavor. Largely absent from the current toolbox of PFAS analytical approaches are methods for quantification of the TOTAL pool of PFAS. Methods for total PFAS will provide a more accurate assessment of the PFAS burden/contamination of the sample/environment, and in combination with targeted methods (e.g. LC/MS/MS) allow for

assessment of the “missing” PFAS – i.e. what are we not quantifying with our targeted methods. The scale of the “missing” fraction can be used to prioritize additional discovery efforts. Here we report on our progress in developing methods for total PFAS using Combustion Ion Chromatography (CIC) to provide levels of total organic fluorine. Key CIC performance metrics for both aqueous and solid matrices will be presented and placed into context with targeted PFAS levels. Once the CIC methods are in place, we will communicate to a broad array of agencies, especially Public Health Labs, the potential (and limitations) of these new tools for total PFAS characterization.

Presenter: Martin Shafer, mmshafer@wisc.edu

Enhancing Swine Diagnostics for the Detection and Differentiation of ASF and CSF from Other Septicemic Diseases: Development of Novel Multiplex High Throughput Taqman qPCR Panels and Point-of-care Isothermal Diagnostic Platforms

C. Hollis, L. Whittington, A. Kalantari, Y. Mosley, H. Naikare; Tifton Veterinary Diagnostic & Investigational Laboratory

African swine fever (ASF), Classical swine fever (CSF), Pseudorabies virus (PRV), and Porcine Reproductive and Respiratory Syndrome (PRRS) are four viral diseases that can cause major problems for populations of swine. In addition to these viral diseases, other septicemic diseases like Erysipelas, Salmonellosis, Pasteurellosis, Streptococcosis, and Listeriosis can also cause herd fatality and significant economic loss. Because of the economic impact these diseases can have on the swine industry, screening for these diseases is an important part of surveillance and diagnosis. Diagnosis of these diseases becomes a challenge since the clinical manifestations from one disease to another can look quite similar. For this research there are two main objectives. First, to develop multiplex Taqman qPCR panels for the detection and differentiation of ASF, CSF, and PRRS from other economically significant septicemic diseases affecting swine. Currently, there are NAHLN protocols for detecting ASF and CSF in a PCR reaction with an internal control. We propose to have ASF, CSF, and PRRS, in addition to an internal control, in the same PCR reaction. This would result in cost savings, less consumption of reagents, and reduced turn-around-time by having three targets in one PCR reaction. In addition to this panel, two other panels will be created to screen for the septicemic diseases listed above. Development of these three panels will result in diagnostic solutions for multiple swine diseases. The second objective is to develop isothermal helicase dependent amplification (HDA) assays for potential point-of-care testing for ASF and CSF pathogens in resource limited settings. This technology uses an enzyme that can separate DNA strands at a constant temperature of 65 °C. The use of a simple heat block can be used in the place of complex machinery for the reaction. We aim to provide proof of concept for the HDA assay and potentially incorporate this process into lateral flow assay strips. This will serve as a potential point-of-care testing option that can be performed barn side and allow rapid detection and diagnosis of ASF and CSF.

Presenter: Carol Hollis, carol.hollis@uga.edu

Evaluating an Automated Solid Phase Extraction System to Extract Per- and Polyfluoroalkyl Substances from Delaware River Water, Following US Environmental Protection Agency Method 1633

D. Baird, S. Desrochers, L. Zhong, S. O'Leary, T. Fan, C. Xu; New Jersey Department of Health Public Health and Environmental Laboratories

The New Jersey Department of Health aims to implement EPA Method 1633 to extract 40 per- and polyfluoroalkyl substances (PFAS) from river water by solid phase extraction (SPE) and quantify them by liquid chromatography-tandem mass spectrometry. The SPE method utilizes weak anion exchange (WAX) SPE cartridges conditioned with methanolic ammonium hydroxide and formic acid, followed by loading 500 mL of sample, rinsing with water and buffer, and eluting with methanolic ammonium hydroxide. A vacuum facilitates flow through the cartridge on the manual SPE. Our lab has successfully extracted 39 of the 40 analytes within 70-130% accuracy using this method. However, it requires continuous monitoring and adjustments, making it time consuming and labor intensive. Our goal is to reduce this burden by automating the SPE method using the Promochrom SPE-03 liquid handler. The automated SPE uses the same protocol, however, it draws the liquids into the cartridge using syringes, and the samples flow through the cartridge by gravity. As a result, it has introduced complications such as cartridge clogging, leaks, and poor elution due to solid particulates and algae in the river water samples. These lead to inadequate analyte recovery, incomplete extraction, and inconsistency between samples. We have improved the automated protocol by changing various conditions such as SPE cartridges, solvents, sample pre-treatment, and sample volume. The Agilent Bond Elute WAX cartridges were more successful in completing the extraction compared to two other WAX cartridges due to larger particle sizes. Two alternative WAX SPE protocols, EPA method 533 and a method published by Coggan et al. at Agilent Technologies, were also tested. While these methods experienced less clogging, they suffered the loss of additional analytes. Lastly, the samples were centrifuged to separate the solids from the bulk of the liquid to allow a majority of the sample to pass through without clogging, before adding the solids at the end. This prevented the samples from clogging during loading, but they subsequently clogged during rinsing and elution, preventing the SPE run from completing. Reducing the sample volume to 250 mL significantly reduced the clogging issue for most samples but sacrificed the sensitivity. Thus far, the optimized method achieves recoveries between 60-150% for all analytes. However, the clogging is still not completely avoidable. In conclusion, automated SPE has yet to be an effective approach for EPA method 1633 for river water in our lab, primarily because it relies solely on gravity for loading and eluting. Further experimentation will involve incorporating vacuum power, which may overcome the sample clogging issue and achieve method requirements.

Presenter: Devon Baird, devon.baird@doh.nj.gov

Evaluating Chemical Exposures from "Non-toxic" Nail Polishes

P. Callaway, K. Anderson; National Institute for Occupational Safety and Health, US Centers for Disease Control and Prevention

In response to consumer concerns over nail product toxicity, the nail industry introduced n-free polishes—polishes that claim to be free of n-number of chemicals. The original n-free (3-free) polishes claimed not to contain formaldehyde, toluene or dibutyl phthalate (DBP), chemicals that have been associated with respiratory health problems, cancer, and poor reproductive health outcomes. Now, there are a wide variety of n-free polishes with many claiming to be 10 or more free. Despite the proliferation of these products, there is evidence to suggest that some n-free polishes contain chemicals they claim to be free of. Some of these chemicals can cause adverse reproductive outcomes and pose a risk to the high proportion of nail salon workers who are women of childbearing age. While the development of n-free polish was largely consumer driven, nail salon workers have higher exposures and thus are likely at greater risk for negative health outcomes from these nail products. Understanding what chemicals nail salon workers are exposed to, and at what levels, can help establish risks associated with nail product use and can enable workers to make informed decisions about product use. In this study, we measured chemical exposures generated while painting nails with 10 brands of n-free polishes using real-time and time-integrated air sampling. Real-time measurements were conducted using a photoionization detector (PID, Tiger, Ion Science) to measure total volatile organic compounds (TVOCs) and a FTIR (Fourier-transform infrared spectroscopy) gas analyzer (DX4040, Gasmeter) to measure individual compounds. Time-integrated samplers were used to measure formaldehyde (OSHA Method 1007, UME passive badges, SKC), DBP (OSHA Method 104, OVS tubes, SKC) and triphenyl phosphate (TPHP, NIOSH Method 5038, mixed cellulose ester filters, SKC), a common substitute for DBP thought to have similar health effects. Airborne concentrations were measured while two coats of polish were applied to artificial nails on a mannikin in an exposure chamber and for two hours after application. Results from approximately 40 trials indicated little variation ($\pm < 1$ ppm) in mean TVOC concentration between polishes and no correlation ($\rho=0.18$, $p=0.22$, Spearman's) with number of n-free claims. Mean TVOC concentrations ranged from 0.22 to 0.86 ppm with peak concentrations ranging from 2.69 to 44.26 ppm, depending on polish brand. Despite all polishes in this study claiming to be 3-free, preliminary results suggest over 80% had measurable formaldehyde concentrations and over 70% of polishes had measurable toluene concentrations. However, neither DBP nor TPHP were detected. This study highlights that despite industry claims, n-free polishes may still contain chemicals associated with negative health effects and that more studies are necessary to understand the true chemical exposures of nail salon workers.

Presenter: Perri Callaway, tqa8@cdc.gov

Implementing Automated Extraction for High Throughput Environmental Testing

M. Wills, Z. Cao, A. Frost, X. Hu, S. Urban; Maryland Department of Health Laboratories Administration

Per- and polyfluoroalkyl substances (PFAS) have become so pervasive in our water that the Biden Administration recently pledged \$5 billion to states addressing the contamination. With this call to action comes the need for robust technology to keep pace with the increasing scale of sample testing. We compared manual extraction to an 8-channel automated solid-phase extractor (SPE-03) over three EPA PFAS methods (537.1, 533, and 1633). Our

results show enhanced elution of compounds, yielding increased analyte recoveries and reduced carryover. The extractor's controlled flow rate diminished human error and improved reproducibility of results. We further adapted the SPE-03 to EPA method 525.2 for semi-volatile organic compounds, further extending the extractor's utility and versatility. Adoption of the SPE-03 could benefit other laboratories working to meet the testing demands of the PFAS contamination crisis.

Presenter: Maggie Wills, mawills1124@gmail.com

Improvements to Sensitive Pesticide Detection in Produce via Gas Chromatography-Tandem Mass Spectrometry (GC-MS/MS) using Analyte Protectants

J. Dickie, A. Lehner, J. Buchweitz; Michigan State University Veterinary Diagnostic Laboratory

Pesticides play a beneficial role in agricultural production, but these chemicals can be toxic when ingested by non-target organisms in high concentrations. Because ready-to-eat commodities like fresh fruit and vegetables may retain residual amounts of pesticides, government agencies regulate the use of these compounds by establishing commodity-specific tolerances. In this study, pesticide residues were extracted from produce using the QuEChERS technique (Quick, Easy, Cheap, Effective, Rugged, Safe). Multiple Reaction Monitoring (MRM) methodologies were used to effectively scan and differentiate fifteen synthetic pyrethroid residues. Initially, chromatography performance worsened with decreasing standard concentrations; gradual shifting in Retention Time (RT) and skewing of peak shapes were observed and hindered ease of quantitation. To address this issue, the concept of Analyte Protectants (APs) was applied, where a mixture of four compounds were introduced to pesticide standards prior to each injection onto the GC column. APs are known to transiently bind to column active sites that otherwise retard or distort the chromatography. Limits of Detection (LOD) and Limits of Quantitation (LOQ) for individual compounds improved by at least an order of magnitude with standard curves [ranging 20 to 50,000 ppb (ng/mL)] that yielded an average coefficient of determination, $R^2 = 0.99$. The extended application of APs to other pesticide classes, including organophosphate and organochlorine compounds, will be presented. A survey of pesticide content in local, Michigan-grown produce will also be conducted; public policies and resources, including the Code of Federal Regulations (CFR), will be used to contextualize the findings.

Presenter: Julia Dickie, juliamdickie@gmail.com

Microbial Source Tracking for Detecting Human and Non-human Fecal Contamination in Drinking and Surface Waters

D. Wright-Foulkes, S. Chmura, T. Hattenrath; New York State Department of Health, Wadsworth Center

Fecal contamination of recreational and drinking waters by human and non-human sources is of significant concern for the safety of communities. Potential sources of contamination include raw sewage, urban stormwater runoff, wastewater effluents, fertilizer usage, or domestic farm animals and wildlife. Consumption of contaminated water containing pathogenic microbes can

lead to outbreaks of severe illness. Microbial Source Tracking (MST) methods present solutions to determine sources of fecal contamination which can assist public health officials with monitoring and remediation. This research project was designed to launch an MST program in the New York State Department of Health (NYS DOH) Laboratory of Environmental Biology (LEB) utilizing EPA Method 1696.1: Characterization of Human Fecal Pollution in Water by HF183/BacR287 TaqMan® Quantitative Polymerase Chain Reaction (qPCR) Assay. Laboratory standard operating procedures (SOPs) were created, a laboratory quality assurance program was developed, and the assays were validated for reliability and performance. Amplification efficiencies, limits of detection, and reproducibility were determined using DNA reference material. Additionally, to improve detection capabilities, various sample volumes were analyzed, and two qPCR thermocyclers were used, QuantStudio 5 and 7500 Fast (Applied Biosystems, MA, USA), to determine the comparability of the assays across different platforms. A validation package will be submitted to the New York State Environmental Laboratory Approval Program (ELAP) for accreditation consideration. The second aim of this project was to develop assays to detect non-human sources of fecal pollution (i.e., ruminants and wildlife) to provide additional information to public health officials. Literature was reviewed for non-human targets that could determine if animals from agricultural communities or wildlife native to NYS played a role in the contamination. The data gained from this part of the project will determine other potentially abundant sources of pollution, which could help determine steps for remediation.

Presenter: Dorothy Foulkes, dmwrigh1@outlook.com

PFAS Detection in Drinking Water Method Development

J.F. Fennessey; Colorado Department of Public Health and Environment

Per- and polyfluorinated Substances (PFAS), synthetic chemicals used throughout industry, including in numerous nonstick and water resistant products, have been linked to cancer, high cholesterol levels, weakened vaccine response in children and numerous other health problems. Despite increased public awareness of PFAS toxicity, the chemicals remain a major health threat. Since the 1950s, PFAS has contaminated virtually every living thing and ecosystem on the planet. The carbon-fluoride bonds of PFAS renders them extremely resistant to destruction, making remediation extremely difficult. Even PFAS that have been phased out are still being detected in water and blood samples. Although PFAS can't be easily removed from drinking water, the types and amount can be determined through liquid Chromatography-mass spectrometry (LCMS). As PFAS are used in the production of Teflon, used in various laboratory materials, PFAS testing requires specialized equipment to minimize laboratory contamination. The CDPHE Laboratory purchased a custom Solid Phase Extraction instrument, EZPFC, and is awaiting the installation of the State of Colorado. There are currently two EPA drinking water methods, EPA 537.1 and EPA 533, which will standardize our procedure and validate for use on these instruments in our laboratory. Once we are able to accurately and precisely measure PFAS in water, this method will be available for use on water samples collected across the state. We can then use the wastewater method EPA 1633, which can detect

a wider range of PFAS and test other matrices, such as food or soil. On this poster you can learn how our method development is progressing.

Presenter: John Fennessey, john.fennessey@state.co.us

PFAS Primer: What is PFAS? Where are They? How Do We Test Samples for PFAS Contamination in a Public Health Lab?

T. Astill; PerkinElmer

PFAS compounds are a group of man-made chemicals that have been used in a wide variety of industries around the world since the 1950s. This class of chemicals are incredibly stable so they are persistent and accumulate in the environment. Growing health concerns regarding PFASs and their prevalence in consumer goods and the environment indicate a critical need to monitor levels of PFAS in the environment. Understanding how to test for this contaminant is a critical health topic. In this poster, analytical workflows will be presented that Public Health Labs can leverage to quickly implement this testing capability in their lab.

Presenter: Toby Astill, toby.astill@perkinelmer.com

Pilot Program of Tennessee Department of Health - Private Well Water and Emergency Response Test Kit Program

K. Dunaway, P. Leathers; Tennessee Department of Health Laboratory Services

The Tennessee Department of Health (TDOH) has developed a new method to conduct quality assessments of private water supply by providing water test kits by mail. The TDOH Division of Laboratory Services, in partnership with TDH Communicable and Environmental Diseases and Emergency Preparedness (CEDEP) Waterborne Program, began this initiative in the late summer 2022. This initiative has two goals: to help screen well and spring water quality for citizens in Tennessee and to act as quickly as possible in response to a large scale natural or manmade disaster. This program will provide a service to Tennesseans who may not be using a municipal water supply due to cost or lack of availability. Residents that use a private water supply and are near areas with agricultural runoff, manufacturing, and natural deposits of contaminants of concern may be at risk of consuming harmful chemicals or pathogens. The lack of well water testing regulations in the state means that citizens may not know if their well or spring is contaminated, and the water quality may not meet the safe drinking water standards afforded to those with municipal, public water supply. During natural disaster response, TDOH's goal is to be able to staff disaster resource centers with testing supplies and provide water safety resources more efficiently. This small-scale pilot is currently funded by the CDC Environmental Health Capacity (EHC) program. This is being facilitated through the use of premade kits which the lab can provide to local health departments, picked up at TDOH Laboratory Services or shipped directly to residents at no cost. The grant nearly funds the cost of testing by providing the funds for the kits (coolers, bottles, bags), the tests performed and the shipping. Currently there is a small cost to the resident of providing ice to maintain shipping requirements of the collected water sample. This grant will be a multi-phase project

with additional outreach to rural, minority or socioeconomically disadvantaged communities. As data is collected, the TDOH will be able to plot water quality results and determine if remediation efforts are needed. Lastly, use the data to help inform law makers on legislation for well and spring regulation to promote the health of the citizens of the State of Tennessee.

Presenter: Patrick Leathers, patrick.leathers@tn.gov

BIOMONITORING AND CHEMICAL THREATS

Genomic Surveillance to Monitor and Identify Emerging SARS-CoV-2 Variants in University Populations in Milwaukee, Wisconsin

A. Bauer, M. Khubbar, S. Scott, J. Lentz, J. Weiner, K. Akinyemi, A. Skillman, N. Balakrishnan and S. Bhattacharyya, City of Milwaukee Health Department

Introduction: Milwaukee Health Department Laboratory (MHDL) has been partnering with two local universities' student health clinics since March 2020 to provide testing and surveillance of the student and staff populations for SARS-CoV-2. In-person learning, on-campus housing and sporting events create the potential for rapid transmission of emerging variants of SARS-CoV-2. Due to the national and international populations on these campuses and their locations in downtown Milwaukee, there were concerns with the spreading of novel SARS-CoV-2 variants. MHDL began sequencing specimens from these two universities to identify variants and help prevent the spread of disease into the broader community. **Methods:** RNA extracts from clinical specimens positive for SARS-CoV-2 using FDA EUA assays were sequenced. Whole genome sequencing (WGS) was performed on the Clear Labs (Clear DxTM WGS SARS-CoV-2) platform, as well as utilizing the ARTIC network workflow on MinION (Oxford Nanopore Technologies) and/or MiSeq (Illumina). Specimens that were successfully sequenced in accordance with in-house QC metrics were uploaded to GISAID, the open-source global repository for the SARS-CoV-2 genome.

Results: From September 2020 to December 2021, SARS-CoV-2 genomic surveillance was conducted on a total of 2,141 specimens at the Milwaukee Health Department Laboratory (MHDL). Of 2,098 specimens submitted to GISAID, 322 (15.3%) were from the two university campuses located within the MHDL jurisdiction and the remaining 1,827 (84.7%) were from the broader community. Of 322 specimens sequenced from universities, 53.7% were the Delta variant, 8.4% Alpha, 3.4% Epsilon, 2.2% Omicron and 32.3% were other lineages, including Zeta, Eta, Iota and Mu. Omicron was detected in community sequencing 12 days prior to first university detection, indicating that spread was potentially from community to university rather than the other way around. **Conclusions:** Due to existing partnerships with the two local universities, MHDL was able to quickly implement genomic surveillance for SARS-CoV-2. University policies of weekly COVID-19 testing for unvaccinated students and staff members ensured adequate surveillance samples for WGS. MHDL surveillance efforts assisted in the identification of outbreak clusters on campus and helped the universities monitor and track suspected cases. Sequencing

specimens from these university populations improved our understanding of the transmission patterns of SARS-CoV-2 variants circulating on a local community level, the level of transmissibility amongst high-risk populations and provided timely actionable data for contact tracing.

Presenter: Amy Bauer, City of Milwaukee Health Department, ambauer@milwaukee.gov

An Introduction to the Iowa Biomonitoring Program at the State Hygienic Laboratory at the University of Iowa

M. Schueller, A. Mattson, S. Dai; State Hygienic Laboratory at the University of Iowa

Biomonitoring is defined by the US Centers for Disease Control and Prevention (CDC) as an assessment of nutritional status and the exposure of the US population to environmental chemicals and toxic substances. The Iowa Biomonitoring Program, initiated in October 2019, is a public health surveillance project conducted at the State Hygienic Laboratory at the University of Iowa (SHL). The three-fold purpose of the Iowa program is to: 1) enhance laboratory capacity and collaborations to perform biomonitoring analyses, and to increase laboratory service coverage across the state, 2) extend the CDC national study scope to Iowa, develop an Iowa-specific output database, establish the background level characterization of the Iowa rural population exposure and compare that to CDC reports, and 3) communicate and educate exposure risks, promote biomonitoring awareness, implement protective measures, and support informed policy development. The SHL is currently assessing the levels of environmental exposure to heavy metals, neonicotinoid insecticides, and phenols (a group of compounds identified as endocrine disruptors). The Iowa program is focused on two specific sub-populations – pregnant women and Iowa residents from 11 counties using private well water as their primary source of drinking water. The specific matrices that are being evaluated to fulfill the assessment of human exposure are urine and well water. SHL has collected 998 urine specimens from 343 expectant mothers at multiple points in time during their pregnancy, and 1,000 urine specimens and 1,000 well water samples from private well water users. A partnership with the University of Iowa Hospitals and Clinics Maternal Fetal Tissue Bank provides the needed maternal urine specimens. The private well user water samples and urine specimens were originally designed to be obtained in partnership with the local public health specialists as collectors. However, the pandemic required that SHL change directions in recruitment and rely on a series of mailings to well owners/users identified in the Iowa Department of Natural Resources (IDNR) Private Well Tracking System (PWTS). The active recruitment period for private well users spans from September 2021 through February 2023 with approximately 70% of the targeted goal achieved through October 2022. These water samples and urine specimens are self-collected by the participants. Analysis of urine and water samples is ongoing at this time. Selected findings from both study groups will be shared with attendees at the APHL Annual Meeting poster session.

Presenter: Michael Schueller, michael-schueller@uiowa.edu

Automating Solid Phase Extraction of PFAS in Human Serum for LC-MS/MS Analysis using a Liquid Handler

Z. Quan Li, C. Xu, D. Mathes, C. Lestician, A. Lin, S. O'Leary, T. Fan, L. Zhong; New Jersey Department of Health Public Health and Environmental Laboratories

Per and Polyfluoroalkyl Substances (PFAS) are a large group of manufactured chemicals that are persistent and potentially hazardous. PFAS is tested in human serum with high performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS), typically after solid phase extraction (SPE). Although it can be challenging, resource intense and time consuming, SPE is deemed necessary in this instance. In our lab, SPE is performed online system. A major limitation of this approach is that sample preparation occurs sequentially before sample injection, therefore limiting throughput. The main goal of this project is to improve the sample throughput of the SPE system by offline automation, where samples are prepared concurrently using a 96 well plate format using a Janus automated liquid handler. The proposed off-line SPE has been successfully deployed by others on various platforms. Sample pre-treatment was deemed the most crucial in eliminating analyte recoveries. The SPE will be conducted on weak anion extraction (WAX) stationary phase. The automated procedure includes sample pre-treatment: Combine 50 μ l, 150 μ l Acetonitrile and internal standard, load the supernatant to the SPE plate after conditioning and washing before sample elution. The new automated offline SPE sample preparation method is expected to enable the lab to prepare PFAS samples from 48 aliquot of 50 μ l human serum in less than two hours for analysis of the 18 PFAS. Meanwhile, the accuracy of the further LC-MS/MS analysis must be within $\pm 30\%$ of nominal value or 3 times of STD value.

Presenter: Zhan Quan Li, zhanquan.li@doh.nj.gov

Biomonitoring Results Reporting to Participants in a State-level Population Surveillance Study (NJHANES)

C. Donohoe, C. Ho, T. Fan, E. Bind, E. Cook; New Jersey Department of Health Public Health and Environmental Laboratories

NJHANES is a population surveillance study conducted by the NJ Department of Health to examine the health and nutritional status of NJ residents through questionnaires and biomonitoring various toxic metals and organic pollutants. In environmental population studies, individual results reporting is challenging; therefore, NJHANES designed the reports to help participants interpret their results in a way that enables them to reduce exposure and improve their health outcomes. Specifically, the team determines elevated exposure levels, delivers those levels in lay terms, and must satisfy CLIA research regulations. The NJHANES reports, including partial reports with metals and final reports with all analytes, were designed to address above challenges. NJHANES reporting guidelines were based on the latest NHANES datasets to establish elevated exposure levels. While the partial report contains results for metals with established health limits, the participants are alerted if their level is three times higher than the 95th percentile of NHANES due to concerns of health risks associated with this range. The 95th percentile estimate was calculated for sex-age stratification to confirm results since exposure levels differ among age groups and/or between sexes.

Data from participant questionnaires are carefully reviewed by in-house subject matter experts (SMEs) to help deliver a personalized interpretation for reducing personal exposure without invoking panic in the participants or losing scientific credibility. We closely followed CLIA guidelines to choose the language used in the report to avoid misuse considering our unique position as a state laboratory. Of the 106 consented participants to date, 66 partial reports have been distributed, and ~4.5% of those who received their partial report have responded with interest in how their exposure levels may influence their health, body burden levels, and potential exposure sources around the household. We followed up with two elevated levels that were higher than the health limits by initiating a conference call with the participant and SMEs to explain the levels observed, go over participant's potential exposure sources documented in the questionnaire survey, and mandatory reporting to state agencies required by state law. NJHANES has yet to receive a negative response following the dissemination of participant results. We are developing a graphical interpretation among both the NJHANES population and the reference US population to aid participant understanding and will be included in the final report packet and presentation. A post-survey will be added to NJHANES to evaluate participant attitudes towards all aspects of the study, including the reporting format. These approaches for the report packets were carefully designed to encourage interest, build trust, and promote the public health on environmental exposures.

Presenter: Colleen Donohoe, colleendonohoe3@gmail.com

Determining the Effects of Hemolysis on PFAS Concentrations in Blood Samples

J. Lynch, B. Clark, E. Mullin; New York State Department of Health, Wadsworth Center

Background: Per- and polyfluoroalkyl substances (PFAS) are man-made organic compounds. They are widely used in consumer products and persist in the environment. There are thousands of PFAS, and studies have shown that exposure to some PFAS may be harmful to humans and animals. New York State Department of Health, Wadsworth Center has an analytical method for analyzing several PFAS in human serum. Hemolysis is a common problem in clinical samples. It is the result of the breakdown of red blood cells. In vivo, hemolysis can occur due to medical abnormalities or dehydration. In harvested plasma/serum samples, it is more common for hemolysis to occur because of sample collection or storage errors. The current laboratory protocol rejects grossly hemolyzed samples. A quantitative level of hemolysis that effects accuracy of PFAS results is not established. Studies have shown that hemolysis can either artificially dilute analyte concentrations or cause matrix interference. The Center for Disease Control has a hemolysis reference palette to determine quality of plasma/serum samples based on visual inspection. This method can be subjective and there are no specific rejection criteria for PFAS analysis.

Project Aims: The aims of the project are to develop a quantitative method for measuring hemolysis level in serum samples and to establish hemolysis rejection criteria for serum PFAS testing.

Methods: A method was developed and optimized to measure the extent of hemolysis. Linear calibration from 0 – 2000 mg/dL was established by multipoint standard addition in human plasma.

Heme concentrations were determined colorimetrically at 540 nm following sample incubation with Drabkin's reagent. Archived and de-identified whole blood samples were harvested for plasma. Hemolysis levels were measured, and plasma was analyzed for target PFAS using extraction and instrumentation methods currently utilized for routine testing at Wadsworth Center. Briefly, sample matrix was spiked with mass labeled internal standards. Samples were extracted to remove phospholipids and proteins and were analyzed using liquid chromatography tandem mass spectrometry. **Next Steps:** PFAS concentrations from paired whole blood, serum, and plasma samples with varying levels of hemolysis will be analyzed. The effect of hemolysis on PFAS concentration will be investigated. A rejection criterion for hemolysis will be established based on statistical analysis of paired PFAS results.

Presenter: Janet Lynch, janet.lynch@health.ny.gov

Establishing a State Public Health Testing Specimen Bank for Future Analysis of De-Identified Clinical Data and use of Associated Datasets

E. Mercado, M. Chatterjee, B. Wallace, M. Josefiak and K. Bush; New Hampshire Department of Health and Human Services

Biomonitoring is the measurement of chemicals in the human body from environmental sources such as air, water, soil and food. This has been an important branch of study at the New Hampshire Public Health Laboratory (NH PHL) due to specific chemical contaminants from the state's local geology, land-usage, historical and current industrial activities. In investigating these chemicals, biomonitoring can help explain the relationship between chemical exposure, body burden and human health. The NH PHL has over 300 donated specimens from the 2019 New Hampshire Tracking and Assessment of Chemical Exposures (TrACE) Study, a surveillance study that tested for 50 different chemicals in whole blood, urine and serum in residents across the state. By the end of summer 2023, BiomonitoringNH intends to launch a public health testing specimen bank at the NH PHL. To guide the development of a de-identified specimen bank, a policy and procedure is necessary. Participants indicated on their TrACE study consent forms whether they wanted their specimens to be destroyed at the end of the study or retained for future scientific use. To act on these elections and to ensure participant confidentiality and proper handling of all specimens and associated data, the policy and procedure created will outline specimen de-identification, tracking, storage and disposal as well as data retention guidelines. The creation of a public health testing specimen bank for the state of NH will support the generation and integration of high-quality environmental and public health datasets. This will include not only biomonitoring data, environmental testing data, epidemiological assessment data, and health indicator data, but infectious disease surveillance data as well. The existence of a state public health testing specimen bank and related datasets will provide other programs and organizations, such as neighboring states and academic partners, an opportunity to explore public health data in new ways as needs and interests arise in public health.

Presenter: Edith Mercado, edith.mercado@affiliate.dhhs.nh.gov

Exploring Environmental Risk from a Rural Health Perspective: A BiomonitoringNH and New England Public Health Training Center (NEPHTC) Initiative

V. Ciulla, M. Josefiak, N. Shonka, M. Chatterjee; New Hampshire Department of Health and Human Services

The New Hampshire State Biomonitoring Program aims to better understand and prevent exposures to environmental chemicals and substances by collecting exposure data, testing clinical samples, and educating NH residents of the findings. As a collaborative effort with NEPHTC, this project focused on preparation of study data and creating an analysis plan using a rural health lens to identify potential differences between rural and non-rural residents' exposure to water contaminants and body burden. The EMPoWER-U (Evaluating Metals in Private Wells and people for Exposure Reduction– Uranium) Study (conducted by BiomonitoringNH in collaboration with the US Geological Survey) focused on homes that use private (unregulated) wells as the primary source of drinking water and were located in areas of New Hampshire with a higher risk of uranium contamination in the groundwater. Along with home water samples, participants provided a urine sample, collected a home air radon sample and completed an exposure questionnaire. Water samples were tested for (among other analytes) uranium, arsenic, barium, beryllium, cadmium, cobalt, lead, manganese, molybdenum, strontium and thallium. Among other chemicals, urine samples were also tested for these chemicals, creating paired clinical and environmental data. Many of these homes sampled in the EMPoWER-U Study were located in rural and/or medically underserved areas of NH. In response to the results of the EMPoWER-U Study, this project focused on a more in depth analysis of rural and medically underserved areas of NH. Data was organized to create a "Master List" of all unique identifiers for all study results enabling rural/non-rural designation, and geocoding for census tracts to be assigned for each participant and household. A data brief was developed to summarize high-level data analysis of study population demographics. Based on the demographics of the data, an analysis plan that focused on rural health and/or medically underserved areas was developed.

Presenter: Victoria Pedersen Ciulla, victoriapciulla@gmail.com

Improving NJHANES Recruitment Strategies

E. Lax, T. Fan, E. Bind, C. Ho, E. Cook; New Jersey Department of Health Public Health and Environmental Laboratories

The New Jersey Department of Health (NJDOH) is conducting a probability-based population surveillance study that collects and analyzes health information, nutritional status and various environmental contaminants in New Jersey residents. The recruitment approach for the New Jersey Health and Nutrition Examination Survey (NJHANES) is undergoing modifications to reach 500 participants. From September 2021 through June 2022, NJHANES averaged five enrollments a month, below the target of 21 per month. The COVID-19 pandemic, government mistrust and holiday season busyness possibly contributed to initial recruitment setbacks. The recruitment packet, containing an enrollment letter, language service request, return card and prepaid envelope, is mailed to 4,800 randomly selected households throughout the two-year study period. As of December 2022, 129 respondents expressed interest via website link or QR code, or by calling NJDOH

or mailing the prepaid return card. In May 2022, a postcard was approved to follow up with any of the 4,800 households that missed the invitation. Ninety-two respondents are enrolled. Eligible respondents schedule a Microsoft Teams consent meeting and enroll by Eventbrite calendar link. Participants can enroll in person if technology is difficult. Questionnaires are administered virtually through REDCap, a HIPAA-compliant software. A mobile phlebotomy service (MPS) or certified NJDOH phlebotomist collects participants' biological samples at their households. The specimens are couriered to NJDOH for testing. Of the NJHANES population, 44% are over 60, compared to 24% of the NJ population. Of the NJHANES population, 43% have a graduate degree, compared to 16% of the NJ population. Seventy-one participants have been sampled; 15 samples collected from MPS were invalid due to low sample volume, clotted blood or incorrect collection container. Sixty-three participants completed the questionnaires and physical exam. Results and thank you gift cards are sent securely. To eliminate sampling errors, the contract with the MPS is terminated. NJHANES will temporarily rely on the NJDOH phlebotomist and investigate new MPSs. In June 2022, the incentive doubled to \$100, and the response rate increased to 20 enrollments per month. An additional 4,800 households will be invited next year. Other factors affecting the response rate will be investigated to provide practical approaches for state-level population surveillance studies.

Presenter: Emily Lax, emily.lax@doh.nj.gov

Improving the Quality of Pediatric Lead Test Results Through Enhanced Specimen Collection and Analysis Practices

J. Nassif, J. Liebreich, K. Granger; Association of Public Health Laboratories

The recent decrease in the CDC Blood Lead Reference Value (BLRV) to 3.5 ug/dL has highlighted the need for systematic changes that some clinicians and laboratories may need to implement to assure the quality of reported pediatric lead results. Building upon the recommendations of the Blood Lead Reference Value Workgroup, APHL has developed recommendations and resources designed to assist medical professionals and laboratory scientists minimize the likelihood of contamination when collecting and testing blood specimens. Also included are analytical practices that can improve precision, accuracy, and sensitivity of pediatric lead assays. Clinician office practices, public health and commercial clinical laboratories will all benefit from implementing these strategies.

Presenter: Julie Nassif, julianne.nassif@aphl.org

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

J. Gallardo, P. Behniwal, J. She; California Department of Public Health—Environmental Health Laboratory Branch

Volatile organic compounds (VOCs) are a group of chemicals that originate from both natural and anthropogenic sources. Cattle farms, wildfires, tobacco smoke, paints, solvents and gas-burning stoves are all examples of environmental 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 VOC urinary metabolites provides easy sampling (non-invasive) and data complementary to other modes of sampling for VOC exposure. In our method, we measure stable VOC mercapturic acid metabolites, formed with a high degree of specificity via the glutathione pathway, making them unique biomarkers. Our analytical method under development utilizes reversed-phase, ultra-high performance liquid chromatography (RP-UPLC) coupled with electrospray ionization triple quadrupole mass spectrometry in negative mode (negative ESI-MS/MS) to simultaneously identify and quantify 31 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, tetrachloroethylene, vinyl chloride and xylene in urine. Our objectives are to improve the chromatographic separation of the metabolites, in addition to enhancing their MS sensitivity through optimization of the parameters of the RP-UPLC separation, ionization source and MS analyzer. A 15 mM ammonium acetate aqueous mobile phase is commonly used to improve chromatographic separation and overcome the issues associated with false ion suppression or enhancement. For some VOC metabolites, such as those from acrylamide, ethylbenzene, N,N-dimethylsulfide, and styrene, the use of a 15 mM ammonium acetate mobile phase did not result in an optimal S/N. Lower concentrations of mobile phase additive increase S/N, albeit with a decrease in peak resolution. We tested ammonium acetate solutions of various concentrations (15 mM, 8 mM, 3 mM and 1 mM) and determined that 3 mM ammonium acetate mobile phase provided symmetrical peaks, adequate peak resolution and enhanced sensitivity. The optimization of the parameters of the ion source and the analyzer, and the results will be presented and discussed.

Presenter: Jonathan Gallardo, jonathan.gallardo@cdph.ca.gov

Method Validation of Per- and Polyfluoroalkyl Substances (PFAS) in Human Plasma using High Pressure Liquid Chromatography Mass Spectrometry (LC-MS/MS)

L. Zhong, D. Mathes, C. Xu, T. Fan, S. O'Leary; New Jersey Department of Health Public Health and Environmental Laboratories

The properties, utility, environmental impact, and potential adverse human health outcomes of Per- and polyfluoroalkyl substances (PFAS) are well documented. The quantitation of PFAS in human serum liquid chromatography tandem mass spectrometry (LC-MS/MS) method is also well established. This project aims to expand the scope of the already validated in-house method for testing PFAS in human serum to include human plasma. The quantitation of PFAS in human serum and plasma by the same (LC-MS/MS) method is reported and the presence of or absence of clotting factors does not affect the accuracy of the method. The only two differences between serum and plasma are that plasma contain clotting factor and EDTA from the collection tube. During the validation we will evaluate if these differences effect the accuracy and precision. These factors can stem from known concerns associated with this analysis include matrix enhancements, suppressions, and most importantly contamination from cartridges, solvents, and other materials. Our preliminary results show excellent agreement

of 12 target PFAS analyte concentrations between serum and plasma with a coefficient of variance between to 0.0104 to 0.189 in two specimens. The precision and accuracy will be assessed by repeated measurements (n=8) at two different concentration levels spiked, targeting ~1 ng/mL and ~10 ng/mL of PFAS concentration levels respectively. The accuracy must be within $\pm 20\%$ of nominal value and the calculated RSD is 0.99.

Presenter: Linbin Zhong, linbin.zhong@doh.nj.gov

NH PHL New Methods: Biomonitoring and Air Quality Assessment for a Targeted Study in Berlin, NH

G. Santos, M. Chatterjee, B. Wallace, M. Josefiak, K. Bush, C. Snow; New Hampshire Department of Health and Human Services

The New Hampshire State Biomonitoring Program (BiomonitoringNH) plans to launch a targeted biomonitoring study to investigate linkages between environmental exposures and the corresponding body burden in residents of Berlin, NH. This town is home to a highly vulnerable population and several sources of potential chemical exposures, including some that specifically contribute to worsened air quality. Due to the potential of air inversion events, a large biomass power plant and an increased prevalence of wood-burning home heating systems, indoor and outdoor air quality in Berlin may pose an increased risk of exposure to certain contaminants and associated health risks for residents. With emphasis on implementing new methods for both clinical and environmental air quality analysis, BiomonitoringNH aims to increase testing capabilities and collect data to promote actionable change for exposure reduction in Berlin. Accomplishing this goal requires new data collection methods at the New Hampshire Public Health Laboratories (PHL) including the use of newly acquired air monitors and validation of a method to identify polycyclic aromatic hydrocarbon (PAH) metabolites in urine, as well as outlining potential data analysis to determine associations between the two datasets. For collection of environmental data, small batches of 5 to 10 PurpleAir air quality sensors will first be co-located in the same environment to determine if all of the sensors yield similar results. This quality assurance step will help to evaluate each sensor's efficacy for use in the citizen science component of the Berlin study. For clinical results, PAHs remain the primary analyte of interest to evaluate the presence of PM_{2.5} – an invisible, yet harmful type of particulate matter found in air pollution. Participants will provide urine samples to be analyzed using a method to quantify PAH metabolite concentrations. Validation of this method at NH PHL will involve collaboration with New Jersey PHL and Centers of Disease Control and Prevention. Lastly, participants will also be asked to complete an exposure questionnaire to aid in identifying potential routes of exposure, highlighting key contaminants unique to Berlin and evaluating the potential chemical exposure risks from their everyday actions. Based on prior BiomonitoringNH study results and reports, a data analysis plan and an outline for an exposure questionnaire will be developed for the Berlin study. The outcomes of this work will guide the execution of the Berlin study over the next year.

Presenter: Grace Santos, grace.v.santos@affiliate.dhhs.nh.gov

Quantitative Analysis of Metals in Dried Blood Spots (DBS) by Inductively Coupled Plasma Mass Spectrometry

V. Chandra, A. Steffens, H. Arman, T. Fan, D. Haltmeier, E. Bind;
New Jersey Department of Health Public Health and Environmental Laboratories

Dried blood spots (DBS) have been used in analytical chemistry for decades due to certain advantages including stability, convenience, and noninvasiveness. DBS are a primary tool for newborn screening programs and have the potential to offer significant untapped data beyond the routine screening conducted on them. Advancements in analytical techniques in recent years have broadened the scope of DBS in clinical medicine. Notably, prenatal exposure to toxic metals affects brain development and other health outcomes, including autism. Here, we describe the validation of a DBS analytical procedure to detect and quantify toxic metals. The laboratory conducted method development to address known concerns associated with testing for metals in DBS including extraction, contamination, reproducibility, and accuracy. In this method, blood samples were spiked with the target analytes at different concentrations, spotted on DBS collection paper, and later analyzed by ICP-MS after various processes, which include drying, extracting with diluent, shaking, centrifuging, and decanting. Targeted metals consisted of aluminum, cadmium, mercury, lead, copper, manganese, selenium, thallium, and zinc. The reportable ranges for these metals on DBS were established by using publicly available population concentration data from NHANES and based on the detection limits from preliminary data using this extraction method. The parameters that were used to validate the method are linearity, accuracy, precision, and linear dynamic range (LDR). Those parameters were evaluated using preliminary data collected in the laboratory. Conditions optimized during development include diluent components, spiking procedure, spotting technique, and more. The method is robust enough to recover the desired analytes in range that is acceptable to meet the study objectives. Aluminum was dropped from the method due to contamination of the filter paper. Linearity for all remaining analytes is strong ($r > 0.995$) using a weighted curve ($1/x^2$). Accuracy for the method is $\pm 30\%$ (except for low-level Hg and Mn, which is $\pm 50\%$ recovery). Precision of the method is within $\pm 30\%$ RSD, with most falling within $+20\%$. LDR exceeds targeted reportable range for all analytes. The method is in the final stages of validation. Data collected using this technique will be compared to biomarkers related to autism from deidentified blood spots provided by the NJ Newborn Screening Program. Preliminary analytical results will be presented in addition to the method development data.

Presenter: Hosen Arman, hmarman535@gmail.com

The Road to the Measurement of PFAS

A. Burditt¹, J. Hover-Jeansonne²; ¹Texas Department of State Health Services, ²Texas Department of State

As part of the Newborn Screening Program in Texas, blood spots are collected on protein saver cards and analyzed for various life-threatening conditions. The newborn population is not screened for exposures relating to environmental chemicals at this time. A better understanding of environmental chemical exposures is needed to build knowledge of this population, their exposure during the fetal development period and in the first 21 days of life. In this study, we are formulating and validating a method to detect PFAS (per-

and poly- fluoroalkyl substances) captured on protein saver cards. Early exposure to toxic chemicals in a very vulnerable population can have life-impacting long-term consequences. This work has involved building an instrument method and sample preparation method for an Ultra-High Pressure Liquid Chromatography Tandem Mass Spectrometer and going through a clinical method validation process (accuracy, precision, regression/goodness fit, carryover, specificity, sensitivity, limit of detection, analytical measurement range, matrix effects, linearity, and interference assessment). The development process for the detection and quantitation of PFAS compounds from protein saver cards is intended to produce reliable, robust, reproducible, and rugged data. Upon completion of the method validation (data collection/review/analysis, report, standard operating procedure), the project will move to Phase 2. One of the chief goals of Phase 2 is to begin data collection for select PFAS compounds within the population of archived specimen protein saver cards, whose parent/guardian has elected to allow the remnant samples to be used for research and method development. The demographic features of the representative population selected will align, as best as possible, with the population of Texas and will hopefully allow further insights and correlations with birth weight, sex, and location with PFAS compounds detected. Ultimately, this biosurveillance will provide baseline exposure information for one of our most vulnerable populations and allow understanding between disease and environmental chemical exposure during fetal development and the first 21 days of life.

Presenter: Ashley Burditt, ashley.burditt@hotmail.com

BIOSAFETY AND BIOSECURITY

Developing Laboratory-specific Risk Assessment Tools for Emerging Infectious Diseases

A. Causey, O. Adair, N. Epie; South Carolina Department of Health & Environmental Control

As public health laboratories are the front lines for the diagnostic testing of newly emerging or reemerging infectious diseases, laboratory professionals must quickly adapt biosafety and biocontainment plans to safely meet unique situational and site-specific challenges. The utilization of frequently shared risk assessment tools provide a basic framework to quickly conduct hazard identification, risk evaluation, and mitigation selection for a new work process or hazard, however; the modification of these tools is often needed to address the variety of hazards and processes present in a laboratory setting. South Carolina Department of Health and Environmental Control Public Health Laboratory (SCDHEC PHL) created a procedure and template for facilitating change management incorporating the following elements; brainstorming possible physical, chemical, or biological hazards in a laboratory setting; utilizing a "what-if" hazard analysis structure and a risk matrix to conduct risk evaluation; establishing expected actions based on initial risk ratings; selecting mitigation strategies based the hierarchy of controls; and documenting the evaluation process. In addition, often overlooked support items were incorporated (ex. materials storage, occupational health needs, training program modification, waste management, etc). This procedure and template was then implemented as a guide for assessing the initial safety and support needs of a new process and

carried through to the next step, and continual process, of the Plan, Do, Check, Act change management and process improvement cycle. SCDHEC PHL has successfully adapted and utilized a Laboratory Risk Assessment Tool (LABRAT) to guide the risk management process in a laboratory setting. This LABRAT utilizes a holistic hazard identification process, a “what-if” hazard analysis structure, and incorporates risk matrix to guide the selection of mitigation strategies prioritized based on a hierarchy of controls.

Presenter: Andrea Causey, causeyam@dhec.sc.gov

Ebola Biosafety Response: Then and Now

M. Marsico¹, M. Pentella², S. Campbell¹, N. Miller², N. Cornish⁴; ¹Association of Public Health Laboratories, ²State Hygienic Laboratory at the University of Iowa, ³Yale University School of Medicine, ⁴US Centers for Disease Control and Prevention

The previous and current Ebola Virus Disease (EVD) outbreaks in 2014-2016 and 2022-2023 have called attention to the climate of biosafety and biosecurity in laboratories around the United States (US). During the 2014-2016 Ebola outbreak, significant gaps were identified in US laboratory biosafety practices, specifically around laboratory instruments to test patient specimens along with issues on the use and disinfection of these laboratory instruments. The concerns around instrument safety led laboratories to delay or refuse specimen testing due to the fear of exposure to EVD. In 2022, similar concerns were raised around testing potential Ebola samples. The 2022 experience, as in 2014, identified gaps in understanding the biosafety impact throughout clinical laboratories testing samples from patients suspected to be infected with ebolavirus. This was confirmed by anecdotal evidence and a small survey of clinical laboratories. The gaps identified in both outbreaks demonstrate the need to improve knowledge, practice, and capabilities regarding clinical laboratory biosafety to be prepared for future emerging threats. Biosafety practices need to improve for both the laboratory community and equipment manufacturers. The poster will highlight biosafety gaps from both outbreaks including recent surveys distributed from state public health laboratory agencies to clinical laboratories highlighting Ebola testing concerns along with potential solutions for future outbreaks.”

Presenter: Michael Pentella, michael-pentella@uiowa.edu

Piloting the ISO 35001 Standard: Cruising towards Biorisk Management

M. Marsico¹, J. Baron²; ¹Association of Public Health Laboratory, ²Science and Safety Consulting, LLC

This poster will introduce the International Organization for Standardization (ISO) 35001: Biorisk Management for Laboratories and Other Related Organizations to viewers along with outlining the APHL/CDC ISO 35001 Implementation Project across two public health laboratories. APHL, in collaboration with the CDC have developed a strategy to provide guidance for laboratories interested in ISO 35001 implementation. We are currently piloting the implementation of ISO 35001 at two public health laboratories through 2023. This poster will outline the standard and why it is important along with key takeaways by those laboratories involved in the pilot project and ideas for future projects.

Presenter: Michael Marsico, michael.marsico@aphl.org

Public Health Laboratory Construction and Renovation Helpful Tips

L. Wallace¹, M. Downey², M. Marsico³, S. Escott², C.J. Woolverton⁴; ¹West Virginia Office of Laboratory Services, ²ABSA International, ³Association of Public Health Laboratories, ⁴Kent State University

Post COVID-19, funding has increased for public health readiness and infrastructure. Many laboratories are improving/expanding BSL-2 and BSL-3 containment laboratories to increase the capacity for work on emerging infectious diseases, such as SARS-CoV-2, as well as re-emerging diseases such as Poxvirus, Poliovirus, Ebola, and Mycobacterium tuberculosis. These improvements should be undertaken with input from all stakeholders (e.g., Biosafety, Safety, Maintenance, Security, Quality), an appreciation for the unique requirements of containment (e.g., directional airflow, waste handling, equipment and room decontamination), and consideration of validation and verification of equipment and the facility at the conclusion of the project. The aim of this poster is to provide guidance for biosafety professionals or those with biosafety responsibilities to guarantee that they are part of the early planning process and involved in developing project specifications. This will help ensure that contracts are awarded to experienced laboratory engineering and commissioning firms that are able to meet all regulatory requirements. The poster will include testimonials from laboratories involved in rebuilding and/or renovation projects, construction references, helpful tips for surviving a construction project and FAQs about the process.

Presenter: Lisa Wallace, lisa.m.wallace@wv.gov

CANNABIS, OPIOIDS AND OTHER CONTROLLED SUBSTANCES

Can Commercially Available Field Tests Reliably Detect Trace Methamphetamine?

W. Naviaux^{1,2}, C. Hedman³, H. Barkholtz^{1,4}; ¹Forensic Toxicology Section, Environmental Health Division, Wisconsin State Laboratory of Hygiene, ²Pharmacology and Toxicology Program, School of Pharmacy, University of Wisconsin-Madison, ³Wisconsin Department of Health Services, Division of Public Health, Bureau of Environmental and Occupational Health, ⁴Pharmaceutical Sciences Division, School of Pharmacy, University of Wisconsin-Madison

Currently there are several commercial methamphetamine surface contamination detection devices and colorimetric tests marketed to first responders, public health officials, and individuals. However, none of these devices or tests have peer reviewed research supporting the manufacturer’s stated methamphetamine detection limits. This work seeks to fill those gaps. These tests are used at critical times from identifying a need to remediate a property to child custody decisions. Without solid evidence to back these tests up the confidence put into these tests is concerning and doesn’t allow for informed decision making. Common colorimetric tests and handheld devices utilized by Wisconsin’s public safety

agencies were identified for testing. These devices were tested at the manufacturer's stated limit of detection, when available, or the EPA recommended level of 1.5 µg/100cm² when no detection level was noted by the manufacturer. Simulated methamphetamine contaminated surfaces were prepared by placing known amounts of methamphetamine in methanol on glass petri dishes. These surfaces were dried and tested for surface contamination per colorimetric test manufacturer directions. Experiments were repeated at increasing concentrations (in triplicate) until the device or test reliably detected methamphetamine. Seven fields tests were examined including (advertised LOD in parentheses): MethChek 100 Immunoassay kit (0.5 µg/100cm²), AccuMeth 1.5 (1.5 µg/100 cm²), MobileDetect DME Meth/MDMA Test (n/a), MobileDetect MDT Multi-Drug Test (n/a), Mistral D4D Pen Test (n/a), Mistral Meth-X Pen Test (n/a), and ID2 Handheld Meth Scanner (1.5 µg/100 cm²). Results have identified the MethChek as reliably meeting the manufacturer stated level of detection (0.5 µg/100cm²). Ability to detect methamphetamine at or below the EPA level of 1.5 µg/100cm² was rare, with most commercially available colorimetric tests requiring higher concentrations to achieve a positive indication. Based on these findings there are several tests that are used by first responders, public health officials and others who are attempting to make informed decisions on methamphetamine contamination clearance decisions, that have limits of detection greater than the EPA's suggested detection limit of 1.5 µg/100cm². Information about the performance and reliability of commercially available tests is vital to the health and safety of those living and working in former or potential former clandestine laboratories.

Presenter: William Naviaux, willnavx@gmail.com

Developing an Analytical Method for Confirmatory Testing of Common Medications for Opioid Use Disorder at the District of Columbia Public Health Laboratory

K. King, O. Jackson, G. Taylor, J. Jacob; DC Public Health Laboratory - Clinical Toxicology Unit

Methadone and buprenorphine are two commonly prescribed medications for opioid use disorder (MOUDs) when healthcare providers utilize a medication-assisted treatment approach. There are unique advantages and disadvantages to both medications; therefore, the current recommendation is for healthcare providers to take a patient-centered approach when choosing a MOUD. Confirmational testing for prescribed medication can be done using solid-phase extraction (i.e., sample preparation and clean-up) followed by liquid chromatography-tandem mass spectrometry (SPE-LC-MS/MS) or solid-phase extraction followed by gas chromatography-mass spectrometry (SPE-GC-MS). However, LC-MS/MS is often the preferred method of analysis, given that it can identify a wider range of compounds, provides more sensitivity and selectivity, and tends to have a shorter analysis run time. Currently, our laboratory uses SPE-GC-MS to confirm and quantitate methadone and its metabolite, 2-Ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP). However, as we seek to expand our MOUD monitoring program to test for buprenorphine and its metabolite, norbuprenorphine, we anticipate developing and validating a novel analytical method using SPE-LC-MS/MS. When validating the method, we will examine the following performance characteristics: precision, accuracy, sensitivity, specificity, total

allowable error, reportable range, reference interval, carryover, matrix effects, interference, and analyte stability.

Presenter: Kaitlin King, kaitlin.bratt@gmail.com

Simultaneous Analysis of Nicotine and Cannabis Metabolites in Serum by Isotope Dilution UPLC-MS/MS Method

Y. Li, J. DeGuzman, P. Behniwal, S. Wang, H. Ip, J. She; California Department of Public Health—Environmental Health Laboratory Branch

Tobacco and cannabis smoking have harmful effects on human pulmonary, cerebrovascular and neurological functions. In this presentation, we report a method developed for quantitation of the toxic chemicals that enter the human body through tobacco/cannabis smoking. We measured cotinine (COT), delta-9-tetrahydrocannabinol (Δ9-THC), cannabinol (CBN), and 11-hydroxy-delta-9-tetrahydrocannabinol (OH-THC). In brief, 100 µL of serum was cleaned up by a 96 well plate under positive pressure hybrid solid phase extraction. Sample analysis was then performed with an ultra-high pressure liquid chromatography coupled with an electrospray ionization-tandem mass spectrometry (UPLC-ESI-MS/MS) in positive mode. The method performance was evaluated though measuring the parameters including the accuracy, precision, detection limits and repeatability. The evaluation results demonstrate that the developed method is selective and sensitive for analyzing the compounds mentioned above in human serum. In details, the sample pre-preparation and clean up procedures include: 1) denaturing peptides/proteins, protein precipitation (PPT), breaking down protein binding with COT and THC, and cleaving glucuronide-analytes conjugations; 2) clean up with a SPE cartridge of strong cation exchange and weak reverse phase properties; and 3) SPE eluted with NH₄OH-ACN and then dried through a gentle stream of N₂ and finally reconstituted with ACN. Samples were introduced to UPLC through an Agilent MultiSampler in MultiWash mode and the analytes were separated with a Phenomenex EVO C18 column under weak basic condition. Post Column Addition with 0.1% formic acid-methanol enhanced ionization efficiency of MS. The method performance and application will be presented and discussed.

Presenter: Ying Li, ying.li@cdph.ca.gov

COMMUNICATIONS, ADVOCACY AND PUBLIC RELATIONS

Barriers to using an APHL Consortium Developed Toolkit in Jurisdiction-Branded Media Publications

A. Berlon, Indiana Department of Health

Public health laboratories across the country continue to experience challenges in recruiting and hiring qualified candidates to fill laboratory vacancies. This poster explores the barriers of using a developed media toolkit in various state publications (i.e., Facebook, Twitter, Instagram, LinkedIn). After working for several months to gain the appropriate approvals of the materials, the initial organic feed post made 963 impressions in the first two hours, which can

be an impressive contrast of impressions compared to sending lab staff to a college fair or career fair for the same amount of time. Therefore, to appropriately respond to the staffing needs of public health laboratories, especially in matters of public health emergencies, the identified barriers need to be addressed by a cross-divisional team within each jurisdiction to better promote career opportunities at public health laboratories.

Presenter: Amanda Berlon, aberlon@health.in.gov

DATA AND ANALYTICS

Automating Data Processing and Data Management in a High-throughput Public Health Laboratory using Google Colaboratory

S. Baird, M. Hetherington-Rauth, A. Rossheim, N. Pysnack, L. Bankers, S. Matzinger; Colorado Department of Public Health and Environment

High-throughput biotechnology allows public health laboratories to sequence large numbers of pathogen genomes, but it also increases the burden of data processing and data management tasks on laboratory and bioinformatics staff. Python is a versatile programming language with rich data science libraries, like Pandas, that can fully automate such tasks. Python code can be executed in Jupyter notebooks, which are interactive documents for incorporating code and text in one place. Jupyter notebooks can be created, shared, and run in Google Colaboratory (Colab), a secure cloud-based web platform within Google Drive. Colab has advantages over other Jupyter notebook platforms because, like Google Docs, it requires no software installation except a web browser, and different users can access the same Colab notebook, ensuring all users can see all edits. Colab can also directly read and write files from and to Google Cloud Platform and Google Drive, allowing for automatic data transfer. Sequencing and bioinformatics staff in the Colorado State Laboratory at the Colorado Department of Public Health and Environment use Colab to automate multiple tasks, saving time and reducing human error. In the Sequencing Unit, we developed a Colab notebook that reads a set of COVID-19 PCR result files, then generates files used by a liquid handling robot to select and consolidate positive samples for sequencing. Generating these files using a previous method was often an hours-long and error-prone process of manually copying and pasting between multiple spreadsheets for each batch of samples. Now, using Colab, this process takes less than five minutes. In the Bioinformatics Unit, we implemented a Colab notebook that reads COVID-19 sequencing result files from Google Cloud Platform and flags variants of concern and quality control failures. Then, it writes the sequencing result files directly to Google Drive. Using this notebook, we re-analyzed initially manually inspected sequencing result data. This automated analysis revealed previously unnoted SARS-CoV-2 lineages within our laboratory, and it also revealed cases where sequencing coverage was missing in a key area of the genome, demonstrating improvements in the depth and speed of analysis. While Colab is a powerful platform, Python programming is an unfamiliar skill to many staff who would benefit from the ability to create and edit code in Colab notebooks. To help develop this skill at our laboratory, we are using APHL's DataCamp training program. Such workforce training programs will aid in the development of

automation tools to keep up with the increasingly high-throughput world of laboratory technology.

Presenter: Samuel Baird, sam.baird@state.co.us

Biotin Supplement Use in the United States, January 2017–March 2020

J.R. Leachman¹, S. Mishra¹, J. Gahche², B. Kit¹, R. Storandt¹; 1US Centers for Disease Control and Prevention, 2National Institutes of Health

Background: Biotin is an essential water-soluble vitamin that is naturally occurring in food and available in dietary supplements commonly used for hair, skin and nail health. In 2017, the Food and Drug Administration issued a communication to alert the public of the possibility that biotin-containing dietary supplement use (BDSU) can interfere with clinical lab tests and mask serious clinical conditions. For example, high biotin blood levels can yield falsely low troponin results, which could lead to missed diagnoses of heart attacks. Biotin interference is of particular concern with BDSU $\geq 1\text{mg}$. We assessed BDSU by sociodemographic characteristics among US children, adolescents and adults. **Methods:** Prevalence of any BDSU and BDSU $\geq 1\text{mg/day}$ and $\geq 5\text{mg/day}$ were analyzed from 15,548 participants in the January 2017–March 2020 pre-pandemic National Health and Nutrition Examination Survey using the 30-Day Dietary Supplement Questionnaire data. Interview survey weights and Taylor series linearization were used to produce nationally representative estimates and to account for the complex survey design. Sociodemographic differences were tested with t-tests.

Results: Approximately 29% of US children, adolescents and adults reported any BDSU in the past 30 days. Females had higher BDSU than males (31.6% vs 26.2%, $P < .001$). BDSU was highest among adults 60+ years and lowest among adolescents 12–19 years (41.4% vs 15.7%, $P < .001$). Non-Hispanic (NH) white (33.6%) had higher BDSU compared to NH Asian (28.7%), NH black (21.3%), and Hispanic people (19.5%) (each $P < .01$). BDSU $\geq 1\text{mg/day}$ and $\geq 5\text{mg/day}$ in the past 30 days overall was 2.9% and 0.8%, respectively, and increased with age (4.8% $\geq 1\text{mg}$ and 1.7% $\geq 5\text{mg}$ among adults 60+ years) (linear P trend $< .0001$). **Conclusions:** Overall, these results warrant detailed history-taking of supplement use especially among older adults who have the highest prevalence of BDSU, particularly given the increased rates of evaluation for heart disease in this group.

Presenter: Jacqueline Leachman, tqd0@cdc.gov

COVID-19 Variant Dynamics Among Individuals with Repeat Whole Genome Sequencing Tests in South Carolina

S. Sarkar, J. Scott, G. Goodwin, D. Drociuk; South Carolina Department of Health & Environmental Control

Abstract: With the rapid development of whole genome sequencing (WGS) for COVID-19 variant identification, the use of these results by public health is also in development. In order to evaluate the ability and functionality of using WGS results, an exploratory analysis of a public health surveillance system, the South Carolina Infectious Disease and Outbreak Reporting Network (SCION), was performed. This analysis evaluated WGS reporting dynamics, including repeat WGS testing, time between WGS testing, and proportion of individuals with WGS results indicating multiple COVID-19 variants among the SC population. **Methods:** Laboratories from across

South Carolina, including our state Public Health laboratory, submit COVID-19 WGS results to SCION. Results with specimen collection dates between March 27, 2020, and December 19, 2022, were obtained using unique identifiers from SCION. LOINC codes, indicating WGS variant results, were selected for analysis. Lineages and sub-lineages of all variants were identified and classified by the main variant classification using outbreak.info, while Omicron sublineages were further classified using CDC's Variant Proportions within the COVID-19 Data Tracker. Invalid and duplicate results were removed from analysis. Microsoft Access/Excel and SAS were used for descriptive analysis. **Results:** A total of 31,154 unique individuals (1.7% of total confirmed and probable cases reported to public health in SCION) yielded 31,694 WGS results. Of those, 508 (1.6%) individuals had multiple WGS results. A majority of WGS results identified Delta (42.8%) and Omicron (52.7%) variants. Among the 508 individuals with multiple WGS results, the average time between specimen collection dates was 33 days, with most (95.1%) individuals having 2 WGS results and the remainder having up to 5 results. Multiple infections with different variants or differing Omicron sublineages were reported among 86 (16.9%) individuals (total of 87 infections due to some individuals having more than 2 infection events), with an average time between specimen collection dates of 168 days. Among these, 54 (62.1%) had different variant infections and 33 (37.9%) had differing Omicron sublineage infections. **Conclusion:** This analysis assessed the current state of variant dynamics among South Carolina COVID-19 cases with WGS results. A small percentage (1.7%) of confirmed and probable cases had WGS results and may not be representative of the population. The large proportion of Delta/Omicron discordant pairs is likely due to the Omicron surge and its greater infectivity and transmissibility. Within-host diversity may have contributed to the short time duration between many of the differing Omicron sublineage infections. These findings prompt advanced investigation into variant dynamics. This analysis is ongoing, and updated results will be available for the May APHL Conference.

Presenter: Sunanda Sarkar, sarkars@dhec.sc.gov

Developing Resources to Facilitate Expanded Application of Non-Targeted Analysis Methods

J. McCord¹, S. Nason², Y. Feng³, J. Sobus¹, C. Fisher⁴, R. Marfil-Vega⁵, A. Phillips¹, J. Sloop⁶, S. Bayen⁷, G. Johnson⁸; ¹US Environmental Protection Agency, ²Connecticut Agricultural Experiment Station, ³Health Canada, ⁴US Food and Drug Administration, ⁵Shimadzu, ⁶Oak Ridge Institute for Science and Education, ⁷McGill University, ⁸University of North Carolina - Chapel Hill

Non-targeted analysis (NTA) using gas or liquid chromatography coupled with high resolution mass spectrometry is a powerful analytical technique that is increasingly used to study organic contaminants in situations where it is impossible or impractical to have analytical standards for all analytes. Common applications include environmental monitoring, food safety evaluation, human exposure and biomonitoring, forensic science, epidemiology, and chemical threat detection, and there is enormous potential for increased use in many analytical contexts. However, methods for ensuring quality control, accuracy, and reproducibility of NTA results are still in development for this emerging technique. The Benchmarking and Publications for Non-Targeted Analysis working group (BP4NTA) is focused on increasing use of and improving the quality of NTA. We have recently published several products

that contribute to this goal including the NTA Study Reporting Tool (SRT), a manuscript that proposes performance metrics for NTA, and a website that includes substantial NTA reference content. As additional BP4NTA efforts develop and progress, the Stakeholders Subcommittee is working to engage with data producers and users from various fields through a series of meetings and surveys with a main goal of learning about drivers and barriers of NTA adoption and data use. Results from this effort will be used to inform and prioritize future BP4NTA activities, ensuring that BP4NTA-generated resources will be of value to the broader scientific community. To date, we have hosted three separate stakeholder meetings, with representatives from food and agriculture laboratories, medical devices regulators, and rapid responders. Future stakeholder meetings will include employees of commercial environmental laboratories, regulatory risk assessors, epidemiologists, students/trainees and others. Preliminary stakeholder survey results indicate high interest in the use of current BP4NTA resources such as our website and proposed performance metrics, demand for data processing tools such as databases and libraries, and enthusiasm for additional strategies to demonstrate data quality and study reproducibility. Continued feedback from NTA-adjacent stakeholders, including those in the Association of Public Health Laboratories (APHL) community will help us to further refine our efforts towards the most useful tools and resources for broadening NTA implementation and use.

Presenter: James McCord, jmccord314@gmail.com

How to Make Informed Decisions to Build Healthcare Resilience for the Future: The Criticality of Data Synchronization Between the Frontlines and Supply Chain Operations

T. Coleman, J. Franck; Deloitte Consulting LLP

Numerous supply chain articles discuss the potential for technology such as blockchain and artificial intelligence (AI), to turbocharge supply chain resilience. They delve into how technology will transform an organization's supply chain to become more agile and resilient. However, in practice, many technology projects do not achieve their promised return on investment and target resilience outcomes. Deloitte recently analyzed 55 survey responses and interviewed over 50 supply chain leaders in the private and public sector across a range of industries, including life sciences, government, health care providers, academic institutions, humanitarian non-governmental organizations (NGOs), retail and technology to better understand key determinants for resilient health care supply chains. Our recently published research indicated that organizations, i.e., health care entities, are not achieving supply chain resilience by leading with technology. Instead, they are increasing resilience through organizational commitment from leadership and staff, and conscious spending by identifying and addressing root causes of health care supply chain issues. We discovered that technology proves far less effective in crisis when the members in the same supply chain ecosystem do not have standardized definitions, communication methods and roles, with the lack of clarity slowing response and hindering operations. Standardizing the basics, like data sharing, authorities and role of the entities involved, is key to maintaining a healthy supply chain ecosystem. Our latest round of research examined how private and public sector frontline workers overcame recurring obstacles to maintain efficient operations and promote supply chain resilience.

Our objective was to understand the perspective of the frontline and identify common root causes for prominent organizational challenges they faced in daily operations. We gathered insights on how frontline workers overcame those challenges, their priorities, lessons learned, and opportunities for improvement that directly impacted resilient supply chain operations across the private and public health care sectors. We employed a multi-pronged approach by interviewing both supply chain leaders and frontline workers across private and public hospitals to understand their respective views. We also conducted supplemental research consisting of follow-up interviews with the supply chain leaders whom we previously engaged to gain additional insights on the persistent organizational challenges that impact supply chain resilience and what has changed over the past year across people, processes and technologies. This supplemental research served to provide a multi-industry viewpoint via case studies that amplified the significance of key industry-agnostic organizational factors that impact overall supply chain resilience and performance.

Presenter: Sarah Slomski, sslomski@deloitte.com

Improving Visibility of Quality Indicators Through Automated Dashboarding: An Effective Way to Enhance Laboratory Quality Management System

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Background: Maintaining compliant and robust Quality Management Systems traditionally involve many laborious and manual processes. Manual data collection and analysis activities require dedicated staffing resources, lead to system inefficiencies, and may result in compliance gaps. One example is provided by personnel competency assessment, which is one of the top deficiency findings cited during accreditation inspection associated with Clinical Laboratory Improvement Amendments (CLIA) and topped the list in 2021 at 18.96% (CMS, 2021). Tracking and documenting competency assessments is a challenging task for busy laboratories with hundreds of laboratory staff and extensive test menus. This poster presents development of automated interactive dashboards as a tool to empower Directors and Supervisors to access and view competency assessment status of their staff and take meaningful actions to meet key regulatory requirements. **Methods:** In 2022 a secure file transfer of weekly backup data for laboratory staff competency assessment records was established with our enterprise quality management program provider (MediaLab). Using a SQL query, backup files were compiled into a single database which has been utilized to develop dashboards. Dashboards were developed for each laboratory section with specific focus on Directors and Supervisors as the intended audience. Priority of data displayed was given to competency status (due soon, in progress, complete, overdue) of each staff member. Further metrics (% completed and % finished on time) were included to highlight trends and potential areas for quality improvement. The data model and dashboards were developed using Microsoft PowerBI (PBI). Dynamic charting and slicer features in PBI were used to allow users to intuitively interact with the dashboard and quickly navigate to key critical data.

Results: Automated dashboards allowed Directors and Supervisors to quickly view the high-level status of competencies and drill into details on a particular competency assessment or see the status

of an individual staff member's competencies. While not a metric specifically targeted for dashboarding, an additional outcome of the dashboard allowed Supervisors to identify competencies mismatched to their staff. **Conclusions:** Development of automated dashboards eases the burden of laborious manual data collection, analysis, and reduces data errors associated with reporting competency assessment information allowing Directors and Supervisors to focus on staff compliance activities. Specifically, metrics such as % finished on time, enables quick identification of problem areas affording rapid actions to improve compliance.

Presenter: Nevada Ruehlen, nevada.ruehlen@doh.wa.gov

DIAGNOSTICS AND NEW DISEASE TECHNOLOGIES

A Landscape of Infectious Disease In Vitro Diagnostic Devices: From 2000 to Present

E. Bradford, K. Yeh, G. Olinger, R. Soong; MRIGlobal

In vitro diagnostic (IVD) devices encompass a broad range of tests that include infectious diseases and have been used to detect and diagnose pathogens for decades. Recent outbreaks such as mpox and the Sudan ebola virus have led to greater need for IVDs that bring the point of care out of the clinical setting and directly to the user, as exemplified during the COVID-19 pandemic. These point of care (POC) devices are ideally portable, functional in low resource environments, easily interpreted, and have limited logistical or storage requirements from manufacturer-to-user. However, this high bar for functionality is supported by novel bioengineering and assay chemistry developments. The rapidly expanding market for infectious disease POC IVDs highlights the need for a practical approach to development that considers a wide scope of factors to include POC characteristics, detection technology, and market assessments including public health to support forward leaning product development. Together, these many factors underscore the need for a clearly defined path from conceptualization to market launch. In this review, we illustrate how Infectious Disease IVD development has evolved while highlighting POC device-specific considerations.

Presenter: Elaine Bradford, ebradford@mriglobal.org

A Panther Fusion Clade II Mpox Lab Developed PCR Assay - Optimization Using the Open Access System

R. Villa, J. Benfer; State Hygienic Laboratory at the University of Iowa

Background: Mpox is a double-stranded DNA virus belonging to the Orthopoxvirus genus. In early May 2022, mpox was reported outside of Central/Western Africa for the first time since 2003, signifying the beginning of a global outbreak where the U.S. has been leading in cases and deaths. To respond to the outbreak, the CDC recommended the Non-variola Orthopoxvirus Generic Real-Time PCR Test for public health labs to test clinical samples and aid in the detection of mpox. Though this test has reliably served as an important diagnostic tool for many public health labs during the mpox outbreak, there are some aspects- timeliness, labor costs, convenience, and workflow efficiency- that require improvement in

the case where high-volume testing may be necessary. Here, we describe the process for developing and optimizing a laboratory-developed assay (LDA) for clade II mpox on the automated Panther Fusion platform and Open Access software. **Methods:** Various concentrations of reagents in a primer probe mix were tested to optimize the LDA. Then, the LDA was validated using 10 positive and 10 negative human mpox samples previously characterized by the CDC method to test for accuracy, precision across two Panther Fusion machines and two days, sensitivity, and specificity among HSV-1, HSV-2, and VZV human samples. Several spiked media extensions (the Aptima Multitest Swab Transport Media and kit, RTM4, an in-house Universal Transport Medium, and dry swabs) were also validated by testing two positive spiked mpox samples and two negative samples across two Panther Fusion machines and two days. **Results:** The LDA was successfully optimized with respect to lower Ct values, greater precision, and high RFUs for PCR standard curves. The LDA achieved the standards for CLIA validation with 95% accuracy, 100% precision, and 100% specificity among human samples, and the media extensions achieved 98% accuracy and 100% precision. **Conclusion:** The methods outlined in the poster for developing an LDA using the Panther Fusion Open Access system can be useful for public health laboratories to prepare for increased testing volume. The automated extraction saves time and labor costs and increases reproducibility of results. The LDA does not require specimens to be processed under BSL-3 conditions, which improves workflow efficiency. The LDA detects clade II mpox specifically, so samples do not need to be sent to the CDC for confirmation, enhancing convenience and timeliness of results. These methods can be applied to the rapid optimization and development of LDAs for many possible pathogens of public health importance.

Presenter: Rebecca Villa, rvilla@uiowa.edu

Comparison of Nucleic Acid Extraction Methods for Detection of *Candida auris* Assessed using RT-PCR and Next Generation Sequencing

L.A. Sealey, E. Zelaya, A.P. Cabello, M. Mann, V. Irani, J. Steyer, W. Bchara, E. Vaughn, J. Doss, A. Murthi, D. Edwards, D. Payne, J. Hauser; District of Columbia Department of Forensic Sciences, Public Health Laboratory Division

Candida auris is a highly transmissible multi-drug resistant yeast with a high mortality rate in immunocompromised individuals. *C. auris* outbreaks are of public health concern due to its asymptomatic presentation in infected individuals, infrequent screenings for detection and its propensity to spread in health care settings. Accurate and efficient detection strategies for *C. auris* are important for coordinating timely treatment and infection control measures. In this study, we compare four nucleic acid extraction methods commonly found in state and local public health laboratories. We compare the suitability of extracted *C. auris* nucleic acids for use in downstream applications, including real-time PCR and whole genome sequencing. Samples were cultured from lab strains and clinical isolates of *C. auris* and other fungal species. Nucleic acids were extracted using MagNA Pure 96, MagNA Pure 24, QIAcube Connect and bioMérieux EMAG instruments. Nucleic acid yield and purity, cost per sample, tech time and instrument time were compared for each extraction method. Qualitative

PCR analyses were performed targeting the internal transcribed spacer region 2 (ITS2) of *C. auris*. Ct values were compared across different extraction methods and different strains of *C. auris*. We also performed long- and short-read next generation sequencing analysis to compare genome coverage, cluster density, sequence length and Q30 scores. While all four extraction methods were able to successfully extract nucleic acids from *C. auris*, we observed different levels of performance in various metrics. The results contribute to the implementation and verification of the real-time PCR assay and sequencing of *C. auris* using multiple extraction methods in a public health laboratory. This comparison provides supportive data for the use of multiple methods to process and accurately detect *C. auris* for rapid screening, thus contributing to existing techniques that can help with surveillance and control in health care facilities. These results will allow public health laboratory leaders to make informed decisions about which methods to use depending upon their desired downstream applications, available instrumentation and staffing levels.

Presenter: Leanna Sealey, leannasealey_@hotmail.com

Development and Optimization of *Candida auris* Surveillance Test using a Cartridge-based Assay System

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Background: *Candida auris*, a multidrug resistant fungal pathogen is often misidentified and requires higher technical skills to identify using complex methods. We used a cartridge-based assay system with an integrated sample processing and nucleic acid amplification (NAA). Here, we demonstrate development of a rapid, sensitive, and specific PCR-based surveillance assay to detect *C. auris* in skin swabs, which can be used in near-patient settings. **Methods:** The assay reagents containing the sample processing buffers, wash buffers and PCR reagents were optimized and built into a sealed, ready-to-use cartridge system. These ready-to-use sealed cartridges were used in subsequent experiments to enumerate dynamic range, limit of detection (LoD), inclusivity, and exclusivity. *C. auris* -negative skin swab matrix (SSM, confirmed by PCR) was collected at the Department of Pathology and Laboratory Medicine, the University of California Los Angeles (UCLA). The dynamic range of the assay was evaluated in Amies media, by spiking *C. auris* (ARO388) from 1 CFU/mL through 104 CFU/mL. A 500 µL of sample was added to the sample chamber of the cartridge and processed for DNA isolation and PCR. The LoD was evaluated by spiking *C. auris* ARO388 into SSM at concentrations of 0, 1, 5, 10, 20 and 50 CFU/mL (Nf20) by adding 500 µL of the spiked SSM into the *C. auris* assay cartridge. The assay inclusivity was tested at 2-3X LoD with additional strains of *C. auris* (Nf8) belonging to clades I, II, III, and IV. Exclusivity with non- *C. auris* strains (Nf10) was also verified. **Results:** The assay dynamic range showed a linearity of R²=0.9887, demonstrating that the detection was not adversely affected in presence of higher target concentrations. The assay LOD was 10.5 CFU/mL of *C. auris* ARO388 in SSM. Inclusivity with both aggregative and non-aggregative strains at 2-3X LOD showed 100% detection. The assay specificity was 100% when it was tested

against other non-target pathogens. The time required from sample input to detection is 58 minutes. **Conclusions:** We have developed a completely integrated, sensitive, specific and a rapid < 1h test, which can be used for routine surveillance of *C. auris* in health care facilities.

Presenter: Sukalyani Banik, sb1712@njms.rutgers.edu

Development of Screening and Sequencing Capacity for *Candida auris* Epidemiology in Nevada

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In 2021, the largest outbreak of *Candida auris* in the United States started in Southern Nevada. The outbreak necessitated the development, validation, and implementation of a diagnostic screening test for *C. auris*. Here we discuss a CLIA validation of a laboratory developed test for the diagnostic detection of *C. auris*. Our validated methods include scalable extractions using the Maxwell RSC 48 (Promega) on axilla and groin swabs. *C. auris* DNA is detected using a modified version of CDC's real-time quantitative PCR. Nevada State Public Health Lab has now performed this test on over 2000 specimens, and even used this method to conduct environmental surveillance in a long-term healthcare facility. In addition, we developed, implemented, and validated *C. auris* DNA extraction methods (Promega) and automated library preparation (Hamilton) of isolated *C. auris* cultures for next generation sequencing using the Illumina MiniSeq and NovaSeq platforms. Whole genome analysis of patient isolates has allowed for robust genetic epidemiology of this ongoing outbreak, revealing early on that the outbreak is actually two concurrent outbreaks of distinct *C. auris* clades. As of now, Nevada State Epidemiology requires our PCR screening be performed on all intakes at long-term healthcare facilities, and NSPHL has a goal to sequence every *C. auris* specimen in Nevada to continue this real-time disease surveillance.

Presenter: Danielle Denise Siao, danielle.siao@gmail.com

Development of the First Automated Antibiotic Susceptibility Test System for *Neisseria gonorrhoeae*

S. Puttaswamy, M.D. Newton, A. Isakson, J. McPeake, T. Hadfield and R.R. Swiger; Acenxion Biosystems

Here we describe the development of the first automated Antibiotic Susceptibility Test system specific to *Neisseria gonorrhoeae*. The system consists of hardware; software & firmware; and consumables that are single-use disposable. The hardware consists of expandable or scalable instrumentation providing environmental conditions that house individual reader units. The software & firmware ensure reliable real-time monitoring of system parameters and results including real-time data display and automated report generation. The consumable is a single-use, disposable, multiwell microfluidic cassette permitting simultaneous testing of multiple antibiotics and concentrations together with controls. The results of analysis of gonococcal strains are typically generated in under 6 hours and are based on phenotypic and microbroth dilution methodology and analysis. The microfluidic cassettes can be formatted to acquire results from minimal inhibitory concentrations (MIC) or susceptibility/intermediate/resistant (S/I/R) analysis. We

show results from analysis of strains including those from the CDC/FDA Antibiotic Resistance (AR) Bank.

Presenter: Sachidevi Puttaswamy, sachi@acxbio.com

Early Detection and Diagnosis of Acute Human Immunodeficiency Virus, Type 1 Infections Using a Pooling Algorithm — Dallas, Texas

M.C. Ty¹, C. Lopez², C. Nwankwo², S. Berno², D.A. Silva², J. Stringer², L. Short²; ¹US Centers for Disease Control and Prevention, ²Dallas County Health and Human Services Public Health Laboratory

Background: Acute Human Immunodeficiency Virus type 1 infection (AHI) contributes to 40% of new HIV transmissions; early detection of AHI is necessary to reduce new HIV infections. As AHIs cannot be detected by traditional serological markers, Dallas County (Texas) Public Health Laboratory (PHL) previously used the Aptima HIV-1 RNA Qualitative Assay to differentially detect HIV-1 RNA to identify AHIs. Since this assay was recently discontinued, DCHHS PHL will transition to the Aptima HIV-1 Quant Dx Assay, which detects and quantifies HIV-1 RNA in vitro with the automated Panther Hologic system. Given a relatively low local AHI prevalence rate and assay expense, DCHHS PHL developed and validated two serum specimen pooling algorithms to reduce the cost per detected AHI. The use of a more sensitive and fully automated test for pooled immunoassay HIV-1 negative specimens could dramatically increase the number of AHIs detected and contribute to the comprehensive national End the HIV epidemic (EHE) initiative. **Methods:** Two pooling algorithms (pools of five and 10 immunoassay HIV-1 negative specimens) were tested for accuracy, precision, analytical specificity and analytical sensitivity. If a pooled sample yielded a positive result, all samples in the pool were retested individually to identify the AHI specimens.

Results: Validations found that the pooling algorithm for the Aptima HIV-1 Quant Dx were precise and had analytical specificity of 100%. Test accuracy was 98.75% with an individual sample limit of detection of 63.86 copies/mL for pools of five and 295.34 copies/mL for pools of 10 specimens. DCHHS PHL decided to implement the pools of five approach in their clinical testing algorithm. During June–November 2022, a total of 7,651 specimens were tested using the five-pooled algorithm. Of 1,522 pools assayed, seven pools were positive, and 13 AHIs were detected. Two detected AHIs had virus levels below the Aptima HIV-1 RNA Qualitative Assay detection limit and would not have been detected with this previous approach. **Conclusions:** The HIV-1 Quant Dx Assay testing of pooled immunoassay negative specimens is an automated, more sensitive test and will better support Dallas County's efforts to detect AHIs and contribute to the EHE initiative.

Presenter: Maureen Ty, riv9@cdc.gov

Establishing a Workflow for Analysis of the Nasal Microbial and Viral Species, and Host Transcriptome from a Single Human Nasal Swab

K. Griffith, Y. Wang, R. Kumari, M. Sheth, P. Ranjan, J. Pohl, S. Gangappa, S. Sambhara, A. Kumar; US Centers for Disease Control and Prevention

The human microbiome is composed of unique combinations of microbial and a viral species that inhabit human skin and mucosal surfaces, such as the nasal passages and

gastrointestinal and urogenital tracts. Host genetics, age, gender, environment, medications and diet can impact the composition of the microbiome. In addition, the individual species within the microbiome contribute to the host immune responses which can impact the abundance of other species within the microbiome. We are interested in identifying the composition of the microbiome and its impact on host transcriptome within the nasal cavity in influenza infection and vaccination. Current methodologies require three human clinical nasal swab specimens; one to study the microbial populations, one for the viral populations and one for analysis of the host transcriptome. We are optimizing the protocol and establishing the workflow for RNA extraction methods and metatranscriptomics to identify the microbial and viral species, and the host transcriptome from a single nasal swab. The revised workflow significantly reduces the number of samples collected and the samples required for analysis. This will positively impact sample collection logistics and patients' comfort, as well as sample processing time. We will use the new workflow to assess the impact of the route of influenza vaccine administration ([intranasal (Flumist) vs. intramuscular (Flucelvax)], as well as influenza break-through infections, on the composition of the nasal microbiome, host transcriptome and the impact on the adaptive immune responses.

Presenter: Kayla Griffith, uah5@CDC.gov

Establishment of Whole Genome Sequencing to Predict Resistant Strains of *Mycobacterium tuberculosis*

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Diagnostic delay is defined as the time interval between the onset of symptoms and confirmed diagnosis of a disease. Current diagnostic tools for *Mycobacterium tuberculosis* (Mtb) limit the scope of detecting patterns due to this delay. The goal of this project is to develop the detection of Mtb outbreaks, including patterns of Rifampin-resistant Mtb and other local strains present in the population utilizing whole genome sequencing (WGS). This project has been simplified to six steps: identify, culture, extract, sequence, analyze, and educate. New Mtb patients will be identified through standard mycobacteria isolation protocols for further characterization. These specimens are cultured through BACTEC MGIT 960® to provide ample template for extraction and downstream applications. Positive cultures are heat-killed and tested for viability. Relevant MTb complex (MtbC) nucleic acid is extracted from a primary BACTEC MGIT 960+ culture for the purposes of whole genome sequencing. MTbC DNA is prepped from standardized protocols for WGS organisms using the Illumina DNA Flex library preparation kit then subsequently sequenced on an Illumina platform, thus ensuring inter-laboratory comparability of sequencing results. Isolates can then be clustered which may elucidate epidemiologically relevant data. Any epidemiologic relationships identified with this process will be interpreted and informative of cases and potential outbreaks. This interpretation will then be compiled into a format that is understood for the general public for health officials and other medical professionals to utilize to educate the general public that may be most affected by these trends.

Presenter: Whitney Heard, whitneyheard97@gmail.com

Evaluation of Two Matrix Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry Methods for Filamentous Fungal Identification

K. Milloy, M. Scoble, E. Craig; Virginia Division of Consolidated Laboratory Services

Objective: Validate and compare the accuracy of the Bruker BioTyper (MBT) and Biomérieux VITEK-MS MALDI-TOF methods for mold identification at the Virginia Division of Consolidated Laboratory Services (DCLS) Mycology Reference Laboratory. **Study Design:** The Bruker method was assessed by utilizing the direct transfer, extended direct transfer, and extraction method procedures with analysis by the MBT Filamentous Fungi Library version 4.0 (Research Use Only). The VITEK-MS method was assessed by utilizing the VITEK-MS Mould Extraction and Inactivation Procedure and the VITEK-MS Mould Kit with analysis by the VITEK-MS Knowledge Base version 3.2 (FDA approved). Eighty-two isolates were tested by both methods, 13 additional isolates were tested by the Bruker MBT method only, and 34 additional isolated were tested by the VITEK-MS method only, based on the individual species listed in the databases. Precision testing was evaluated by testing five isolates over three days, including duplicate testing and testing by two analysts. **Results:** Bruker MBT was able to correctly identify 72 out of 95 isolates tested for an accuracy of 75.8% and VITEK-MS was able to correctly identify 73 out of 116 isolates for an accuracy of 62.9%. If isolates previously identified to the genus level only are removed, the species level identification for each method was 80.8% (42/52 isolates) and 75.4% (52/69 isolates) for the Bruker MBT and VITEK-MS methods, respectively. Both methods resulted in 100% precision. A correct identification by the method was defined as an identification to the expected identification at the genus, species, or complex/group level. Expected identification of isolates tested during the validation were previously identified isolates using microscopic/macroscopic morphology, DNA probe, biochemicals, and/or DNA sequencing. Discrepant results from the expected identification were observed in 3% of isolates tested for the Bruker MBT method and 6% of isolates tested for the VITEK-MS method. **Conclusion:** The validation testing of the Bruker MBT and VITEK-MS methods for the identification of molds resulted in an accuracy of 75.8% for Bruker MBT and 62.9% for VITEK MS with rare misidentifications. Although the ability of both MALDI-TOF methods for mold identification does not perform as reliably as MALDI-TOF when applied to bacterial agents, as published in the literature, the MALDI-TOF will provide important information to supplement macroscopic morphology, microscopic morphology, biochemicals and other tests, for the identification of molds.

Presenter: Kathleen Milloy, kathleen.milloy@dgs.virginia.gov

Hepatitis C RNA Quantitative Stability Study – Report on Expansion of Nucleic Acid Testing for Diagnosis of Hepatitis C Infection and Treatment

A Mirolo¹, C. Loring², J. Alexander¹; ¹New Hampshire Public Health Laboratory, ²INTEGRA Biosciences

Background: The New Hampshire PHL performs quantitative HCV RNA testing using the Roche Cobas Ampliprep/Cobas Taqman HCV Test v2.0 (CAP/CTM). A challenge faced by the NH PHL is the relatively short acceptable storage time (no more than 3 days at

2-8°C) for samples collected for HCV RNA testing. This requirement can be difficult for providers to meet due to limited access to courier services. Additionally, to align with the recommended CDC testing algorithm, the NH PHL sought to offer automatic reflexing to HCV RNA quantitative testing for samples testing positive for HCV antibody. This service was previously unmanageable due to the short storage requirements for HCV RNA samples. Therefore, the NH PHL evaluated specimen stability of samples submitted for quantitative HCV RNA testing. This study was funded by APHL. **Methods:** Specimens submitted to the NH PHL for routine quantitative HCV RNA testing were used; positive HCV RNA specimens were stored at < 70°C. 30 specimens were selected for the study. They were thawed, aliquoted, and stored at 2-8°C. Samples were tested four times; Day 0, 3, 7, and 10 after thawing (Day 0 representing the day of thawing, used to control for loss of virus while frozen). Day 3 represented the maximum time at 2-8°C and established the “true” viral load for each specimen. This was then compared to results obtained from testing after 7 and 10 days at 2-8°C. **Results:** For our stability validation, the acceptable level of variation across time points was defined as the three day time point detectable viral load, +/- 0.31 logs. This acceptable level of variation was established by a review of College of American Pathologists (CAP) proficiency test (PT) results. Peer group results for HCV viral load quantitative testing using the CAP/CTM v2.0 for the years 2018-2019 were analyzed. The acceptable variation in score for peer group samples during this period ranged from a low of the sample peer group mean +/- 0.23 logs to a high of the sample peer group mean +/- 0.45 logs. We chose +/- 0.31 logs as our acceptable range as it represented the mean of the acceptable low and high ranges. All samples met our criteria for acceptability of results on Day 7 and Day 10, and all samples that displayed a detectable viral load on Day 0 retained positivity through Day 10 (the acceptability range could not be calculated for all samples on Day 3). **Discussion:** This study demonstrates that HCV RNA in serum specimens may be stable for up to 10 days when stored at 2-8°C. We found this to be true for specimens with viral loads ranging from 2.68 to 7.41 logs. A limitation of this study is the relatively low number of samples, particularly samples with very low viral loads. Only one sample with viral load of < 1.0 log was available in sufficient quantity to use in this study, and that sample yielded a result of “Not Detected” across all time points. Inclusion of more samples with low starting levels of viral RNA would bolster these results.

Presenter: Jessica Alexander, jessica.l.alexander@dhhs.nh.gov

Implementation and Validation of a Method for *Legionella* Detection and Enumeration using the QIAcuity Digital Polymerase Chain Reaction Platform

B. Bornu, K. Plemmons, D. Newborn, M. Poole, D. Pettit, S. Shone, C. Goforth; North Carolina State Laboratory of Public Health

Legionella, a genus of pathogenic gram-negative bacteria, that contains the species *L. pneumophila*, is responsible for legionellosis, which encompasses illnesses including Legionnaires' disease and Pontiac fever. With over 50 species recognized, it is widespread throughout many water sources. Transmission of these microorganisms are not person to person but are often the result of poorly maintained cooling towers, decorative fountains, hot tubs, as well as potable water sources. This procedure utilizes a digital PCR assay for the detection of *Legionella* spp., *Legionella*

pneumophila serogroups 1-15, and specifically *L. pneumophila* serogroup 1 from environmental water samples. Using digital PCR allows for DNA quantification, which can assist in determining the effectiveness of *Legionella* remediation strategies. Validation included distinct filtering methods for potable and non-potable water sources, followed by a Kingfisher DNA extraction. The QIAcuity digital PCR system was validated using the 26K Nanoplate with multiplex analysis. This method utilizes three sets of oligonucleotide primers and probes that amplify unique target sequences within the *Legionella* genome. For *Legionella* spp., a target was selected which amplifies a portion of the 23S rRNA gene that is thought to detect all species and serogroups of *Legionella*. The macrophage infectivity potentiator (MIP) gene target is used to detect *L. pneumophila* serogroups 1-15. *L. pneumophila* serogroup 1 is identified via a region of the *wzm* gene. A bicoid inhibitor control is also implemented to identify sources of inhibition. This method, as with other PCR methods, is beneficial due to the possibility of a one-day turnaround time for a presumptive positive result, as opposed to several days for cultural methods.

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Molecular Detection of OXA β -Lactamase Genes in Antimicrobial-Resistant Bacteria

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Antimicrobial resistance (AMR) is a rapidly increasing global health threat driven by the spread of antibiotic resistance genes in bacteria. The CDC estimates that 2.8 million infections and 35,000 deaths in the United States are due to antimicrobial-resistant pathogens annually. Public health laboratory testing is crucial for detecting outbreaks caused by antimicrobial-resistant pathogens and for surveillance of AMR threats. Of particular concern is the emergence of multidrug-resistant microorganisms for which few or no treatment options are available. Carbapenems are a class of β -lactam antibiotics that are typically reserved as antibiotics of last resort due to their potency against both Gram-negative and Gram-positive bacteria and their ability to resist hydrolysis by most β -lactamases. For this reason, the spread of carbapenemase genes which confer carbapenem resistance in bacteria is an urgent public health threat. The New Jersey Department of Health (NJDOH) Public Health and Environmental Laboratories (PHEL) surveils for AMR threats in clinical bacterial isolates by performing antimicrobial susceptibility testing, a modified carbapenem inactivation method (mCIM) for the detection of carbapenemase production, and real-time qPCR testing for surveillance of nine β -lactamase gene families. Except for OXA-48, testing is not currently performed by the NJDOH to detect OXA-family β -lactamase genes encoding class D carbapenemases called oxacillinases. OXA genes can confer resistance to penicillins and some cephalosporin and carbapenem antibiotics. OXA genes are most often identified among isolates of *Acinetobacter baumannii*, a Gram-negative opportunistic pathogen that is a major cause of hospital-acquired infections. In this study, we tested the performance of a commercially available real-time qPCR kit for the detection of 6 OXA gene families in antibiotic resistant bacteria: OXA-23, OXA-24/40, OXA-48, OXA-51, OXA-58, and OXA-143. Bacterial isolates known to harbor OXA genes were provided by the CDC and FDA Antibiotic Resistance Isolate Bank and the Antibiotic Resistance Laboratory Group (ARLG) and were tested

using the Streck ARM-D® OXA real-time qPCR kit. The isolates tested in this study included 47 *A. baumannii*, four *Acinetobacter pittii*, and six *Klebsiella pneumoniae* isolates. The performance of the test was validated at the NJDOH PHEL by measuring the accuracy, precision, and specificity of the test in which PCR results were compared to the known genotype of each bacterial isolate. The addition of the real-time qPCR test for OXA-family genes at the NJDOH PHEL increases the AMR surveillance capabilities of the laboratory, particularly for the detection and genotyping of carbapenem-resistant *A. baumannii* isolates which pose a significant threat in healthcare settings.

Presenter: Eagle Bui, eagle.bui@doh.nj.gov

Optimization of Multiplex RT-PCR Assay for Detection of Respiratory Syncytial Virus, Enterovirus, Human Parechovirus, and Adenovirus using the Panther Fusion System

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Many viral pathogens present the opportunity for influenza-like illness in infants and immunologically compromised adults, including respiratory syncytial virus (RSV), enterovirus (EV), parechovirus (HPEV), and adenovirus (HAdV). Rapid identification of these diseases could assist clinical providers with appropriate therapies to alleviate severity of illness, as well as provide outbreak surveillance data to local health departments. These pathogens can be identified using conventional PCR; however, it can be costly, labor intensive, and inefficient to test samples for one viral target at a time. The Open Access feature within Hologic's Panther Fusion System allows users to fully automate laboratory developed tests (LDTs) for nucleic acid amplification and real-time PCR (RT-PCR). The DC PHL aims to optimize a multiplex RT-PCR LDT to detect respiratory syncytial virus types A and B, adenovirus type 14, pan-human parechovirus, and pan-enterovirus in human respiratory samples. To assess assay performance, nasopharyngeal, oropharyngeal, and nasal swabs will be spiked with positive reference material for each target of interest. Samples will be tested for accuracy, precision, analytical sensitivity (limit of detection) and specificity. Variables to be optimized for this assay include concentration of primers, probes, MgCl₂, KCl, and Tris. Primers and probe sequences used for detection were obtained from published literature and produced by Integrated DNA Technologies. Human RNase P will be used as a positive extraction control.

Presenter: Erika Roloff, rolloff@gwmail.gwu.edu

Piloting Whole-Genome Sequencing to Identify *Mycobacterium tuberculosis* Complex and Associated Antimicrobial Resistance: The North Carolina State Laboratory of Public Health's Experience

A. Yaya, T. Lawson, K. Chase, W. Glover, S. Shone; North Carolina State Laboratory of Public Health

Introduction: The North Carolina State Laboratory of Public Health's (NCSLPH) experience with whole genome sequencing (WGS) of *Mycobacterium tuberculosis* Complex (MTBC) started with an APHL-CDC pilot to generate quality genomic sequences from Mycobacteria Growth Indicator Tubes (MGIT). The developed methods were applied to another pilot project to evaluate the Wadsworth Center,

New York State Department of Health's (NYSDOH) NGS TB Pipeline Service for which historical sequencing data from the CDC and the Michigan Department of Health and Human Services (MDHHS) were also used. **Method:** For the APHL-CDC pilot, NCSLPH extracted and sequenced DNA from 50 MTBC MGITs using the Qiagen EZ1 instrument. WGS library preparation was performed following the laboratory Standard Operation Procedure for PulseNet Nextera DNA Flex Library Preparation. The Wadsworth Center, NYSDOH's NGS TB Pipeline Service, was used for bioinformatics data analysis. Subsequently, 112 MGIT and solid media (Lowenstein Jensen (LJ) and 7H10) local North Carolina (NC) MTBC isolates were processed and sequenced using the optimized DNA extraction and sequencing protocol identified through the APHL-CDC pilot. These sequences, along with 263 historical local NC sequences from the MDHHS were analyzed using the Wadsworth Center, NYSDOH's NGS TB Pipeline Service. Overall, 425 sequences composed of 350 MTBC and 75 Nontuberculous Mycobacterium (NTM) across 287 patients (266 MTBC and 21 NTM) were analyzed. **Results:** The 425 analyzed sequences yielded 13 resistance-associated genes or noncoding regions with 21 unique high-confidence single nucleotide polymorphisms (SNPs) predicting resistance to Rifampin, Isoniazid, Pyrazinamide, Ethionamide, Streptomycin, and Fluoroquinolones. All the mutations identified by WGS were confirmed phenotypically using MGIT (for first-line antimicrobials) or agar proportion (for second-line antimicrobials) based Drug Susceptibility Testing (DST). The bioinformatics analysis also yielded a significant number of unknown and neutral mutations that are yet to be reported in the literature. **Discussion:** These pilots yielded results that proved the value of WGS integration in the NCSLPH diagnostic testing workflow. Our short-term goal is to sequence additional specimens to gain a perspective of currently circulating resistance markers within the NC population. The long-term objective is to develop a validation set composed of a panel of isolates with diverse high-confidence resistant mutation markers. This could be a collaborative effort with the CDC and is critical to jurisdictions with limited resistant strains diversity. During this process, it will be crucial to develop a plan to catalog neutral and unknown mutations that might be of interest for future use. Finally, NCSLPH will be evaluating the workflow algorithm to determine the revision of DST testing.

Presenter: Al-Mounawara Yaya, dr.yayamouna@gmail.com

Rapid, Point of Care Testing using Surface-enhanced Raman Spectroscopy and Deep Learning

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Laboratory based services have been the norm for diagnostic testing procedures. While the results of these diagnostic tests are reliable, the time from when the samples are submitted to when the results are obtained can range anywhere from a few hours to a few days or longer. Herein lies an ever-present issue in diagnostic testing: the ability to obtain accurate results while minimizing turnaround time. This issue was notable during the peak COVID-19 pandemic as many labs became overwhelmed due to the high volume of tests received. This, along with the numerous restrictions and testing guidelines put in place, has increased the need for rapid, point of care testing. One method that may prove to be a viable option would be a portable surface-enhanced Raman spectroscopy (SERS) sensor combining with deep learning for rapid SARS-CoV-2 RNA

detection from human and animal nasopharyngeal swab samples. The SERS sensor will be prepared based on a silver nanorod array (AgNR) substrate by assembling probe DNA to capture SARS-CoV-2 RNA. SERS spectra of nasopharyngeal specimens from positive and negative will be identified in less than 30 minutes. The probe DNA functionalized AgNR array SERS sensor combining with deep learning could serve as a potential rapid point-of-care COVID-19 diagnostic platform for testing of humans and animal samples.

Presenter: Teddy Spikes, trs53790@uga.edu

Reduced Ion Suppression in an Automated Extraction of Vitamins B1 and B6 from Whole Blood for LCMS Analysis

K. Dukes; Biotage

Phospholipids and proteins in blood and plasma sample are a main cause of ion suppression in liquid chromatography-mass spectrometry (LCMS) analysis. In addition to ion suppression, the phospholipids and proteins in blood and plasma samples can cause premature column failures and increase frequency of instrument maintenance. Presented is the work demonstrating the effectiveness of the Biotage ISOLUTE PLD+™ phospholipid removal plate used in the extraction of Vitamins B1 and B6 from human whole blood. The PLD+ plate can be incorporated into an existing method with little or no modification, as the blood or plasma is precipitated directly on the plate and can be easily automated. The Biotage ISOLUTE PLD+™ demonstrated an over 90% reduction in phospholipids and proteins and an increase in signal-to-noise ratio of the endogenous levels of B1 and B6.

Presenter: Kyle Dukes, kyle.dukes@biotage.com

The Development of a Multiplexed Immunoassay for Multi-Stage Lyme Disease Detection

J. Needham, D. Reyes, G. Ireton, A. Vallur; InBios International, Inc.

In the United States, Lyme disease is primarily caused by *Borrelia burgdorferi* sensu stricto, spread via the black-legged tick, *Ixodes scapularis*. Symptoms of early Lyme disease may include fever, rash, headache and fatigue. Later stages of Lyme disease, particularly if left untreated, may lead to complications including arthritis, memory loss and carditis. Lyme disease remains the most widely spread vector-borne disease in North America with approximately 476,000 infections in the US each year causing an annual economic burden approaching \$1 billion; however, the accurate and early diagnosis for Lyme disease infection remains challenging. The low parasite load and early dissemination of the spirochete limit the sensitivities for direct detection assays. Discernable manifestations, such as erythema migrans (EM), may only present in up to ~70% of patients. At this time, serological assays remain the mainstay of laboratory confirmation though it may take weeks to develop a sufficient antibody response. The US Centers for Disease Control (CDC) recommends testing sera with a “standard” or “modified” two-tier testing algorithm (STTA or MTTA) which requires testing a specimen with a sensitive immunoassay followed by either an immunoblot or enzyme-linked immunoassay (ELISA) to confirm the diagnosis. The development of a singular, more robust serological assay that can detect Lyme infection at both early and late stages of the disease will significantly improve clinical management and patient

well-being. Here, we describe the development and preliminary evaluation of the InBios Lyme Detect™ Multiplex ELISA which uses a microarrayed panel of target proteins to simultaneously detect IgG and IgM reactivity independently to each antigen and interprets results through a machine learning (ML) algorithm to properly categorize a given specimen. This assay is performed using an ELISA format to ensure easy adaptation by clinical laboratories. Proprietary software is used to automatically analyze the captured images, determine signals for each target antigen and provide the ML interpretation for each tested specimen. Preliminary testing with 35 confirmed and well-characterized early Lyme (EM positive) disease specimens, including Panels 1 and 2 obtained from the CDC and the Lyme disease biobank of the Bay Area Lyme Society shows that the sensitivity for detecting early-stage infection is improved by approximately 37% when compared to the STTA (48.6% for STTA vs. 85.7% for the Lyme Detect™ Multiplex ELISA) while the specificity with other disease, endemic normal and non-endemic normal human serum samples is ≥98.5%. Furthermore, independent reactivity for IgG and IgM is recorded for each target antigen, providing critically relevant data to the physician regarding the patient's specific antibody response.

Presenter: James Needham, james@inbios.com

The Role of Circulating Influenza Viruses on B Cell Memory

V. Vyas, Y. Wang, M. Sheth, M. Reed, J. Pohl, S. Gangappa, S. Sambhara, U. Shanmugasundaram, R. Kumari, M. Mishina, S. Sharma; US Centers for Disease Control and Prevention

Influenza viruses cause annual seasonal epidemics and have the potential to cause occasional global pandemics due to antigenic shift. In the United States, influenza primarily circulates from August through April, except for the 2009 pandemic, which caused infections in early summer. Subclinical infections may stimulate the immune system and contribute to the persistence of memory post-vaccination. Before the COVID-19 pandemic, there was no way to address whether circulating influenza viruses contributed to the persistence of B cell memory. However, the COVID-19 pandemic presented a unique situation in which influenza circulation was not detectable during the 2020-2021 flu season. In addition, social distancing and wearing masks to mitigate the spread of SARS-CoV-2 also diminished the incidence of influenza, which allowed us to test if circulating influenza viruses impact influenza B cell memory. To address this question, we are developing a comprehensive immune monitoring workflow to characterize the frequencies of H3 hemagglutinin (HA)-specific B cells via high dimensional multiparametric flow cytometry, H3 HA-specific B cell receptor repertoire with 10X Genomics platform, serum H3 HA-specific IgG and IgA antibody repertoire by Liquid Chromatography and Mass Spectroscopy (LC/MS). After establishing the workflow, we will test the clinical samples to assess the impact of circulating viruses on B cell memory in individuals vaccinated in during the 2019-2020 and 2020-2021 influenza seasons.

Presenter: Ved Vyas, uao4@cdc.gov

Validating a PCR Test for the Detection of *Legionella* in Clinical Specimens at the New Jersey Public Health Laboratory

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Approximately 250-350 cases of Legionnaires' disease, a severe pneumonia caused by *Legionella* bacteria, are reported to the New Jersey Department of Health (NJDOH) each year. *Legionella* are found naturally in freshwater environments, however the risk for humans is increased when *Legionella* contaminate human-made waters systems. Legionnaires' disease is contracted by inhaling or aspirating water containing *Legionella*. Thus, outbreaks of Legionnaires' disease are associated with contaminated water systems that create aerosolized droplets such as cooling towers, hot tubs, and fountains. When Legionnaires' disease cases are reported, public health laboratory testing is crucial to rapidly identify a potential outbreak and prevent additional cases. Clinical testing is required to diagnose and connect cases within an outbreak, and environmental testing is required to identify the contaminated source. Currently, the NJDOH Public Health and Environmental Laboratories (PHL) uses culture-based methods to perform environmental *Legionella* testing of potable and non-potable water samples. The NJDOH PHL has not previously performed *Legionella* testing for clinical specimens. Here, we report the optimization of a qPCR test for the detection and differentiation of *Legionella* species, *Legionella pneumophila*, and *L. pneumophila* serogroup 1 in clinical respiratory specimens. For this diagnostic test, DNA is extracted from sputum using either the Roche High Pure PCR Template Preparation Kit or the Qiagen EZ1 Advanced XL system. The extracted DNA is used as template in a multiplex qPCR reaction using previously published primers and probes (Benitez and Winchell, 2013). To analyze the test's performance, a validation study was conducted by testing sputum specimens that were spiked with *L. pneumophila* serogroup 1, *L. pneumophila* serogroup 3, or *Fluoribacter bozemanii* (previously *Legionella bozemanii*). The test detected the expected species and serogroup of *Legionella* spiked into 40 sputum specimens with 100% sensitivity and specificity. The limit of detection of the method was ~250 CFU/mL in sputum. Lastly, a stability study was conducted in which sputum specimens stored at either 4°C or -20°C were repeatedly tested over 14 days. Specimen handling requirements for the test were modified to account for our finding that Ct values for *Legionella* positive specimens remained relatively constant over a two-week period. By validating this qPCR test, PHL now has the capability to detect *Legionella* within both environmental and clinical specimens. An additional validation study has been conducted so that *Legionella* can be cultured from clinical specimens. With these increased testing capabilities, the future goal of the laboratory is to perform whole-genome sequencing of both environmental and clinical *Legionella* isolates to rapidly identify and connect outbreaks of Legionnaires' disease.

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Validation of High-Throughput Screen for Carbapenemase Gene Variants

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Increasing throughput and minimizing result turnaround times are critical to improving detection methods for large screens of patient samples to identify carbapenemase gene variants in multi-drug resistant, carbapenem-producing organisms (CPOs) in healthcare facilities. CPOs are serious threats to public health that have been identified in increasing numbers of healthcare-acquired bacterial infections. Rapid detection of CPOs from patient rectal swabs is key for health professionals to prevent the spread of these antimicrobial resistant phenotypes within the health care system. The currently approved CPO screening method begins with the Cepheid GeneXpert Carba-R PCR detection method, which is only able to detect the presence of five antimicrobial resistance genes (IMP, KPC, NDM, OXA-48, and VIM). Positive samples are then processed by culture-based methods followed by CPO gene target detection by Real-Time PCR, all of which takes 5-10 days. The Cepheid method cannot detect the presence of clinically relevant variants like IMP-14, IMP-18, IMP-27 OXA-23, OXA-24/40, OXA-58, or OXA-235 that are validated for detection by the Real-Time PCR. In addition to detecting more variants than the Cepheid-based method, directly detecting variants from patient rectal swabs is one-tenth the cost/sample compared to the Cepheid-based method.

Here we combined the reliable detection method of carbapenemase gene variants by RT-PCR with an automated DNA extraction system to screen for a broad set of carbapenemase gene variants directly from rectal swabs, eliminating the need for culture-based methods, allowing for same day turnaround time of results. Validation of this method allows healthcare providers to respond quickly to prevent the spread of harmful CPOs in acute and long-term healthcare facilities, while minimizing costs and labor for public health laboratory analysts process patient samples.

Presenter: Nicholas Olivarez, nicholas.olivarez@doh.wa.gov

Validation of MagNA Pure 24 Instruments for Downstream use of DNA in CLIA-regulated and Nonregulated Sequencing

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Background: Whole genome sequencing is a powerful analytical tool that allows public health laboratories to rapidly identify and characterize pathogens and determine genetic relatedness among organisms. The Wisconsin State Laboratory of Hygiene (WSLH) previously used the MagNA Pure LC 2.0 (Roche) to extract DNA for sequencing; the instrument was discontinued at the end of 2022 and WSLH chose to replace it with the MagNA Pure 24 (MP24) system (Roche). Onboarding new automated laboratory instruments requires validation before use, but validation requirements differ based on how data will be used. Data used for patient results or to guide care must meet Clinical Laboratory Improvement Amendments (CLIA) regulatory requirements; whereas results used for surveillance purposes are not regulated. Using both instruments, we extracted DNA from a panel of organisms representative of CLIA-regulated and nonregulated tests and compared quality and

quantity of DNA extracted. **Methods:** For CLIA-regulated tests, DNA was extracted from 12 *Streptococcus pneumoniae* isolates and plated alternating with 12 no template control (NTC) wells to test for sample carryover. For nonregulated tests, we extracted DNA from 3-5 isolates of seven organisms (*Salmonella enterica*, Shiga toxin-producing *Escherichia coli*, *Enterobacter cloacae*, *Acinetobacter baumannii*, *Klebsiella* species, *Pseudomonas* species, and *Escherichia coli*) and 1 negative control (NTC) per run. Extracted DNA quantity was assessed using a Qubit spectrophotometer. DNA quality was assessed with a Nanodrop 2000 spectrophotometer by measuring absorption peaks at 260 and 280 nanometers and calculating peak ratios; a ratio of 1.8 was considered pure DNA and ratios of 1.7–2.2 were considered acceptable. We sequenced DNA extracted from ≥ 1 isolate per organisms using the MP24 and compared resulting quality metrics to those of DNA sequences from the same organisms extracted by the MagNA Pure LC 2.0.

Results: DNA quantity was similar to DNA obtained with the MagNA Pure LC 2.0 (mean 14.78 ng/ul *Salmonella* extracted with MagNA Pure LC 2.0 vs 20.66 ng/ul with MP24). DNA from all organisms extracted by the MP24 had sufficient quality for use in sequencing and all 260 over 280 ratios were within 1.7–2.2. Sample carryover did not occur. DNA recovered from negative controls run with *S. pneumoniae* isolates was too low to detect. All assessed sequencing quality metrics indicated MP24-extracted DNA was suitable for use in downstream sequencing, including CLIA-regulated tests.

Conclusion: This study validates MP24 use as a replacement for the MagNA Pure 2.0 instrument. Our validation plan and data might be useful for other public health laboratories to validate DNA extraction instruments used for CLIA-regulated and nonregulated sequencing analysis.

Presenter: Matthew Martin, tqe7@cdc.gov

Validation of the Roche COBAS® 5800 for Detection of Hepatitis C Virus (HCV)

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Background: The Michigan Department of Health and Human Services (MDHHS), Bureau of Laboratories (BOL) experienced a 30% increase in specimens submitted for hepatitis C virus (HCV) antibody and nucleic acid testing in 2022. BOL evaluated the Roche COBAS® 5800 System, a compact molecular testing platform with increased testing volume capacity that performs an automated nucleic acid amplification test to detect and quantify HCV. Performance and workflow for the COBAS® 5800 System were compared with the COBAS® Ampliprep/COBAS® TaqMan® HCV Test currently in use at BOL. **Methods:** Roche provided an HCV validation panel. A linearity assessment was performed using 24 positive HCV samples with an expected analyte concentration range of 55 – 3×10^7 IU/ml. Precision and accuracy were determined by testing a total of 15 low (1000 IU/ml) and 15 high (1×10^6 IU/ml) analyte concentration samples over five days. The assay's limit of detection (LOD) was tested using 20 low analyte concentration replicates. A method correlation study compared performance of the COBAS® 5800 System HCV Test with the COBAS® Ampliprep/COBAS® TaqMan® HCV Test using 44 deidentified clinical samples previously tested for HCV. Analyte specificity was determined by testing 22 serum samples antibody reactive for *Treponema pallidum*, HIV, hepatitis A virus, or hepatitis B virus. Workflow analysis measured average

time per run and maintenance for both platforms. **Results:** Linear range for HCV samples tested on the COBAS® 5800 System was successfully established with an R² value of 0.99. For precision, all sample results fell within two standard deviations from established means. Precision inter-run coefficient of variability (%CV) for low and high concentration sample results were calculated at 6.17 and 4.72, respectively. Accuracy was determined and all sample results fell within expected intra-assay variation of $\pm 0.5 \log_{10}$. HCV was detected in all 20 LOD panel samples with an observed range of < 15 to 215 IU/ml. Platform comparison results using 38 HCV-positive and 6 HCV-negative samples had 100% agreement. Linear regression analysis of platform comparison results returned an R² value of 0.98. Analyte specificity was 100% in 21/22 samples with no HCV detected; one sample returned an "HCV Invalid" result caused by sample aspiration error. Workflow analysis showed the COBAS® 5800 System provided appreciable time savings at all stages of sample processing and maintenance, compared with the COBAS® Ampliprep/COBAS® TaqMan® HCV Test. **Conclusion:** The COBAS® 5800 System is an acceptable testing platform for HCV detection in clinical samples. Implementation of the COBAS® 5800 System for HCV testing will increase laboratory testing capacity, reduce time required for hands-on sample manipulation, and decrease turnaround time to reportable results.

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DIVERSITY, EQUITY AND INCLUSION

Creating an Inclusive Workplace: Initiating and Implementing a Diversity, Equity and Inclusion Work Group in a Public Health Lab

J. Laine, Minnesota Department of Health

Diversity, equity and inclusion is vital to all public health laboratories as we collectively work to entice a more diverse candidate pool, increase retention of current and future employees, and make our workplace more inclusive and welcoming. The Minnesota Public Health Laboratory (MN PHL) established a Diversity, Equity, and Inclusion (DEI) work group in April 2021, with members representing each area of the PHL; Environmental, Infectious Disease, and Newborn Screening. The goal of the MN PHL DEI work group is to embed Diversity, Equity, and Inclusion practices, principles, and language into everyday work. This poster will share how to implement a DEI work group, built from the ground up to include staff from all levels of the Public Health Laboratory. Items such as creating a charter, establishing goals, planning tools utilized in creating a work group, and defining a vision for the work group will be discussed. We will share initiatives completed in our first year, addressing recruitment and leadership initiatives. Summary questions and results of a division wide survey of employees and their perceptions of DEI in their workplace will be represented. In general, results of the survey indicated that while most respondents felt welcome in the workplace, there was a need for further education regarding definitions of DEI and what embedding DEI in their workplace meant for them as employees of a public health laboratory. Survey respondents felt that they wanted to feel more comfortable discussing DEI initiatives by learning more about these topics and working to ensure a welcoming, open work environment in which to have those conversations. Current work initiatives

for year two of the DEI work group, including discussion of five newly formed committees, that were formed as a direct result of survey responses received, and the work of these committees will be shared. Finally, this poster will indicate considerations for implementing a DEI work group in a public health laboratory setting, and future goals of the work group.

Presenter: Anna Strain, anna.strain@state.mn.us

Inclusive Leadership

N. Balakrishnan¹, M. Freeman², S. Smith³; ¹Georgia Public Health Laboratory, ²Virginia Division of Consolidated Laboratory Services, ³North Carolina State Laboratory of Public Health

How we consider, understand, and interpret the environment informs perception. Perceptions inform decision making and beliefs. For this reason, beliefs about various identities can be biased and affect the treatment of others. The labels unconsciously given to people may negatively impact achieving an inclusive workplace that is supportive of the diversity in people. Several researchers have documented the importance of inclusiveness and described its elements—fairness and respect, value and belonging, confidence and inspiration. Effective leaders must embrace inclusive leadership to promote a healthy working environment free of unconscious bias. Unconscious bias is critically important to understand, acknowledge, and manage microaggressions in our working environment. There are six signature traits of an inclusive leader. They must be cognizant and mindful of personal and organizational biases that narrow their field of vision and precludes objectivity. They must be courageous. This includes not only advocating for self and others, but also demonstrating vulnerability and humility. They must demonstrate commitment to diversity and inclusion, ensuring their objectives align with team personal values. There should be a willingness to collaborate through empowerment. Inclusive leaders should embrace the diversity of the organization and communities and motivate others to respect and honor their differences. They should strive to understand the diversity of their team through the purposeful act of building cultural intelligence. Finally, they should encourage a setting where curiosity is welcomed and encouraged. Have an open mind because differences encourage growth. Embracing inclusive leadership should be a goal for progressive organizations, understanding the importance of the six signature traits. Each leader is encouraged to assess your workplaces to identify gaps, determine areas of improvement, and strategically implement activities that support an unbiased environment. The “APHL Gathering Place: All are Welcome” promotes, embraces, and models DEI within the public health laboratory infrastructure by sharing ideas, observations, and lived experiences while also providing resources supportive of diversity, equity, and inclusion practices. The DEI Task Force has gained insights on the complexities of diversity, equity, and inclusion and uses the awareness to help grow others.

Presenter: Nandhu Balakrishnan, nandhu.balakrishnan@dph.ga.gov

FOOD SAFETY AND SECURITY

Determining the Viability of EPA Method 200.7 as a Replacement for EPA Method 365.1 for the Analysis of Phosphorous in Water Quality Samples

J.J. Chisholm, Indiana Department of Health

The Indiana Department of Health (IDOH) has utilized the EPA 365.1 analysis method for the analysis of phosphorous in water quality samples. This project aims to determine if phosphorous can be analyzed using the EPA 200.7 method that was used to analyze metal content in water samples.

Presenter: John Chisholm, jjchisholm@yhc.edu

Evaluation and Validation of Quantitative PCR for *Listeria monocytogenes* in Foods

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Background: The Food and Drug Administration (FDA) has begun requiring state laboratories performing surveillance under the Laboratory Flexible Funding Model (LFFM) to have the capability of quantifying any samples positive for *Listeria monocytogenes*. The current cultural method for quantification has shown variable recovery during verification trials while also being costly and time consuming. This single lab in house validation project will explore using quantitative polymerase chain reactions (qPCR) to enumerate levels of *L. monocytogenes* in contaminated food products.

Methodology: Testing design was guided by the International Standard (ISO) single lab in house validation protocol (ISO 16140 series). Three food categories were chosen to assess the scope of abilities of the qPCR process to enumerate *L. monocytogenes* under various food related stress conditions (heat-processed milk and dairy products, fresh produce and fruits, and ready to eat/ready to reheated meat products). One type of food was chosen per category, and one item was chosen per type. The three items to be tested include ice cream, sprouts, and cooked deli meats. Five different individual sub studies will be included in the overarching validation. Test to be completed are relative trueness, an accuracy profile, limit of quantification, in-house precision, and inclusivity/exclusivity for detection of the bacteria of interest. **Results:** Results will become available at the conclusion of the study and will be used to evaluate the accuracy, reliability, and consistency of the qPCR enumeration for potential implementation into laboratory methods. **Conclusions:** The findings of this project will be beneficial in understanding how molecular methods can be utilized to enumerate positively screened *L. monocytogenes* samples as an alternative to the current cultural route.

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Reference Materials for Food Safety Chemistry Testing: Importance and Considerations

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Background: The New Hampshire Public Health Laboratories performs food safety chemistry testing as part of the FDA's Laboratory Flexible Funding Model. Through its human food surveillance work, the program tests a wide variety of food products.

Due to the high degree of variability in food matrices, a robust method for selecting reference materials for food chemistry testing quality assurance is critical. This is especially important when results may be used for compliance or regulatory actions. **Purpose and Scope:** This poster describes the methods by which the NH PHL selects reference materials for food chemistry testing, with considerations for matrix matching, analyte matching, processing for high quality results, and addressing processes that can affect the reference material performance over time. In particular, this poster presents a case study on transpiration and its effects on liquid reference materials and certified standards. Transpiration is the process by which liquid evaporates through a closed container. It is influenced by light intensity, time, humidity, and temperature. In a laboratory, the environment can either increase or decrease the transpiration rate. The transpiration rate can also be affected by physical movement or agitation of the container. Transpiration affects the concentration by reducing the volume of the solution, thereby increasing the concentration. This can affect the measured results relative to the Certificate of Analysis, and therefore must be evaluated to determine if this difference has implications for quality control and quality assurance. **Methods:** To evaluate the effect of transpiration on a liquid reference material, this case study used a NIST-traceable multi-element standard solution containing Arsenic, Chromium, Lead, Molybdenum, Selenium, Zinc, Cadmium, Copper, Manganese, Nickel, and Thallium. Over the course of eight months, the solution was weighed before and after being placed into storage to measure mass loss due to transpiration. The mass loss was converted to a percent change in concentration of the target analytes relative to the certified values on the Certificate of Analysis. **Results and Conclusions:** We calculated an overall transpiration loss of 0.2692g, or 0.22% of the starting mass of the solution over a period of eight months. The average daily transpiration rate for the period of eight months is 0.039 mg/day. In contrast, the percent uncertainties on the certified concentrations of target analytes from the certificate of analysis range from 0.4% to 0.8%. We determined that the effect of transpiration on target analyte concentrations in a multi-element standard solution was not significant over a period of eight months. Transpiration is one of the many important considerations in the context of maintaining reference materials for robust chemical analysis.

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INFORMATICS

Asked on Order Entry Questions: Quality and Value of Data for Oregon COVID-19 Surveillance and Response

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Background: The COVID-19 pandemic response identified challenges about how best to gather important epidemiological information to guide pandemic control and response. One tactic employed by the Department of Health and Human Services (HHS) under the Coronavirus Aid, Relief, and Economic Security (CARES) Act was to mandate that providers report epidemiological risk questions (e.g. is this the patient's first COVID-19 test?) to testing laboratories who were then required to report COVID-19

"Ask on Order Entry" (AOE) info on results sent to HHS. The Oregon State Public Health Laboratory (OSPHL) entered AOE info into the laboratory information management system (LIMS) primarily from paper requisition forms submitted by providers. At the time of the mandate, there was no available reporting solution from the LIMS so the OSPHL built an in-house coding solution to enable reporting. Due to the high costs of collecting and reporting this information, the OSPHL set out to determine the quality of AOE data and utility for local epidemiological response. **Method:** This investigation sought to determine the accuracy and completeness of AOE info, including key attributes of interest for state COVID epidemiology: "hospitalized" (either hospitalized or admitted to intensive care unit) and "pregnant" along with an epidemiologic review of how these data were actually used. AOE info from specimens tested for SARS-Cov2 at the OSPHL in May 2021 (pre-Delta surge) and September 2022 (post-Delta surge) were assessed for completeness and epidemiologic utility. **Results:** The OSPHL conducted 5,764 SARS-Cov2 tests in May 2021. Of these tests, only 16 reported a "hospitalized" status, a completion rate of 0.3%, and only 7 reported a "pregnant" status, a 0.1% completion rate. The most consistently reported AOE attributes were "first COVID-19 test" (87.1% completion rate) and "symptomatic" (80.1% completion rate). In September 2022, the OSPHL conducted 1,179 SARS-Cov2 tests. "Hospitalized" status was not provided for any specimen and "pregnant" status was only reported for 1 patient, a < 0.0% completion rate. "First COVID-19 test" (100% completion rate) and "symptomatic" (92.8% completion rate) were the most consistently reported. The comparison of these data to other data sources indicated issues with data quality that complicated their practical use. **Conclusion:** The OSPHL found interesting trends in AOE completeness in two different timeframes. It is not clear if attributes such as pregnancy and hospitalization status were under-reported or if these were not as relevant for the populations being tested. This reporting mandate required a significant level of effort from the OSPHL and well-populated attributes did not guide local response. This reporting paradigm may benefit from replacement with FHIR (Fast Health Interoperability Resources) as that becomes more widely available.

Presenter: Laure Boyd, laurel.boyd@oha.state.or.us

Building a Sustainable Model for Electronic Test Orders and Results and Surveillance Reporting

L. Boyd¹, M. O'Malley Moore¹, M. Yungclas¹, S. Hearn¹, J. Kondamuri², M. Kourbage², A. Prada²; ¹Oregon Health Authority ²J. Michael Consulting

Background: The ability to share data is central to the public health laboratory's mission. Yet maintaining multiple point-to-point connections, each with their own standards and surveillance implications, can be time-consuming and resource-intensive for the public health laboratory to maintain. CDC's Data Modernization Initiative challenges public health partners at every level to create solutions that "move from siloed and brittle public health data systems to connected, resilient, adaptable, and sustainable 'response-ready' systems that can help us solve problems before they happen and reduce the harm caused by the problems that do happen." **Method:** With this DMI goal in mind, the Oregon State Public Health Laboratory (OSPHL) has partnered with J Michael Consulting to design an in-house technical solution that can manage the laboratory's data exchange needs, including surveillance reporting and electronic test orders and results (ETOR)

for communicable diseases and newborn screening. The solution combines an SQL database with Rhapsody's enterprise architecture and web service capabilities to enable a unified pathway for multiple electronic data exchanges. **Results:** The OSPHL solution is now in production for PHLIP messaging, with plans to add LRN messaging. OSPHL will pilot the ETOR component of the solution in spring 2023. This solution is configurable, so it can accommodate multiple HL7 formats and can quickly change to accommodate new tests or workflows or requirements for sharing data. The solution was designed to be LIMS-agnostic, receiving orders and sending results for two separate LIMS, and it will be adaptable should OSPHL implement a new LIMS in the future. The Rhapsody route is a configurable, scalable solution, allowing OSPHL to nimbly respond to new outbreaks, changing reporting mandates, and new testing needs. Furthermore, OSPHL can leverage the data stored in the SQL database, as well as the inherent functionality of the Rhapsody integration engine, to monitor and troubleshoot all messaging activity including providing a means for transactional reporting as paper test orders are replaced by electronic orders, potentially using a broker. **Conclusion:** OSPHL's approach to data exchange realizes many of the goals of DMI and is in line with CDC's commitment to strengthen data reporting, management, and analytics across public health.

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Centralizing Electronic Laboratory Reporting (ELR) Beyond COVID-19: Challenges, Lessons, and Considerations for the Future

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Background: During the pandemic, state-based public health agencies were able to demonstrate the capacity to accept electronic results in the HL7 v2.5.1 ELR standard. With the foundation of centralized ELR built on the AIMS platform for COVID-19, there is an opportunity to use these data pathways to increase the use of electronic means to report laboratory data beyond SARS-CoV-2 test results. APHL, via Datapult, has developed a service that expands its ELR capabilities from one infectious agent to over ten (10), and has plans to expand to over 90 by the end of 2023. Expanding beyond COVID-19 is not easy: for the service to achieve the goal of reducing burden on public health agencies and laboratories, it must tailor reporting to the rules and requirements of each jurisdiction. Datapult, working together with Clinical Architecture, developed the ELR process that will allow for rule-driven, tailored reporting. This poster describes the challenges and lessons learned when deploying this service, and considerations for further centralizing ELR for any reportable test result. **Challenges and Solutions:** When automating ELR in a centralized manner, the first problem to address is variable reporting requirements. These variations are presented in several ways: the types of test results, the types of infectious agents, and the number and types of supplemental results (like A1C or CD4), among others. The poster describes each of the challenges that the team faced and how these were addressed to achieve a centralized ELR service. **Method:** The poster illustrates the process taken to create a modular framework that can be combined in various ways to mirror any jurisdiction's ELR rules. To develop this modular rule set, the rules were decomposed into the different "reportability flavors" (e.g., positive, indeterminate, negative) and "reportability factors" (e.g., antibody, antigen,

nucleic acid, viral load). These modules can then be combined in any permutation to adhere to each jurisdiction's published rules.

Initial Real-World Results and Lessons Learned: The initial release of this service is planned for March 2023 and the first few months of results will be shared via the poster. These real-world results will include challenges that presented themselves in real-world use and how they were addressed, and quantitative metrics such as the ELR volume stratified by test type. **Considerations:** Laboratories must be ready to report results for any scenario—whether this be reporting for endemic diseases or reporting for emerging infectious diseases. The poster will provide considerations as visualized questions/prompts that can be considered by individuals who attend the poster session.

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Development of a COVID-19 Test Ordering System at the Colorado State Laboratory

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On March 5, 2020, the first positive COVID-19 case was confirmed in Colorado. At this time, all clinical testing at the Colorado Department of Public Health and Environment (CDPHE) State Laboratory was ordered on paper submission forms. In response to the pandemic, the State Lab became responsible for providing surveillance testing for long-term care, community outreach and outbreak response. The volume of test orders rapidly increased beyond the lab's capacity to perform manual data entry. Electronic test ordering was first implemented using the LabOnline web portal integrated with our Horizon LIMS but was limited to single sample orders. The need to provide testing services to large institutional populations, such as correctional and long-term care facilities, led the State Lab to develop a CSV file-based interface capable of ordering thousands of tests at a time. However, properly formatting CSV files proved to be difficult for clients without direct interface capabilities. To provide customers with a simple, robust means to upload high-volume test requests, the State Lab, in collaboration with OIT (Governor's Office of Information Technology), developed the Colorado Test Ordering System (CTOS) web application. CTOS easily handled more than 55,000 sample submissions per week from more than 350 sites at the height of the pandemic. The lessons learned and technology developed will serve the State of Colorado during future crises, and the CTOS framework has already been deployed to assist with other data acquisition challenges.

Presenter: Justin Nucci, justin.nucci@state.co.us

Electronic Case Reporting (eCR) Data Quality Assessment and Reporting

J. Park, L. Parlier, J Michael Consulting

The APHL Electronic Case Reporting (eCR) Direct Support team works under the auspices of the APHL eCR Program to assist PHAs with the implementation of eCR. eCR provides an efficient and reliable source of encounter based clinical data that is useful in the timely reporting of cases of notifiable conditions to public health departments. The APHL eCR direct support team created a framework and set of tools to assess and report the quality of Electronic Initial Case Report (eICR) messages received

individually and in aggregate. The toolset leverages standard ISO Schematron files specific to eICR CDA documents that include standard conformance assertions and extended assertions for more granular quality assessment. Schematron files are implemented in a Rhapsody Integration Engine route that processes each eICR individually and persists document, assertion failure, and summary information into a database repository for further analysis and reporting.

Key features of the Data Quality Rhapsody Route and Repository are:

1. For each eICR received, capture, and persist key data elements for analysis:
 - a. Unique identifier of the eICR linked to reports and errors
 - b. Key sending provider information
2. Process the eICR through a standard and local schematron validation filter
3. Collect and persist each Schematron assertion failure
4. Log any errors while processing the Schematron validation
5. Group assertions and create summary reports that are configurable by the jurisdiction for custom reporting and monitoring

A default eICR data quality schematron file is provided by APHL but jurisdictions have the option of implementing a local schematron file along with the default. The local schematron can include local jurisdiction specific assertions and configuration options in the route allow for local rules to override defaults in cases where they conflict or are redundant. The data quality toolset is currently implemented in Rhapsody with plans for implementation in Mirth, and Pilotfish. The repository can be hosted in any standard database and has been tested in Microsoft SQL server and PostgreSQL. The primary use of the eICR Data Quality Route is to assess initial data quality of eICR's generated by providers during the eICR onboarding process and for ongoing monitoring of production message feeds. The route has been deployed to multiple PHAs and is used on the AIMS platform to assess message quality during provider onboarding. Jurisdictions, as well as the national team, can find issues in real-time to improve initial quality or discover patterns over time. Electronic case reporting is a critical requirement for modernizing disease surveillance to continuously improve public health.

Presenter: Rachel Shepherd, rachel.shepherd@aphl.org

ETOR: A National Solution

R. Shepherd, D. Shirazi, R. Merrick; Association of Public Health Laboratories

The COVID-19 pandemic has underscored public health's need to modernize the way we exchange data. The ability to electronically exchange test orders and results (ETOR) on a wide scale would revolutionize public health. The sooner laboratories receive and process test orders, the faster they can return results — essential for surveillance, outbreak and public health emergency response and early intervention. APHL has been developing a centralized and national ETOR solution using AIMS as an intermediary that could enable widespread ETOR adoption across public health. Laboratories are urgently striving to develop ETOR solutions and connect with partners, but there is an opportunity to achieve

nationwide growth, capitalize on economies of scale, and to build and maintain a single solution that could benefit everyone (rather than have every entity develop a single and disparate solution). Session attendees will learn how they can take advantage of the proposed solution and services to meet their ETOR needs. They will also have the opportunity to hear from laboratories about how they are engaging in ETOR now, as well as how they plan to in the future. The ability for public health laboratories to seamlessly exchange electronic test orders and results with submitters is an imperative public health need and it is also now a required activity under CDC's Data Modernization Initiative (DMI). Additionally, widespread workforce challenges have made it clear that laboratories do not have the necessary informatics workforce to build and maintain longterm ETOR solutions independently. This collective approach could enable significant ETOR gains by leveraging shared resources and expertise.

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Informatics Workforce Development and Training

V. Olanrewaju, DC Department of Forensic Sciences Public Health Laboratory

Informatics is the study of computational systems. One type of system used within Informatics is a Laboratory Information Management System (LIMS). LIMS lets you effectively control the flow of samples and data associated with them to boost productivity in the lab. LIMS helps to standardize tests, procedures, and workflows while also providing precise process controls. By reducing manual labor, a laboratory information management system LIMS aims to increase productivity, accuracy, and quality control. LIMS provides unique and highly specialized testing and other services for public health laboratories. With post-covid increases in testing and surveillance, it is imperative that public health laboratories operate at top efficiency. In order to do that, public health laboratories must ensure that laboratory staff are adequately trained in the use of LIMS. With LIMS being used wholly and correctly, this will help to ensure the laboratory is satisfactory in testing outputs and workflows. After an annual gap analysis on the Washington, D.C. Public Health Laboratory (DC PHL), it was found that majority of DC PHL's identified areas of need were underutilization of the LIMS for lack of user training. This points to a great need to strengthen workforce management and training in LIMS. This poster will examine some of the obstacles, successes, and necessary enhancements taken at the DC PHL in 2022 and 2023 to revamp current training in order to strengthen the workforce with training from the informatics unit.

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Inventory and Evaluation of Laboratory Informatics Systems: A How-to Guide

M. Akre, B. Van, M. Yost-Daljev, J. Michael Consulting

It has been several years since APHL released the Informatics Self-Assessment Tool, and more than a decade since the publication of APHL's Requirements for Public Health Laboratory Information Management Systems (RPHLIMS). This tool and this document have become indispensable resources for public health informatics projects. In this poster, we explain how to leverage these resources, in combination with remote and onsite discovery, to conduct a

comprehensive LIMS assessment. Our LIMS assessment process documents the Current State of the laboratory, facilitates a gap analysis, and provides recommendation for Future State efficiencies. These inputs empower lab leadership to develop a comprehensive data modernization plan for their organization and make a well-informed decision on whether to maintain, augment, or replace their existing LIMS. Despite the disparate drivers of your evaluation, this process allows laboratories to define what you have, describe what you want, get what you need with documentation to submit to procurement offices.

Presenter: Monica Akre, makre@jmichael-consulting.com

Sharing Data Across the Nation through AIMS

R. Shepherd¹, A. McShane²; ¹Association of Public Health Laboratories, ²Ruvos, Inc.

For over a decade, the APHL Informatics Messaging Services (AIMS) Platform has facilitated public health data exchange for public health laboratories and agencies and their clinical partners. Each day, hundreds of thousands of messages are routed through the Platform. This session will provide an in-depth look at the variety of ways public health laboratories use AIMS, from setting up a new use case and data flow, to actually routing, validating, and parsing the data. How does AIMS keep the data flowing? And how can AIMS help laboratories meet their data exchange needs? Attendees will learn how AIMS is making it easier to share data across local and state lines and with CDC and with clinical partners. They will learn the breadth of public health data and use cases that AIMS supports, as well as the variety of services available on the Platform. Attendees will learn about the safeguards that AIMS has put in place to secure the nation's public health data. They will learn how APHL continues to modernize the AIMS Platform to meet the changing needs of their members. Every state public health laboratory and agency exchanges data with AIMS. It is a cornerstone of public health data exchange today, with significant plans for more services in the future. As PHLs are planning approaches to data modernization, they should leverage these available services and incorporate AIMS capabilities into their design."

Presenter: Rachel Shepherd, rachel.shepherd@aphl.org

LESSONS LEARNED FROM PAST DISEASE EVENTS

An Evaluation of Testing Access in the Laboratory Response Network (LRN) during the First 10 Weeks of the Mpox Response, May 17 – July 31, 2022

K. Thomas; US Centers for Disease Control and Prevention

Background: This evaluation sought to identify the successes and challenges that LRN member laboratories faced during the initial 10 weeks of the public health response to the 2022 mpox outbreak. Authors explored how prior investments and programmatic support enabled the initial testing response and identified barriers that

limited testing and testing access within LRN laboratories. Findings will inform and improve future planning and responses. **Methods:** CDC's Division of Preparedness and Emerging Infections led an evaluation using a qualitative approach. Key partners were identified for unstructured interviews using purposive sampling. Eleven interviews were carried out with CDC Subject Matter Experts (n=5), select state and local public health laboratories and epidemiologists (n=5), and the Association of Public Health Laboratories (APHL). Participants were selected to obtain diverse viewpoints from those that have been most involved in this response, while also collecting information in a timely manner. Discussions were carried out using four guiding questions:

1. What investments in preparedness supported the response to mpox?
2. What were some of the main successes in the first 10 weeks of the mpox response?
3. What were some of the main challenges in the first 10 weeks of the mpox response?
4. How can the LRN improve their response to infectious disease outbreaks in the future?

We conducted a thematic analysis of the subsequent discussions.

Results: Through our discussions with key LRN partners, we identified themes which informed the development of seven recommendations for improved LRN response in the future. These recommendations build upon the successes and challenges identified in discussions.

1. Increase and sustain necessary public health laboratory infrastructure and connections with clinical and commercial laboratories.
2. Create and exercise surge plans.
3. Update approved protocols to include automated extraction methods when possible.
4. Update electronic test ordering and reporting systems for LRN member laboratories.
5. Increase training for laboratory staff, providers and partners in working with public health laboratories.
6. Improve communication between CDC, LRN member labs and providers.
7. Plan for expansion to commercial and clinical laboratories as part of outbreak response.

Conclusions: The LRN systems plays an integral role in the US's outbreak preparedness, including helping to provide a critical initial response until testing capacity can be expanded to commercial and clinical sites. The findings presented in this paper will be used as a starting point for discussions with CDC and partners about the future and potential improvement of the LRN. In addition, testing readiness for future responses will require a systemic approach that includes providers, commercial and clinical laboratories and other partners in addition to public health and LRN laboratories.

Presenter: Katharine Thomas, twu2@cdc.gov

Gotta Catch 'Em All: Defining Cost-Effective Variant Surveillance Through Retrospective Analysis of SARS-CoV-2 Whole Genome Sequencing

R. Cox, A. Smith, J. Freeman, G. Godfrey, G. Goodwin, C. Aroh, O. Adair, N. Epie; South Carolina Department of Health & Environmental Control

New SARS-CoV-2 (SC2) variants are continually arising that modify viral infectivity, vaccine efficacy, and the public health response. The goal of variant surveillance is the detection of viral diversity in a community, allowing for the rapid identification of new variants of concern and enabling the implementation of new pharmacological or non-pharmacological interventions. Establishing a surveillance program that captures the broadest array of variants in a sustainable and cost-effective manner remains an ongoing priority given heightened public interest during epidemics. Here we perform control-matched retrospective analysis of data from PCR testing and sequencing workflows to determine the impact of selective sequencing of samples on the breadth of variants detected, both from the laboratory data and statewide sequencing data. Specifically, we define the impact on variant capture and reagent cost of establishing a cycle threshold limit for sequencing PCR positive samples, modifying sequencing workflow with an Agilent TapeStation quality control reflexed from Qubit QC, and correcting loading concentration to achieve optimal Illumina MiniSeq run metrics. It is hoped that the lessons learned from this pandemic will enable data-driven decisions for establishing an adequately broad variant surveillance program for the next epidemic.

Presenter: Greg Goodwin, goodwigr@dhec.sc.gov

Maximizing Lessons Learned from Past Outbreaks to Develop a Sustainable Laboratory Cross-training Program

C. Williams, D. Payne, Jocelyn Hauser; District of Columbia Department of Forensic Sciences Public Health Laboratory Division,

Although public health laboratories range in complexity and size, all face the unexpected in the form of outbreaks and other public health emergencies. Laboratories must be able to manage the unexpected without disrupting day-to-day operations or reducing the laboratory's efficiency. We performed a retrospective analysis of how our laboratory adapted to accommodate the increased testing demands during both the COVID-19 pandemic and the mpox outbreak of 2022 to develop a sustainable cross-training plan to ensure our laboratory is better prepared for future outbreaks. Here, we describe steps taken to strategically plan a laboratory-wide cross-training program at the District of Columbia Public Health Laboratory (DC PHL). DC PHL's cross-training model is centered around coordinating preemptive cross-training in all units to ensure proper coverage for future emergencies. In reviewing our mpox response, it was evident that training staff during the outbreak resulted in substantial lag time and caused added strain for our testing team, as they had to train and test suspected samples. As part of a strategic cross-training plan, we developed a plan that included proactive cross-training which would allow our lab to increase testing capabilities and efficiency while reducing the strain on staff, further improving overall preparedness. Our cross-training plan includes a new hire bootcamp to review basic concepts, such as pipetting, quality control, accessioning and LIMS. Training ensured a baseline competency and reduced the amount of time

required for individuals to cross-train in other units, allowing us to maximize the number of cross-trained staff. Having staff available to assist a unit for an unexpected outbreak or emergency will maximize our laboratory's emergency preparedness exponentially, creating a resilient laboratory system our nation's capital can count on.

Presenter: Channyn Williams, channyn.williams@dc.gov

NEWBORN SCREENING AND GENETICS

Impact of Birthweight on TREC-Based SCID Screening

C. Weaver, South Carolina Department of Health & Environmental Control Public Health Laboratory

Newborn screening for Severe Combined Immunodeficiency (SCID) improves clinical outcomes by identifying high-risk individuals prior to symptom onset. These patients may then receive confirmatory diagnostic testing and early therapeutic interventions which have been proven to dramatically improve clinical outcomes. The genetic causes of SCID are complex so newborn screening is based on the quantification of T-cell Receptor (TCR) Excision Circles (TRECs). TRECs are a byproduct of T cell maturation wherein TCR gene rearrangement leads to the formation of T cell receptor antigenic diversity. During this process, some regions of the genome are excised and circularized into TRECs. Since SCID is characterized by the absence or severe depletion of T cells, patients also exhibit low TREC counts. Although TREC-based SCID newborn screening is effective, decreased TREC counts are also found in preterm neonates whose T-cells have not fully matured. This complicates the interpretation of TREC results and characterization of high-risk groups. The South Carolina Public Health Laboratory (SCPHL) recently validated the PerkinElmer NeoMDx assay for SCID newborn screening. The impact of birthweight on results interpretation of the TREC-based test method was evaluated using data from the initial three months of testing. Regression analysis revealed very weak linear correlation between TREC copy number and birthweight. The correlation was not markedly improved when birthweights were binned into broad classes. Despite lack of direct correlation, qualitative screen results were affected by birthweight. Very low (< 2500 grams) and low (1500-2499 grams) birthweight neonates were found to comprise only 15% of the population but 87% of abnormal screens. Compared to the population at large, very low birthweight babies were over 20-fold more likely to screen abnormal. In contrast, normal birthweight neonates (≥2500 grams) were tenfold less likely to screen abnormal. The effect of birthweight on screen results is likely a shift in the TREC frequency distribution as median TREC copy numbers were lowest among very low birthweight neonates. Finally, retesting of abnormal specimens yielded normal screen results in just 36% of very low birthweight individuals compared to 60% from those of normal birthweight. Taken together, these results support the hypothesis that performance of TREC-based SCID screening is impacted by birthweight. Findings presented here suggest that neonates whose abnormal screens are not attributable to low birthweight may represent a high-risk group for SCID. Future studies will incorporate specimen recollection data and clinical follow-up to provide a more comprehensive risk profile for this group.

Presenter: Cory Weaver, weavercj@dhec.sc.gov

MS/MS X-ALD Cutoff Establishment and Onboarding

C. Kirwan, C. Porter, G. Bonn; Colorado Department of Public Health and Environment

X-linked Adrenoleukodystrophy (X-ALD) is a peroxisomal disorder originating in the ABCD1 gene located on the X-chromosome. Producing a transmembrane transport protein, the gene facilitates transport of very long chain fatty acids across the membrane for beta-oxidation and subsequent metabolic production. Impaired function originating from lack of transport proteins or gene mutations leads to the accumulation of very long chain fatty acids (VLCFAs), such as C26:0-lysophosphatidylcholine (C26:0-LPC) and C24:0-lysophosphatidylcholine (C24:0-LPC) analytes. The accumulation of these analytes leads to detectable screening testing through the use of MS/MS mass spectroscopy for initial newborn screening testing conducted by the Colorado Newborn Screening Program (CONBSP) at Colorado Department of Public Health and Environment (CDPHE). Following the validation of PerkinElmer NeoBase 2 and MS/MS QSight instruments in July 2022, CONBSP initiated the induction of X-ALD and C26:0-LPC testing, bringing Colorado and Wyoming in line with the new Recommended Uniform Screening Program (RUSP) requirements for screening of disorders. A validation plan was designed to address accuracy and precision of establishing C26:0-LPC analyte on two of the PerkinElmer MS/MS QSight 210MD instruments. Utilizing CDC Quarter 1 and Quarter 4 QC and PT samples, for accuracy and precision, respectively, determination of statistically significant differences and lack thereof established confidence in CONBSP capabilities in C26:0-LPC screening in population testing. Establishment of an initial conservative cutoff of 0.9 $\mu\text{mol/L}$ for C26:0-LPC was validated due to the rarity of the disorder and determination of cutoff, taking into consideration data trends and literature in more than 23,000 patient samples run routinely Sunday–Friday over the course of four months followed with confirmation testing. A 98th percentile cutoff was established from these patient samples and correlated to PerkinElmer's reference study cutoffs. Cutoff adjustments will be made post onboarding through the use of PerkinElmer Biochemical for LC/MS secondary testing in review. Additionally, during the study, the analysis of CDC quality control samples stored (>3 months) long term versus short term (<3 months) was conducted in addition to those stored in the fridge (2-8°C) versus freezer (<-10°C) in regards to the stability of C26:0-LPC for MS/MS testing.

Presenter: Cullan Kirwan, kirwancullan@hotmail.com

Validation and Implementation of a Custom Second Tier Next-Generation Sequencing Assay for Improved Accuracy of Cystic Fibrosis Screening at DCLS

J. Chavez, L. Lion; Virginia Division of Consolidated Laboratory Services

The current Cystic Fibrosis (CF) screening assay at DCLS includes a biochemical test for elevated Immunoreactive Trypsinogen (IRT) followed by a Luminex 39-mutation gene panel for those samples that fall within the daily top 4% of IRT levels. The Luminex panel currently in use detects 39 common CF variants, enabling us to refer babies for diagnostic testing with a high level of sensitivity.

However, this panel is known to lack the heterogeneity necessary to adequately screen the allelic diversity of CF Transmembrane Conductance Regulator (CFTR) gene mutations in Virginia. Variant detection is significantly lower in non-white newborns due to the Eurocentric composition of the Luminex-39 panel. The DCLS Newborn Screening Group and the Sequencing and Bioinformatics Group are developing and validating a targeted next-generation sequencing (NGS) assay for the expansion of the current CF second-tier screen. The NGS assay will be run using Illumina MiSeq instruments and the corresponding Illumina library prep kit. This assay will include DNA extraction from dried blood spot cards, library preparation, DNA sequencing and comparison of sequencing results to the variants listed in CFTR2. This assay aims to identify most variants classified as “CF-causing,” “varying clinical significance” and “unknown significance” in the CFTR2 database, totaling over 400 variants. This presentation will outline gaps in the current assay and the benefits of implementing the NGS assay in its place, then discuss key aspects of the validation of this assay. While the assay is not expected to be implemented before the APHL 2023 Conference, this poster will include a prospective analysis of its accuracy, precision and impact on the screening of CF in the VA population.

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Validation of a Multiplex Assay for SCID/SMA Newborn Screening

C. Weaver, South Carolina Department of Health & Environmental Control Public Health Laboratory

Severe Combined Immunodeficiency (SCID) is a group of disorders characterized by reduction of T-cells leading to a fatal loss of adaptive immunity. Molecular-based assays can identify SCID patients prior to symptom onset and expedite therapeutic intervention. The PerkinElmer NeoMDx assay accomplishes this by detecting DNA fragments produced during T-cell maturation called T-cell Receptor Excision Circles (TRECs). The multiplexed NeoMDx assay also screens for Spinal Muscular Atrophy (SMA) which is a disease characterized by progressive loss of motor neurons leading to immobility, respiratory failure and death. Successful therapies are available but are most effective before symptom onset. As with SCID, newborn screening has allowed for expedited SMA treatment. The NeoMDx assay targets the SMN1 gene because both copies are deleted in > 95% of SMA patients. The South Carolina Public Health Laboratory (SCPHL) recently validated the PerkinElmer NeoMDx assay for SCID and SMA newborn screening. The assay proved to be > 98% accurate for both targets (TREC and SMN1) and the internal reference (RPP30) over three levels of control material. Intra- and inter-day precision were well within acceptable limits of 15% coefficient of variance. SCPHL tested 5571 deidentified, residual specimens to define internal controls and diagnostic cutoffs. Using specimens from known SCID and SMA cases, diagnostic sensitivity was found to be 100% for both SCID and SMA while specificity was 99.8% for SCID and 100% for SMA. Based on data presented here, SCPHL approved NeoMDx as a high complexity CLIA regulated test and went live with the method in September 2022.

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Verification of the Qsight 225 MDTM for the Screening of X-linked Adrenoleukodystrophy (X-ALD) and Adenosine Deaminase Severe Combined Immunodeficiency (ADA-SCID) in Alabama

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Across the United States, newborn screening (NBS) programs test the newborn population for the presence of rare genetic and metabolic diseases every day. State NBS programs will choose which conditions to include on their panels based on the recommended uniform screening panel (RUSP). Of the RUSP conditions screened for by the Alabama NBS program, many are identified via tandem mass spectrometry. Having previously used the NeoBase™ kit on the Waters Acquity™ Tandem Quadrupole Detector (TQD), a verification study is being conducted for use of the NeoBase 2™ kit on the Qsight 225 MDTM tandem mass spectrometer. In addition to ensuring accurate and precise measurements of analytes for current disorders, this newly verified method will allow for the measurement of key analytes for two new disorders: ADA-SCID and X-ALD. Both X-ALD and SCID are conditions found on the RUSP, however the variant ADA-SCID specifically is not. Out of all instances of SCID, the ADA cases make up approximately 10–15%. As a result of an adenosine deaminase deficiency, there is a toxic build-up of metabolites such as adenosine and 2'-deoxyadenosine, ultimately impacting the immune system in ways that are traditionally observed in pure SCID cases. The NeoBase 2™ kit utilizes these high concentrations of adenosine to allow for the early identification of ADA-SCID. Upon completion of the study, the Alabama Department of Public Health's Bureau of Clinical Laboratories will be able to maintain screening efficiency and expand the state's panel to include the new conditions.

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at Aegis Sciences Corporation. RPIP uses target capture-based enrichment for detection, quantification and characterization of 282 pathogens directly from respiratory samples. NGS data were analyzed with the Explify RPIP Analysis app in BaseSpace (Illumina), and the results were paired with non-identifying patient demographics and self-reported symptoms. Most participants (n=3338; 81%) reported ≥1 respiratory symptom but SARS-CoV-2 was only detected by RT-PCR in 22% (n=740). In contrast, RPIP detected respiratory viruses in 55% (n=1856) of participants; SARS-CoV-2 was the most frequently detected virus (86% agreement vs RT-PCR). Among symptomatic individuals testing negative by RT-PCR (n=2583), RPIP identified a possible etiology in 1,219 (47%). Viruses detected frequently by RPIP in this group included targets of national viral surveillance, such as influenza A virus subtypes, H3N2 and H1N1 (n=120; 4% & n=19; 0.6%, respectively), human parainfluenza viruses (HPIV)-1 (n=12; 0.4%), HPIV-2 (n=6; 0.2%), HPIV-3 (n=78; 2%) and HPIV-4 (n=32; 1%), seasonal coronaviruses (n=107; 3%), respiratory syncytial virus (n=70; 2%), human metapneumoviruses (n=35; 1%), and adenoviruses (n=9; 0.3%). Human rhinovirus A (n=333; 10%), B (n=97; 3%), and C (n=117; 3%) were also frequently detected. Notably, RPIP detected viruses that are not routinely tested for influenza C virus or human parechovirus, some of which are of public health concern, e.g., enterovirus D68 & mumps virus. Genome coverage was sufficient to infer type directly from the sample via the RPIP analysis app in many cases. As bottlenecks to routine implementation of metagenomic NGS are addressed via technical advances, cross-sector collaboration, data standards development and sustained funding & policy support, there is untapped potential to improve understanding of disease on the population level. Scalable sequencing of respiratory samples from symptomatic outpatients seeking testing can improve situational awareness and provide early warning of increased local transmission of pathogens beyond SARS-CoV-2 to inform public health action.

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NEXT GENERATION/LONG-READ SEQUENCING, METAGENOMICS AND BIOINFORMATICS

A Scalable Model for Metagenomics-based Surveillance for Influenza-like Illness in the Community

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The SARS-CoV-2 pandemic accelerated the integration of genomics into pathogen surveillance and demonstrated the value of private-public cooperation to scale testing. Commercially available technology could broaden routine surveillance from one pathogen (SARS-CoV-2) to multiple causes of influenza-like illness (ILI). This study presents feasibility for community-based ILI surveillance via high throughput next-generation sequencing (NGS). Residual, self-collected upper respiratory samples (n=4104) from individuals across all 50 US States and Puerto Rico seeking SARS-CoV-2 testing at a national retail pharmacy between 2022 Epi Weeks 18–46 were tested with the Respiratory Pathogen ID/AMR Panel (RPIP, Illumina – For Research Use Only. Not for use in diagnostic procedures)

Comparison of Two Bioinformatics Pipelines for Antimicrobial Resistance Analysis

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Next-generation sequencing (NGS) coupled with reliable bioinformatics pipelines is considered essential for surveilling the antimicrobial resistance in healthcare associated infections (HAI) of Carbapenem-resistant Enterobacteriaceae (CRE). The validation of bioinformatics pipelines is of great importance to ensure consistent findings, which can negatively influence antimicrobial resistance surveillance for patient care. For validation purposes, the comparison of a new bioinformatics pipeline, PHoeNix (Portable Healthcare Nextgen Informatics), was compared against the current Abricate (github.com, Torsten Seemann) workflow analyzing antimicrobial resistance markers. The ability of PHoeNix pipeline to find antimicrobial resistance genes and virulence markers was evaluated while considering user experience (captured by time for analysis relative to the current workflow). Parallel evaluation of 138 total isolates were run through both pipelines producing nearly identical results for the five major beta lactam resistance genes blaNDM (100% similarity (82/82)), blaVIM (100% similarity (2/2)), blaIMP (100% similarity (1/1)), blaKPC (100% similarity (11/11)), and blaOXA (97.2% similarity (72/74)). Of the total isolates

(n=138), 119 were further analyzed for virulence markers mpA (50% similarity (1/2)), mpA2 (100% similarity (2/2)), iroB (100% similarity (1/1)), iucA (94.1 % similarity (16/17)) and peg-344 (100% similarity (2/2)) using both pipelines. Worth further investigation, the PHoeNix pipeline interpreted additional genes such as ampH, penicillin binding protein, bla_{NMC} and bla_{Zn} that were not detected by Abricate. Outputs generated by the PHoeNix pipeline were also furnished with QC and contamination results. Unlike analysis by Abricate, the PHoeNix pipeline can be conveniently run on a Linux server (kernel.org) using FASTQ files generated by the industry standard, Illumina sequencers (Illumina, Inc., San Diego, CA); and as an open-source program, the software is available free of cost including upgrades. The PHoeNix pipeline is more efficient with time and raw data quality checks. And further, the PHoeNix pipeline provides additional useful analysis for identifying antibiotic resistance genes and virulence markers in surveillance isolates.

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Establishment of Next Generation Sequencing for SARS-CoV-2 Utilizing Wastewater Samples from Rural Northeast Texas

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Background: One of the greatest hurdles to timely public health response, as exemplified by the COVID-19 pandemic, is disease monitoring and reporting. The rapid evolution of the SARS-CoV-2 variants makes it difficult to assess the evolution of the pathogen in a timely manner; hence hindering public health response. Currently, the Public Health Laboratory of East Texas (PHLET) service area includes 35 counties within East Texas; many of these counties are primarily rural in nature. The majority of these counties are largely underserved making them especially vulnerable to exposure of rapidly emerging diseases. Therefore, PHLET is validating the first wastewater based NGS (next generation sequencing) for rapid detection of SARS-CoV-2 variant circulating within the population near Smith and Gregg counties. **Objectives:** The primary aim of this project is to monitor and analyze the circulating and emerging SARS-CoV-2 variants in East Texas through utilization of regional wastewater samples. Additionally, we will create a pipeline for the data and share it with local, state and federal collaborators. Finally, PHLET plans to expand to other pathogens such as influenza and respiratory syncytial virus (RSV). **Methods:** The method followed the standard amplicon-based Illumina MiniSeq system, which can sequence up to 100 samples. For the validation phase, over 40 clinical samples containing SARS-CoV-2 were chosen spanning from March 2020 to August 2022. They were extracted using the Qiagen Non-DSP Viral RNA kit. Next, the samples were prepared using the Illumina COVID Seq Assay (96 samples). Sequencing was performed on the MiniSeq using the MiniSeq high-output reagent kit (300 cycles). Finally, data was analyzed using the DRAGEN COVID Lineage App. **Conclusions:** Next generation sequencing of SARS-CoV-2 will enable the lab to expand variant detection and coordinate with our local partners to implement appropriate public health measures in response. In addition, by utilizing the Illumina instrument software for base-calling, the laboratory can import sequencing data for future collaboration with partners for bioinformatics pipeline development, as well as contribute the basic science of COVID-19 evolution through analysis of the data.

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Evaluating Whole-Genome Sequencing as an Approach for Antimicrobial Susceptibility Testing with AREScloud

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Antimicrobial resistance is a growing public health threat that has been exacerbated during the SARS-CoV-2 pandemic, primarily due to the continued misuse of antimicrobials. The primary method to identify antimicrobial resistance and inform appropriate and targeted treatment is through antimicrobial susceptibility testing (AST). Determining the antimicrobial resistance profile is critical to patient care and antimicrobial resistance surveillance. Clinical and public health laboratories typically perform AST using broth microdilution or Etest and sometimes follow up with PCR and whole-genome sequencing (WGS) to further characterize the resistance mechanism. More recently, new methods are emerging that enable whole genome to be an effective alternative in antimicrobial susceptibility testing. Using machine learning models, WGS-AST methods can predict a wider variety of resistance phenotypes than possible in many laboratories and can provide a greater amount of additional data, including pathogen and resistance mechanism identification. Herein we compared the AREScloud web application (Ares Genetics), a cloud-based WGS-AST tool that utilizes machine-learning models established for various bacterial-drug combinations to the phenotypic AST profiles generated at the Wisconsin State Laboratory of Hygiene. We used AREScloud to compare the genotypic AST profiles for 16 public health reportable bacterial isolates, including five *Acinetobacter baumannii*, one *Enterobacter cloacae* complex, four *Escherichia coli*, three *Klebsiella pneumoniae*, and three *Pseudomonas aeruginosa*. Using phenotypic AST profiles as the reference, error designation and accuracy statistics were performed for 152 bacterial-drug combinations with an overall categorical agreement of 82.2% and very major, major, and minor error rates of 2.6%, 4.6%, and 10.5%, respectively. Here we demonstrate a strong potential for WGS-AST to be used as an effective complement to phenotypic AST and its use as a component in the overall effort of antimicrobial resistance surveillance.

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Exploring Viral Genetic Diversity by Deep Sequencing of Whole Genomes Following Target Enrichment: A Case Study of Universal Typing of Human Papillomavirus

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Introduction: The public health importance of information on viral genetic diversity has been highlighted by the COVID-19 pandemic. Intraspecific genetic variations (strains, variants, integration and lineages) may impact transmissibility and pathogenicity. While metagenomics has been the most widely used approach to generate genetic information on viruses, it requires extensive bioinformatics support, and in human samples with low viral load may lack required sensitivity, and specificity. In addition, PCR-based enrichment risks skewing the representation of viral genetic variations. RNA bait-based enrichment for all known types of a pathogen following whole genome deep sequencing (eWGS) is an alternative approach. Using Human Papillomavirus (HPV) as

a model, we demonstrate the effectiveness of target enrichment combined with next generation sequencing. HPV is a family of more than 200 DNA viruses, is the most common sexually transmitted infection, and the etiologic agent of cervical and other anogenital and head/neck cancers. Current assays focus on the 14 high risk types associated with most HPV-related cancers, but identification of the full spectrum of HPV types and variants could provide insights on tropism and disease associations. eWGS was used in this study to explore the genetic diversity and potentially identify novel and unknown sequences of HPV. **Methods:** Samples (total nucleic acid [TNA] extracts of exfoliated cervical cells) were indexed and pooled prior to target enrichment. The pools of samples were enriched by hybridizing to RNA bait composed of 25,239 RNA probes targeting 202 HPV types and 12 probes targeting the human beta-globin gene. The Agilent SureSelect protocol (XT2) was used for library preparation, and sequencing was performed on a HiSeq 2500. An in-house bioinformatic pipeline was used for automated HPV typing and identification of full-length sequences. **Results:** The bioinformatic pipeline identified 2,376 HPV types (2,320 alpha, 9 beta and 45 gamma genomes) in the cervical samples (n=761). The prevalence of HPV types in the alpha genomes were HPV16 (12.5%), HPV31 (5.8%), HPV51 (4.8%), HPV66 (4.7%), HPV52 (3.8%), HPV90 (2.24%), HPV39 (3.9%) and HPV18 (2.5%). Of 2,320 alpha genomes, 1,374 genomes (59%) met the definition of full-length sequences (>1,000 mapped reads with >80% genome coverage). This suggests that up to 41% of the HPV types could be integrated or have lost segments of their genome. HPV 16 (218/291, 75%) and HPV52 (72/87, 82%) had the highest proportion of intact genomes and HPV39 had the lowest (47/90, 52%). **Conclusions:** eWGS allows the full spectrum of HPV genetic diversity to be explored in one assay. This approach could be adapted for a wide variety of pathogens of public health importance.

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Genome Sequence of Serodino Virus, a Novel Oliveros-like Clade E Mammarenavirus

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A novel Mammarenavirus genome was identified within a suspect hantavirus sample collected from a rodent in Maciel, Argentina. Metagenomic deep sequencing analysis revealed Mammarenavirus-like reads in addition to those belonging to a species of hantavirus. The genome for the novel Mammarenavirus-like virus was initially built using de novo assembly. BLAST analysis of the partial genome yielded hits within the Mammarenavirus family. A guided de novo assembly was performed to complete the novel Serodino genome. Mammarenaviruses are negative-sense RNA viruses that are strongly associated with rodent species as their typical reservoir and contain a small (S) and large (L) segment. The S segment encodes the viral nucleoprotein (NP) and glycoprotein precursors (GPC), while the L segment encodes the viral polymerase (L) and RING-finger domain (Z). Mammarenaviruses are divided into two major serocomplexes: Old World and New World; the New World serocomplex is further segregated by Clades A-D. Serodino virus is most closely related to Oliveros virus; an arenavirus of Argentinian origin, discovered in 1990. Oliveros virus was first detected in the town of Oliveros, Argentina, located north of the Argentinean

Hemorrhagic Fever (AHF) endemic area that was undergoing rodent survey between 1987 and 1990 in response to a Junín virus outbreak. Phylogenetic analysis revealed that the Serodino S segment is 90.1% identical to Oliveros virus, however, the L segment is 70.4% identical. At the protein level, the S segment proteins (NP and GP) are at or above 99% similar and greater diversity is observed for the L segment proteins (L and Z) at 88.2% and 83.0%, respectively. Presence of these segments by RT-PCR has been confirmed and confirmatory sequencing will ensue. The International Committee on Taxonomy of Viruses (ICTV) mandates that new viral species may be classified by a specific host species or reservoir, specific geographic location, a cause of human disease, significant differences in antigenic cross-reactivity or a minimum of 12% amino acid sequence dissimilarity to existing viruses within the genus. Based on the observed L segment diversity and ICTV species-level designations, we propose that Serodino virus is a distinct New World Mammarenavirus and a member of a unique Clade “E” species.

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Implementation of a Targeted Nanopore Sequencing Assay for Antimicrobial Resistance Detection in *Mycobacterium tuberculosis*

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The time and cost associated with the diagnosis and treatment of *Mycobacterium tuberculosis* (MTB) infections can be considerable, specifically in cases involving multi-drug or extensively-drug resistant (DR) strains. Due to the slow growth rate of this bacterium, traditional (phenotypic) methods for determining antimicrobial susceptibilities require weeks to months to complete. Meanwhile, patients may be placed on inappropriate therapies leading to treatment failure and further resistance development. To improve turnaround times for resistance detection, a targeted Next Generation Sequencing (tNGS) assay was developed at the New York State Department of Health that can profile MTB antimicrobial susceptibilities direct from primary specimens. This assay utilizes multiplex PCR to amplify thirteen loci implicated in resistance to first and second-line antimicrobials. Amplicon libraries are sequenced on an Oxford Nanopore device and analyzed in real-time using a custom bioinformatics pipeline. The assay cost, including reagents and consumables and excluding labor, is approximately \$75 per sample. tNGS was attempted on 45 specimens previously determined to be MTB-positive. A subset of these samples yielded sufficient DNA for sequencing and aided in the establishment of sample quality criteria for referring samples to tNGS. These criteria include a positive AFB smear and/or a real-time PCR Ct value of ≤34.0. With these parameters met, tNGS accurately provided susceptibility profiles and lineage identification for all samples sequenced, which included 28 pan-susceptible and two multi-DR strains. Comparison of turnaround times showed a mean improvement of 13 days (and a maximum of 31 days) for direct specimen tNGS when compared to whole genome sequencing performed on the resulting patient isolate. These results demonstrate the utility of tNGS to offer early comprehensive antimicrobial resistance detection for MTB.

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Implementing Influenza Sequencing for Molecular Epidemiological Surveillance of Influenza Strains Throughout the 2022-2023 Season in New Jersey

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Influenza viruses are single-stranded, negative-sense RNA viruses that pose a serious risk to human health. Infections from the influenza virus can range in severity, varying from mild illness to death. The influenza genome is comprised of eight RNA gene segments that are prone to changes, through the processes of antigenic shift and drift, which may have an impact vaccine effectiveness. The two types of influenza viruses that cause seasonal epidemics are influenza A and B, where A is further divided into subtypes and B into lineages. Influenza A virus subtypes are based on the combination of hemagglutinin (H) protein (18 types) and neuraminidase (N) protein (11 types) in the virus. Whole genome sequencing of influenza can better inform scientists about the evolution of the influenza virus and in turn, provide epidemiological data for vaccine development and maintenance. One of the important public health needs addressed at New Jersey Public Health and Environmental Laboratories (NJPHL) is the surveillance of seasonal circulating influenza viruses utilizing whole genome sequencing. In order to meet this need, a protocol for whole genome sequencing of influenza viruses was implemented. RNA extracted from clinical samples, deemed positive for influenza by qPCR testing, was used for sequencing. Complementary DNA (cDNA) was generated from the RNA and amplified by PCR. Primers used for amplification targeted a conserved region of the gene segments in the influenza A virus and each individual segment of the influenza B virus, as suggested by the CDC. Resultant amplicons were purified and used to prepare libraries with the Illumina Nextera DNA library preparation kit. Short-read next generation sequencing was conducted on the Illumina iSeq. Sequencing data were used to generate assemblies for each of the eight gene segments to determine the lineage and subtype of each genome, and to identify Single Nucleotide Polymorphisms (SNPs) against the appropriate subtype reference. Several steps of the protocol were optimized, including increasing the starting RNA volume, modifying the cDNA synthesis approach, and adjusting the loading concentration for sequencing. Sequencing results provided a comprehensive look at the clade-specific genetic changes of H3N2, the most prominent subtype of influenza A recorded during the 2022-23 season. Utilizing the assemblies generated, a phylogenetic tree was created to visualize genetic differences in each of the eight gene segments across specimens collected in New Jersey during the current influenza season. From this work, NJPHL now has the capability for whole genome sequencing of influenza that will be continued as a surveillance method, and sequencing data will be reported to the CDC via the National Center for Biotechnology Information. Implementing this method will help to bolster vaccine development and support national influenza surveillance efforts.

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Improved Quality Control Analysis of Illumina Sequencing Data

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Sequencing data generated from high throughput sequencing (HTS) methods requires rigorous quality control steps to ensure the accuracy of downstream analysis. The importance of quality control to evaluate sequence data is well established in bioinformatics, and while general guidelines are in place, they are actively changing with the introduction of new tools and data requirements. Due to the vast quantity of data produced by SARS-CoV-2 sequencing efforts, the time dedicated to ensuring sequence quality has increased. Additionally, the need to classify sequence lineages requires added quality checks to keep mixed infection and potential contamination out of downstream analyses. To tackle these challenges, we have introduced new processes to our workflow by adding MultiQC and expanded our quality control dashboard to include a quality visualization of the plate layout. Our current process uses FastQC (Babraham Bioinformatics) as a first step in quality assessment. FastQC is a widely used tool for assessing the quality of short-read data. FastQC returns a full report for each sequence, making the evaluation process time consuming. Therefore, we have added MultiQC to our quality assessment. MultiQC takes the output from a variety of available tools, including FastQC, and provides a single report summarizing the data for easier interpretation. We customized the MultiQC report to show sequences failing at specified thresholds, such as those with mean quality scores 5, and adapter content > 5%. MultiQC also generates data that can be used in downstream analyses to create additional metrics. With this data, we created a table that calculates a score for each read between 0 and 3, based on where the mean quality along a read drops below a certain threshold. Reads with a score below 1 are flagged, and a score of 0 fails. This previously manual process has now been automated using a python script. To flag potential mixed infections and contamination across the 96-well plate used for sequencing, we have expanded the use of our quality control dashboard. The dashboard is used to visualize sequence depth across the genome, check for amplicon dropouts, coverage levels, and overall depth of sequence samples. We added to the dashboard a visual of the sequencing plate layout, with each well labeled and color scaled with the mean allele frequencies for each sample. If these frequencies vary across the genome, that sample is flagged for follow-up. This visualization is especially useful for detecting cross-contamination as neighboring wells are the most likely candidates for liquid spills. This additional QC step helps to ensure the integrity of our pangolin (-analysis-mode usher) lineage calls for SARS-CoV-2. These changes to our workflow have resulted in a simplified quality assessment process with clearer visualizations and fewer reports and has reduced the time spent on quality assessment by half.

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Maximizing MAGs from Long-read Metagenomic Assemblies: A Post-assembly Pipeline with Completeness-aware Binning

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The use of metagenome-assembled genomes (MAGs) in microbial surveillance has increased in popularity. Metagenome assembly can allow for fine-scale resolution of epidemiological tracing and improve recovery of antimicrobial resistance (AMR) genes. Highly accurate long reads provide major advantages for metagenome assembly. PacBio HiFi sequencing of metagenomic samples with the Sequel IIe system regularly produces reads 8–15kb in size with a median QV ranging from Q30–40 (99.9–99.99% accuracy). With the development of new metagenome assembly algorithms specific to HiFi reads (including hifiasm-meta), it is now possible to reconstruct full metagenome-assembled genomes (MAGs) for many high abundance species. However, discontinuous assemblies routinely occur for lower abundance taxa. Post-assembly tools incorporating binning methods are therefore required to identify these MAGs from the assembled contigs. Here, we present the newest version of HiFi-MAG-Pipeline, a comprehensive workflow that automates major steps including binning, quality filtering, and taxonomic identification. The outputs include high-quality MAG sequences, associated metadata, and visualizations of key MAG characteristics. Importantly, the pipeline uses a custom binning strategy that is tailored to long-read assemblies. To demonstrate the performance of this pipeline, we assembled and analyzed several publicly available HiFi metagenomics datasets. We show that complete, circular MAGs are routinely produced from HiFi metagenome assembly, and that HiFi-MAG-Pipeline recovers more total high-quality MAGs than other standard methods. Overall, our results suggest that HiFi sequencing is an effective strategy for obtaining large numbers of high-quality MAGs, which can be used to improve microbial surveillance efforts.

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Monitoring COVID-19 Variants in Wisconsin Using SARS-CoV-2 Whole-Genome Sequencing in Wastewater

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SARS-CoV-2, the viral agent of COVID-19, poses challenges to the clinical surveillance systems due to large numbers of asymptomatic and therefore untested individuals, the increased use of at-home tests, and the limited access to testing facilities for some populations and regions. In Wisconsin, these challenges also affect the number of clinical specimens sequenced, which can lead to a skewed picture of the COVID-19 variant situation, which impedes the ability of the public health community to take appropriate action. Wastewater-based epidemiology (WBE) overcomes many of these challenges by testing integrative wastewater samples representative of entire communities served by municipal wastewater treatment facilities. Since 2020, WBE has demonstrated its utility to serve as a complementary and independent epidemiological tool to assess COVID-19 prevalence and help prevent the spread of COVID-19 in communities. While the monitoring of SARS-CoV-2 concentrations has been popularized within public health laboratories, the use of genomic sequencing of wastewater to monitor COVID-19 variants is far from becoming mainstream. The Wisconsin State

Laboratory of Hygiene (WSLH) initiated in January 2022 a statewide wastewater SARS-CoV-2 genomic surveillance program to monitor the prevalence of COVID-19 variants in the population using a tiled amplicon whole-genome sequencing strategy. We demonstrated that wastewater-based prevalence variant data mirrored the successive Omicron waves documented in clinical samples. Thus, it could serve as an early warning system. To make these data accessible to larger audiences, we developed interactive visuals for a [public-facing dashboard](#). We also developed comprehensive and detailed data visualization tools for internal use to assess the quality of sequencing data, identify emerging mutations, and detect potential cryptic variants. Our bioinformatics workflows rely on free and accessible databases and software and are all publicly available to help other public health laboratories interpret their next-generation sequencing data.

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Multi-State Evaluation of LRN Rapid BSL-3 *Bacillus anthracis* Whole Genome Sequencing for Antimicrobial Resistance Markers and Bioengineering Detection for Anthrax Preparedness

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During a public health emergency involving *Bacillus anthracis*, detection of antimicrobial resistance (AMR) will inform decisions about anthrax treatment and post-exposure prophylaxis to save lives. *B. anthracis* is a bacterium subject to select agent requirements (42 CFR Part 73). CDC's BioDefense Research and Development laboratory completed a multi-state pilot study evaluation of same-day whole genome sequencing (WGS) for *B. anthracis* in collaboration with the Association of Public Health Laboratories and Laboratory Response Network (LRN) laboratories, including Arizona Department of Health Services (Phoenix, AZ), Wadsworth Center, New York State Department of Health (Albany, NY), Texas Department of State Health Services (Austin, TX) and the Division of Consolidated Services (Richmond, VA). LRN scientists conducted rapid BSL-3 WGS, with Oxford Nanopore MinION Mk1B sequencers using genomic DNA from agar cultured *B. anthracis*. Nucleic acid was prepared from culture isolates and sequenced in the same biological safety cabinet, thereby minimizing the time to results and reducing laboratory biosafety risks. Each laboratory completed at least four single-plex and one multiplex sequencing runs. Genomic data sets were analyzed on-site using custom, open-source bioinformatics software called PiMA—a bacterial genome analysis pipeline. The software was installed on the same laptop that powered the BSL-3 sequencer and performed quality control analysis, de novo sequence assembly and identification and characterization of AMR features, including the detection of plasmids, integration sequences and mutations. Both single-plex

and multiplex MinION sequencing runs were successfully completed on-site by LRN laboratory scientists after minimal remote training. For 100% of the runs in this study, scientists sequenced and assembled the bacterial chromosome and associated plasmids from *B. anthracis* strains, and each yielded the expected predictive genomic AMR profile with no evidence of bioengineering detected in as few as five hours. Data analyses conducted by the LRN scientists were completed in <2 hours and matched CDC findings with no differences observed in the quality of assemblies generated or in the detection of AMR features. Following analysis, chromosomal single nucleotide variants (SNVs) related to fluoroquinolone resistance were interrogated, and correctly identified. However, an SNV located in a homopolymeric region that confers *B. anthracis* penicillin resistance was not reliably resolved by MinION sequencing alone. In this study, laboratory scientists from all four states successfully profiled plasmids, mutations and genetic signatures associated with AMR in under five hours, demonstrating that trained laboratory scientists could rapidly sequence and analyze *B. anthracis* in a BSL-3 laboratory using a portable sequencer, strengthening public health preparedness for an emergency anthrax response.

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Next Generation Sequencing Strategies for Public Health Surveillance and Outbreak Investigation of SARS-CoV-2 and Mpox Infections

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Introduction: The COVID-19 pandemic has greatly impacted public health preparedness and brought to light the crucial role of advanced molecular investigation and surveillance of genetic mutations in effectively responding to infectious outbreaks. While COVID-19, caused by the SARS-CoV-2 virus, remains the leading cause of death by a single infectious agent, the emergence of mpox as a threat in the US and worldwide further emphasizes the need for accurate detection of pathogen variants for public health initiatives and outbreak management. The objective of the Dallas County Health and Human Services (DCHHS) next generation sequencing (NGS) program is to establish and provide sequencing-based pathogen clade analysis and genomic strategies that could effectively assist in outbreak investigation of respiratory pathogens, including influenza and SARS-CoV-2 and the emerging mpox infections. **Methods:** The workflows for both SARS-CoV-2 and mpox have been designed and developed from Illumina COVIDSeq protocol by differing the genome-specific primer pools. The most updated ARTIC community V4/V4.1 primer pools (for SARS-CoV-2) and a custom design pool from Yale School of Public Health (for mpox) have been employed for highly accurate detection of mutations in the respective viral genomes. The workflow includes steps for viral RNA/DNA extraction, library prep, sequencing in the MiSeq, analysis and report generation. The sequence analysis steps include raw read assembly and consensus sequence mapping to reference genomes and variant identification using Bioinformatics platform, including Illumina BaseSpace, NextClade, Pangolin web tools and CLC Genomics Workbench. **Results:** COVIDSeq protocol has been validated through cross-analysis verification of 40 de-identified samples provided by another public health lab with 100% agreement on positive Alpha/Gamma/Delta/Omicron variants and

QC precision samples. About 100 COVID patient samples have been successfully sequenced and detected for ~20 Omicron sub-lineages in which BA.5 was the most prevalent in July–November 2022, and BQ.1 spiked in December 2022. These reports are also in direct agreement with other agency labs, including CDC. Using the parallel workflow, we have sequenced over 170 mpox samples and identified B.1 lineage of West African Clade IIb as the most dominant variant during the peak surge of mpox in July through November 2022. The viral genome sequences have been uploaded to global databases (GISAID, NCBI). **Conclusion:** The DCHHS NGS program has successfully created streamlined and modified protocols for the efficient detection of genetic mutations in both SARS-CoV-2 and mpox. These strategies not only aid in real-time surveillance and outbreak investigation of these infectious agents, but also provide valuable educational and technical resources for promoting public health interventions.

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Pathogen and Antimicrobial Resistance Detection in Wastewater using Hybrid Capture Enrichment Provides a Method for Tracking Emerging Infectious Diseases and Antimicrobial Resistance Genes

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Monitoring wastewater for pathogens and antimicrobial resistance has emerged as an important epidemiological approach to assist public health professionals in tracking and potentially predicting trends in community disease prevalence. Increased pathogen levels in wastewater often precedes increases in clinical case metrics, providing an opportunity for non-invasive early warning of outbreaks. The use of quantitative PCR methods to detect and quantify pathogens in wastewater has become routine and provides a sensitive and targeted means to track viral dynamics and infection burdens in communities. This approach has distinct advantages for both sensitivity and throughput, however, it is limited to a single (or if multiplexed, at best a small number of) pathogens for any one assay. Next-generation sequencing (NGS) may provide a powerful alternative approach for monitoring large numbers of specific pathogens. Successful implementation of NGS in wastewater depends on applying robust methods for capture and concentrating the microbes and extracting the nucleic acids from concentrated samples. There is currently limited knowledge on how modern concentration and extraction techniques affects the recovered number and types of microorganisms in wastewater. Here we provide an evaluation of two concentration methods and multiple extraction methods using representative samples from Colorado and Wisconsin. The performance of two concentration approaches (1) a Concentrating Pipette (InnovaPrep) and (2) Nanotrap Microbiome Particles (Ceres Nanosciences) were evaluated. Furthermore, four different extraction techniques were assessed using wastewater samples obtained from college dorms and wastewater treatment facilities in Colorado and Wisconsin. The extracted nucleic acid was sequenced with Shotgun metatranscriptomics-which depleted ribosomal RNA (rRNA) using RiboZero Plus™ Microbiome (Illumina) coupled with total RNAseq library preps to profile the entire microbial content in the samples and the Urinary Pathogen

ID/AMR Panel (UIP) and Viral Surveillance Panel (VSP)-two precision metagenomics assays using biotinylated DNA probes to enrich microbial target sequences. UIP targets 174 genitourinary pathogens and >3700 AMR markers, while VSP targets 66 viruses. Shotgun metatranscriptomics confirmed viruses known to be abundant in wastewater, such as hCoV-OC43 and Rotavirus A. Precision metagenomics with UIP and VSP allowed for more in-depth strain identification as well as discovery of a greater number of less abundant pathogens, such as various noroviruses and enteroviruses. The results from these studies provide important guidance on how collection and concentration methods can impact the types and quantities of pathogens detected in wastewater samples and highlight the benefits of NGS approaches for advancing the potential of wastewater surveillance.

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Performance Evaluation of Genomic Analysis Tools for *Shigella* and Enteroinvasive *Escherichia coli* Species Identification and Serotype Prediction

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Shigella and enteroinvasive *Escherichia coli* (EIEC) are genetically and phenotypically closely related. They also have similarities in the pathology of infection and can cause diarrhea, fever and stomach cramps. Commercially available or laboratory-developed culture independent diagnostic tests that cannot differentiate between *Shigella* and EIEC are commonly used; additionally, isolation and differentiation of the two organisms can be challenging due to biochemical similarities and O-antigen cross-reactivity. Correct identification at the species and serotype levels is essential for public health laboratory surveillance and epidemiological investigations. In this study, we assessed the performance of ShigaTyper, ShigEiFinder and Mykrobe genomic analysis tools for species identification and serotype prediction of *Shigella* and EIEC. Whole genome sequencing data of 42 reference strains and more than 300 clinical strains of *Shigella* and EIEC from our collection were included in the analyses, covering the predominant serotype submissions to the California Department of Public Health Microbial Diseases Laboratory (CDPH-MDL). Additionally, all strains also had species and serotype designations determined through conventional testing schemes by our laboratory. *Shigella* is classified into four species and more than 50 serotypes based on biochemical tests and lipopolysaccharide O-antigen serotyping. Currently, the CDPH-MDL performs conventional *Shigella* serotyping for diagnostic reporting and conducts whole genome sequencing of *Shigella* for surveillance and epidemiological investigation. Challenges associated with conventional *Shigella* serotyping include subjectivity of interpretation, inability to type rough strains and recognize newly emerged serotypes, potential cross-reactivity and safety concerns due to manipulation of highly concentrated *Shigella* suspensions for which the infectious dose can be as low as 10–100 organisms. For these reasons molecular serotyping was evaluated as a potential replacement for traditional methods. In our study, the serotyping prediction generated by ShigaTyper, ShigEiFinder and Mykrobe provided robust and discriminatory typing of diverse *Shigella* serotypes, demonstrating that the evaluated analysis workflow would be acceptable for surveillance of *Shigella* infections. In

addition, the combination of the three genotyping analysis programs demonstrated accurate identification and differentiation of *Shigella* and EIEC. An analysis of the potential cost savings of transitioning to molecular serotyping for diagnostic reporting was conducted to further support future implementation of these genomic analysis tools.

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Rapid Implementation of Mpox Virus Whole Genome Sequencing Protocol, Using Multiple Sequencing Platforms, Allowed for Real-Time Surveillance of Outbreak

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Mpox virus is a member of the orthopox virus genus. The virus is spread by close skin-to-skin contact, and infection with the virus can lead to symptoms that include fever, myalgia, and skin lesions. The WHO declared a global outbreak of mpox virus in the summer of 2022. The first patient specimen that tested positive with mpox virus in New Jersey was identified at the New Jersey Public Health and Environmental Laboratories (PHEL) on June 16th, 2022. To test for mpox virus, DNA was extracted from skin lesion swabs in a Biosafety Level 3 laboratory with either a manual protocol (Qiagen column) or automated approach (Qiagen EZ1), using CDC protocols. Whole genome amplicon-based sequencing was applied to positive specimens (Ct value < 35) using both short-read sequencing (Illumina MiSeq V2/500 cycles) and long-read sequencing (Oxford Nanopore Technologies). Amplicons ranged in length from 1587 bp - 2497 bp. A total of 56 specimens were mpox positive using qPCR at PHEL, and all of which were sequenced using short-read sequencing technology. The range of Ct values for the positive specimens sequenced was 15.0 - 31.4 cycles. Additionally, 14 specimens were also sequenced using a long-read sequencing method. Using assemblies generated from each short-read sequencing dataset, a whole genome alignment was conducted to generate a phylogenetic tree. Quality Control (QC) metrics were also compared in these two approaches. The QC analysis shows that the percentage of the reference genome covered and the mean depth of coverage were greater, on average, when performing short-read sequencing. The subsequent Nextclade analysis showed that all specimen sequences were identified as Clade IIb, which was the main circulating lineage of the outbreak, and associated with less severe symptoms than Clade I. There were consensus lineage calls between sequencing approaches for 12/14 specimens, while one specimen had discrepant lineage calls and one specimen was only identified to the lineage level using long-read sequencing, but the sub-lineage was identified with short-read sequencing. Genome sequence data were submitted to the National Center for Biotechnology Information (NCBI) as Biosamples and raw sequence data (fastq files). Whole genome sequence assemblies that met internal QC thresholds were also submitted to Genbank. Specifically, the short-read sequencing data and resulting assemblies from 47 specimens were submitted. While Mpox infection levels in the community dropped off drastically in the fall of 2022 and remain low in early 2023, PHEL has demonstrated preparedness to implement whole genome sequencing of Mpox. Furthermore, the laboratory's expanded next-generation sequencing infrastructure

demonstrates the ability to quickly develop and implement protocols for sequencing novel pathogens with multiple sequencing technologies and produce high quality results for surveillance and scientific query.

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The Impacts and Considerations for Usage of Automated Liquid Handling Robots for Sample Preparation for Next Generation Sequencing in Small Public Health Laboratories

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With the rise in antibiotic resistance amongst human bacterial pathogens and in the wake of the SARS-CoV-2 pandemic, the necessity for rapid and cost-effective sequencing technology to identify bacterial and viral strains is readily apparent to those involved in the fields of patient care and public health. The advent of second and third generation sequencing technology provided a means for public health scientists to investigate the genomes of pathogens at decreased cost and time investment for laboratory personnel compared to earlier sequencing strategies. As sequencing technology has improved and the output of genetic information increased, so has the demand for the frequency and number of pathogens sequenced by laboratory scientists. Despite current advancements and lowering of cost compared to early sequencing methods, the molecular biology inherent in sequencing strategies still requires a major investment of public health laboratory resources both in reagent costs and health laboratory personnel lab hours. Additionally, health laboratory personnel's responsibilities by their very nature are consistently in flux as they respond to the mercurial nature of public health matters, increasing the strain on laboratory personnel. The introduction of robotic liquid handlers was one means to ameliorate the strains on laboratory staff and increase throughput for the number of sequenced specimens. The usage of liquid handling robots allows for laboratory scientist to assign automated repetitive tasks to liquid handling instruments, increasing or maintaining sample throughput but decreasing dedicated personnel hours to those steps. In heavily populated areas under the purview of large public health systems with dedicated sequencing facilities, the usage of these robots has allowed for increased sample throughput and alleviated some of the inherent issues with dedicating lab hours/staff to a task. However, the problems faced by the public health laboratory staff in more rural states with lower population density differ from those referenced above. Are there similar benefits in labor time and throughput garnered from the introduction of such automated liquid handling systems in public health laboratories in more rural or less populous states? We at the South Dakota Public Health Laboratory (SDPHL) sought to compare associated reagent costs, number dedicated laboratory staff hours, plastic waste and increased sample output between liquid handling automation and manual sequencing preparation by lab testing staff. This poster outlines our findings regarding the above mentioned as well as provides some considerations that might assist other public health labs when using liquid handling automation.

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Time and Motion Study of a Fully Automated Isolate Whole Genome Sequencing (iWGS) Platform for Surveillance of Foodborne Pathogens

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Background: PulseNet, developed by the US Centers for Disease Control and Prevention, established whole genome sequencing (WGS) as the gold standard for the surveillance of foodborne pathogens. The MiSeq-based sequencing platform currently used for WGS has drawbacks that limit use for rapid epidemiological applications of this technology. Clear Dx™ Microbial Surveillance WGS (Clear Labs, San Carlos, CA) has developed a platform that addresses some of the recognized limitations. This study was done to compare the Clear Labs sequencing process with the current process used by PulseNet. **Methods:** Time and motion studies to compare the manual MiSeq Illumina workflow currently used in the Nebraska Public Health Lab (NPHL), were done with a beta prototype of the Clear Dx™ platform. Both workflows were independently performed by two technicians. Comparisons included the number of human touch points encountered, the total hands-on time for sample processing including DNA extraction, total run time, and the number of disparate pieces of lab equipment required for the entire workflow. **Results:** The MiSeq-based manual workflow used 4 disparate instruments and 3 kits from different vendors, whereas the Clear Dx™ workflow used one integrated instrument and one kit from Clear Labs. The total workflow time from set up-to-results for the MiSeq-based method was 44 hours, while for the Clear Dx™ was 27 hours. The most significant differences between the two workflows were the number of contiguous human touch points, which were >20 (manual) and 1 (Clear Dx™) and the total hands-on-time, 140 min (manual) and 33 min (Clear Dx™) respectively. **Conclusions:** The fully automated Clear Dx™ platform would improve laboratory efficiency and provide results faster to optimize epidemiological investigating. Additionally, a lower skill threshold for laboratory staff to perform the Clear Dx system would allow for greater flexibility in lab operations. Additional studies are needed to define hardware tolerance and to meet the PulseNet criteria for reporting.

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Tracing Anthrax-like Capsule Biosynthesis Genes in Non-Bacillus anthracis Species through Whole Genome Sequencing

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Bacillus anthracis is the causative agent of anthrax, an acute disease in humans and non-human mammals, and is a Tier 1 select agent in the United States. Molecular tests for *B. anthracis* generally rely on detection of its toxin or protective capsule. PCR methods amplify DNA housed on its two virulence plasmids, pXO1 and pXO2, encoding the tripartite toxin (protective antigen, lethal factor and edema factor proteins in combination), and the antiphagocytic capsule, respectively. It has been reported previously

that non-*B. anthracis* organisms within the *Bacillus cereus* sensu lato group may partially adopt the virulence factors encoded by pXO1 and/or pXO2. However, it is unclear how this occurs in cases where neither plasmid is detected. To explore this phenomenon, we selected archived isolates previously identified through classic microbiological methods as *B. cereus* from the Special Bacteriology Reference Laboratory's historical collection at the US Centers for Disease Control and Prevention, which were originally sampled from infections that are atypical for *B. cereus*. As a preliminary screening, we performed the Laboratory Response Network *B. anthracis* real-time PCR protocol and whole genome sequencing on 10 isolates. No isolates indicated the presence of either *B. anthracis* virulence plasmid. However, two of the resulting genomes were found to contain an apparent homolog to the *capD* gene, a gene normally housed on pXO2 that supports escape from host immune defenses through capsule depolymerization in *B. anthracis*. Work will continue screening the reference archives for similar genetic phenomena and incorporate long read sequencing on identified isolates to clarify the genomic location of *capD*.

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Surveillance Whole Genome Sequencing in Denver County, Colorado

A. Smith, L. Bankers, A. Rossheim, N. Pysnack, E. Austin, A. Wheeler, S. Matziner; Colorado Department of Public Health and Environment

The spread and prevalence of continually evolving SARS-CoV-2 variants is primarily monitored through the testing of symptomatic people and subsequent sequencing of the viral sample. However, test results are not available in real time due to factors such as delayed onset of symptoms, access to testing, and test result reporting. As the pandemic has progressed, the number of clinical samples collected has reduced due in part to the increase in both at-home test use and vaccinations that have helped reduce transmission and symptom severity. Such changes in testing patterns can influence estimates of SARS-CoV-2 variant prevalence. On the other hand, people who are infected with SARS-CoV-2 can shed viral RNA in fecal material, which is detectable in wastewater, regardless of symptoms or testing method, if even performed. Wastewater data obtained from community sources can represent an aggregate sample of the variants currently circulating in the population, whereas submitters to our surveillance efforts are skewed towards samples from symptomatic patients at inpatient healthcare facilities. As such, variant proportions estimated from wastewater whole genome sequencing are expected to be less biased. However, whether this expectation holds, and whether variant proportions estimated from clinical surveillance are congruent with wastewater data, remain open questions. Since March 2021, CDPHE has partnered with wastewater facilities from across Colorado to collect bi-weekly wastewater samples, which are used for detection, quantification, and genomic characterization of SARS-CoV-2. In addition, clinical samples are submitted from across the state for whole genome sequencing as part of our sentinel surveillance program. Here, we analyzed wastewater data from June 2022 through October 2022 in Denver County. SARS-CoV-2 weekly lineage abundances were estimated from whole genome sequencing data using Freyja. SARS-CoV-2 viral concentrations (genome copies per liter) were quantified using ddPCR. We are using

multivariate time series analyses following the Johansen procedure to evaluate whether the time series of lineage frequencies estimated from wastewater and sentinel surveillance sequencing and viral load can form a cointegrating relationship, indicating a correlation between these variables. If so, this would suggest that we are obtaining accurate representations of variant trends and prevalence of the broader community. If not, this shows that variant proportions estimated from our sentinel surveillance system differ from those estimated from our wastewater surveillance data. This may inform whether sentinel surveillance strategies should be adjusted by increasing right-sizing parameters and/or including underrepresented populations. Additionally, this may inform us of ways that wastewater data may supplement our current sentinel surveillance program.

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Validation of Illumina's Viral Surveillance Panel at New Jersey Public Health and Environmental Laboratories

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Next-generation sequencing has become a cornerstone in public health as it allows us to trace origins of outbreaks, understand evolution of pathogens over time and potentially identify novel variants. In late 2022, Illumina released the Viral Surveillance Panel (VSP), which uses hybrid-capture enrichment technology to sequence 66 DNA and RNA viruses—including viruses identified as significant risk to public health. There are many viruses on the panel that are tested at New Jersey Public Health and Environmental Laboratories (PHEL) through other techniques, such as real-time quantitative PCR and whole genome sequencing. The use of the VSP at PHEL would strengthen the sequencing capabilities and viral surveillance program in New Jersey. In order to test the VSP, Influenza and SARS-CoV-2 samples with CT values ranging between 15 and 20 were selected from samples submitted to PHEL. The library preparation process includes the generation of cDNA, tagmentation, capture of hybridized probes and enrichment of the library. The final libraries were run on both the MiSeq and iSeq to determine which instrument would be better for this study. The sequencing results were then analyzed using Genome Detective, DRAGEN Microbial and Kraken. Two library preparations were completed, and it was determined that decreasing the final elution volume from 32 µL to 16 µL dramatically increased the concentration of the library. It was also determined that iSeq would be more appropriate instrument to use for sequencing compared to MiSeq. Overall, the sequencing analysis using Genome Detective, DRAGEN Microbial and Kraken were accurately able to assemble and identify the expected virus with coverage over 95% and confidence fraction over 0.94. In several samples, there were other viruses also found showing the ability of the VSP to detect all viruses present. After confirming the panel worked for the Influenza and SARS-CoV-2 samples, the panel will be tested further with samples of different Ct values to determine accuracy and precision. The panel will also be tested on other viruses in three virus types: Respiratory viruses, Bloodborne viruses and arboviruses to further show the capability of the panel for surveillance of infectious diseases of public health concern.

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What's in the Water? A Prototype Environmental Metagenomics Database and Pipeline for Pathogen Detection, Microbial Composition, and Antimicrobial Resistance Detection

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Environmental water metagenomics remains challenging for public health pathogen detection and characterization due to ecological diversity and lack of relevant, robust publicly available bioinformatics analyses. Currently, there are limited well-curated environmental water metagenomic databases and few bioinformatic workflows available which perform community level comparisons that allow for rapid public health response. Available environmental databases lack critical subtype or strain information relevant to public health. The objectives of this project are to create a well-curated, water-associated microorganism genomic database with a focus on public health-relevant organisms and to integrate the database into a bioinformatic workflow of open-source tools to optimize pathogen and antimicrobial resistance detection from shotgun metagenomic sequencing data of varying depths. Preliminary database curation is completed using genomic sequences of species (Nf8) included in the ZymoBIOMICS genomic community. These sequences are then processed through the ART data simulator to generate an in silico metagenomic read dataset, which is supplied to kmer-based approaches, Kraken 2 and Kalamari. These kmer-based approaches then match the in silico data with the database sequences. The predicted matches serve as an assessment of Kraken 2 and Kalamari. Our first version of the database will use the results from the two kmer-based approaches to build a comprehensive data which includes 11 genres of critical public health interest to the Waterborne Diseases Prevention Branch. Bioinformatic tool development for pathogen detection and characterization from Illumina MiSeq and NovaSeq paired-end data is done using a kmer-based approach. The kmer-based approach is then supplemented with a distance-based sequence similarity method to recover approximate matches to the environmental water database. Sequences that are not classified through these processes are then assembled into contigs. Predicted gene features are then identified from the contigs to provide a potential functional composition of the environmental sample, including comparison to antimicrobial resistance databases for resistome profiling. Tools produced from this work could inform future environmental pathogen detection, source tracking, and outbreak response. Moreover, by evaluating the communities that occur alongside pathogens in environmental waters, these analyses may lead to identification of unknown microbial risk indicators. Finally, a well curated water environmental database will be made available via the Kalamari nomenclature to support more environmental sequence-based research.

Disclaimer: The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the US Centers for Disease Control and Prevention.

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Whole Genome Sequencing Analysis of Multiple *Candida auris* Outbreaks in Southern Nevada

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A *Candida auris* outbreak has been ongoing in Southern Nevada since August 2021. Over 800 patients have tested positive for *C. auris* at 20 facilities with dozens of associated patient deaths. This abstract describes whole genome sequencing of over 800 *C. auris* isolates from this outbreak. Genetically distinct subgroups of *C. auris* were detected from Clade I (2 distinct lineages) and III (1 lineage). Open-source bioinformatic tools were developed and implemented to aide in the epidemiologist investigation. This investigation compares three methods for *C. auris* whole genome analysis: nullarbor, MycoSNP and a new pipeline TheiaEuk. We also describe a novel analysis method focused on elucidating connections between isolates within an ongoing outbreak. This study places the ongoing outbreaks in a global context based on available whole genome sequencing. Lastly, we describe how the generated results were communicated to epidemiologists and infection control to generate public health interventions.

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Whole Genome Sequencing and Transcriptional Analysis of *Pseudomonas aeruginosa* from Patients with Cystic Fibrosis

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Pseudomonas aeruginosa is an opportunistic pathogen and a major cause of morbidity and mortality in people with cystic fibrosis (CF). *P. aeruginosa* is one of the most common causes of nosocomial infections and was recently listed by the US Centers for Disease Control and Prevention as a pathogen of concern, as it can form chronic infections with resistance to many commonly used antibiotics. Therefore, it is important to understand the pathogenesis of *P. aeruginosa* in isolates from patients with CF. Quorum sensing (QS) is a mechanism for cell-cell communication in response to cell population densities that controls gene regulation. QS allows bacteria to communicate and coordinate functions such as biofilm formation and virulence factors. The two main QS pathways in *P. aeruginosa* are the LasI-LasR and RhII-RhIR systems. The signal synthases LasI and RhII produce acyl-homoserine lactones (AHL), also called autoinducers (AI). These AI bind to their respective transcription factors LasR and RhIR, triggering additional QS gene expression. In addition to these Las and RhIR systems, the enzyme PqsE directly interacts with RhIR to control virulence via enhanced binding of the RhIR-PqsE complex to DNA. To understand the pathogenesis of *P. aeruginosa* in CF patients, it is crucial we explore the variation in *P. aeruginosa* genomes, particularly genes associated with QS, and their expression. In this research we are interested in linking common polymorphisms in QS genes with altered transcriptional regulation in clinical isolates from patients with CF. We performed whole genome sequencing and transcriptional analysis of CF isolates to measure QS gene regulation under nutrient rich and nutrient limited conditions and correlated that with changes in the genomic sequences of key QS regulators. We will also insert a noninteracting PqsE variant into the CF isolate strains that is incapable of virulence phenotypes to

determine how the removal of PqsE in this system alters QS gene expression and if it renders these strains non-pathogenic. We hypothesize that the PqsE-RhIR interaction is crucial for QS and disease progression. Our results have identified CF isolates with elevated levels of RhIR and PqsE expression, which correlates to an increase in QS gene expression. By combining transcriptional and genomic data in *P. aeruginosa* isolates from CF patients we can better understand the development of acute and chronic infections and potentially identify targets for future therapies and improve the health outcomes for those with CF.

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Whole Genome Sequencing of Influenza A and B: Assessment of Different cDNA Preparation Strategies

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Background: High throughput whole genome sequencing (WGS) for influenza A/B during peak influenza season allows for rapid response to emerging variants to assist in the public health response for ongoing surveillance. The current national influenza A/B WGS surveillance system effort employs an amplicon-based approach. Here, we investigated the usefulness of specific adaptations to the nationally approved protocol for increased flexibility for surge cases, such as increased sample volume and supply chain issues, as well as more cost-effective options for cDNA preparations. We tested three areas where cDNA preparation could be optimized. (1) We compared the use of two reverse transcriptases—LunaScript (LS) and SuperScript III. LS has been shown to have higher fidelity than SSIII, but it is more expensive. (2) We tested pooling A and B primer sets together, rather than using each primer set separately, to see if we could avoid the need to determine each sample's subtype by PCR before WGS. (3) We assessed whether using a lower primer concentration resulted in similar WGS results to higher primer concentrations, which would reduce reagent costs. We assessed the effect of these three optimization tests by evaluating the average sequencing depth and percent genome coverage. **Methods and Results:** We sequenced 10 clinical A samples with controls in duplicates using LS vs SSIII. The results showed >97% coverage on all eight gene segments when using SSIII compared to inconsistent coverage with LS. SSIII also showed higher sequencing depth. To determine whether pooled primer sets are effective in providing the same percent coverage and depth as unpooled primer sets, three different cDNA preparations were tested: (1) Only A primers, (2) Only B primers, and (3) Pooling A/B primers together. Unpooled primer sets showed higher sequencing depth compared to pooled primer sets. However, the reduced sequencing depth from the pooled primer sets did not affect overall sequence quality and was well within national sequence quality metrics guidelines. To assess the effect of primer set dilution, A/B primers were diluted twice over dilutions recommended in the current protocol utilized for national surveillance efforts. Using a mixture of influenza A/B clinical samples, the sequencing results of the lower limit of dilution still produced >97% coverage on all eight segments. Influenza A samples sequenced better, in terms of coverage and depth, than Influenza B samples. **Conclusion:** Our results demonstrate that to save time and resources, subtyping can be skipped and samples can be quickly sequenced using pooled primers. To be more

cost effective, without diminishing quality, SSIII performs better compared to another commonly utilized enzyme, LunaScript. Finally, appropriately diluted primers may provide a cost-saving alternative that should be explored further.

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Whole Genome Sequencing of Mixed Pathogen Runs to Reduce Turnaround Time and Costs per Sample

D. Mallal, V. Nguyen, A. Alford, A. Lo, M. Heatherington-Rauth, S. Matzinger, A. Rossheim; Colorado Department of Public Health and Environment

The demand for whole genome sequencing (WGS) as an advanced molecular detection method for outbreak detection and pathogen characterization has increased, especially in the post-COVID era. With this increase in demand, there is a need to be able to quickly and robustly onboard new pathogens while also being cognizant of cost and turnaround time. The Colorado Department of Public Health and Environment (CDPHE) Laboratory rapidly expanded sequencing capacity, adding multiple pathogens of public health concern to sequencing repertoire. In order to do this effectively without impacting turnaround time of routine sequencing efforts, including COVID surveillance and PulseNet, we implemented sequencing multiple pathogens on a single Illumina MiSeq run, a method supported by the PulseNet Network for Norovirus WGS. By expanding mixed pathogen sequencing runs to include additional viral organisms, we significantly decreased the cost of sequencing and turnaround time per sample. The maximum loading capacity of a typical PulseNet run on the Illumina MiSeq platform that includes *Shigella*, *E. coli*, or *Vibrio* is 100MB (~20 *Salmonella*, *E. coli*, or *Shigella* isolates) for a v2 500 cycle kit, or 175MB (~35 *Salmonella*, *E. coli*, or *Shigella* isolates) for a v3 600 cycle kit. Although PulseNet confirmed mixed pathogen runs are successful when adding in amplicon sequencing of Norovirus, we found that other viral organisms, such as influenza, can also be appended to a sequencing run without affecting the quality of sequence coverage. Our strategy for sequencing viral genomes, including influenza, mpox, norovirus and RSV (Respiratory syncytial virus) on mixed pathogen sequencing runs is to first generate amplicon-based cDNA followed by the Illumina DNA library preparation method. Next, these amplicons are spiked into a full PulseNet run, varying the volume spiked in based on genome size and depth of coverage requirements of the virus. We successfully performed WGS on 48 influenza (genome size 13,500 bps) samples in addition to a full PulseNet run on both v2 and v3 chemistries without sacrificing quality or depth of coverage of either sample type (For example, influenza maintains an average of >100x coverage depth, and bacterial isolates maintain a >40x average De Novo coverage). This procedure reduces the loading cost per sample from ~\$67 (20 bacterial samples on a v2 kit) to ~\$20 per sample (20 bacterial samples plus 48 influenza samples on a v2 kit). For mpox (genome size 190,000 bps), which has a much larger genome than influenza, we included 16 samples on routine PulseNet runs, and observed excellent coverage (average >400x coverage) of the viral amplicons without any decrease in quality metrics for the bacterial samples on the run. We successfully demonstrated how mixed pathogen sequencing runs can reduce the cost per sample and overall turnaround time while maintaining high quality.

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Whole Genome Sequencing of Mpox: Assessment of Different Library Preparation Protocols

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Onboarding a streamlined, scalable whole-genome sequencing (WGS) workflow for viral pathogens is important when piecing together transmission networks and tracking genetic mutations that might impact pathogenicity. Efficient and consistent WGS surveillance is dependent on the ability to onboard an adaptable and robust workflow that capitalizes on the strengths of the different available sequencing platforms. However, when onboarding new protocols, it is important to compare the efficacy of the available options. That can be achieved by testing whether or not there is a measurable difference in genome percent coverage, mean depth of coverage, total number of reads (raw and filtered), total number of reads mapped to the reference genome and lineage designation. In response to the 2022 mpox outbreak, multiple WGS and next-generation sequencing (NGS) options have been developed. To assess the usefulness of a subset of these newly available approaches for public health response on Colorado mpox clinical specimens, we participated in an early access trial of a tiled amplicon approach from Integrated DNA Technologies (IDT) and assessed the feasibility of using different library preparation protocols for use on the Miseq Illumina platform. Specifically, we compared an mpox amplicon panel from IDT that can be used in combination with either the IDT xGen library preparation or the Illumina DNA library preparation. In order to compare the two different workflows for clinical samples, we ran each workflow on seven clinical samples with Ct values 400, increased total number of raw reads by >1,000,000, and filtered reads by >400,000, with comparable percent reads mapped to the reference genome of >99%, and equivalent NextClade lineage designation. Future plans include assessing hybrid capture as an additional/alternative approach for sequencing mpox clinical samples and wastewater detection. Overall, this comparison highlights diversity in library preparation strategies for WGS and NGS workflows.

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ONE HEALTH APPROACHES

Survey of Abscess-Forming Pathogens Found in Cervid Retropharyngeal Lymph Nodes

E. Palise, Montana Veterinary Diagnostic Laboratory

Caseous Lymphadenitis (CL) is an economically important chronic infection of sheep and goats. The disease occurs when the bacterium *Corynebacterium pseudotuberculosis* (C. pseudoTB) enters the body through breaks in the skin or contact with mucous membranes. The bacteria then travel to the lymph nodes via the lymphatic system and form encapsulated abscesses over an incubation period of one to three months. Although less commonly described in scientific literature, CL can also be found in wild ruminants including deer and other cervids. In previous years, the Montana Veterinary Diagnostic Lab (MVDL) observed a large number of abscessed cervid lymph nodes among hunter-harvested samples

that were submitted for chronic wasting disease (CWD) testing. During the 2021 CWD testing season, most of the abscesses observed in these lymph nodes were found to be caused by C. pseudoTB, and additional work was completed to phylogenetically characterize the C. pseudoTB isolates. The present project aims to continue and expand upon this preliminary work using hunter-harvested cervid lymph node samples submitted to MVDL during the 2022 CWD testing season. The first goal of this project is to catalog and characterize which bacteria, in addition to C. pseudoTB, may be contributing to the observed lymph node abscesses in wild cervid specimens. This information will aid in understanding what types of abscess-forming bacteria may be circulating through wild cervid populations. Since recreational hunting of wild cervids is widely enjoyed amongst the people of Montana, the second goal of this project is to assess the potential threat of zoonotic transmission of bacterial pathogens to hunters and other personnel that may be handling biological material from these animals, given that over 60% of emerging infectious diseases are zoonotic. Ultimately, the work completed during this project serves a One Health purpose, by gathering information that can be used to inform decisions and recommendations for managing human and animal health.

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PANDEMIC PREPAREDNESS, DISEASE FORECASTING AND EMERGENCY RESPONSE

A Nationwide Survey of Clinical Laboratories' Capabilities, Capacity, and Willingness to Support Emergency Response Surge Testing

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Clinical laboratories are often the first line of defense in a public health response as they conduct testing at the request of physicians to diagnose, monitor or treat disease. As part of these functions, many clinical laboratories are responsible for referring suspicious or unusual specimens to public health reference laboratories for further characterization and analysis. More recently, select clinical laboratories with high-throughput testing capacity ensured timely diagnostic testing to inform medical care follow-up for individuals in a large-scale outbreak. As such, clinical laboratories serve as a critical gateway between every day medical care of individuals and public health. Although CDC has considerable information on the capabilities of public health laboratories, the same level of information is not available for the clinical laboratory sector, which includes small, medium and large organizations in hospital, independent and other private sector healthcare settings. To collect this information, the CDC conducted a three-year, multi-faceted study to assess the capabilities, factors that influence capacity, and willingness of the nation's clinical laboratories to support large-scale response to chemical, biological, radiological and/or emerging threats. The outcome of this study resulted in a significant yield of information from 1,295 laboratories spread out across the 10 HHS regions that will inform CDC and its state and local public health stakeholders how to better leverage and integrate clinical

laboratories into large-scale public health emergency responses, as well as identifying critical gaps or vulnerabilities that need to be considered for overall public health emergency response and preparedness.

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Comparative Data of Clinical Specimens from Southern Nevada Public Health Laboratory on SARS-CoV-2 Variant Detection in Clark County, Southern Nevada with Wastewater Surveillance

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Shortly after the first reporting of the Omicron variant in the United States, the variant began to dominate Clark County, Southern Nevada by mid-December 2021. Due to the increased transmissibility of the Omicron variant, the Southern Nevada Public Health Laboratory (SNPHL) of the Southern Nevada Health District significantly expanded the implementation of Next Generation Sequencing (NGS) for SARS-CoV-2 RNA extracts from clinical specimens. During this time, our team increased its capacity to identify the variants of interest and variants of concern from 48 specimens/week to 192 specimens/week for 2022. By February 2022, the government had increased access of free at-home test kits to the community. This impacted the public health sector in identifying variants of interest and concern circulating in Southern Nevada due to the reduction of PCR testing. To consolidate the investigation of the Omicron lineages circulating in the Southern Nevada community, SNPHL collaborated with the University of Nevada, Las Vegas (UNLV) on wastewater surveillance for SARS-CoV-2. When comparing the data with the wastewater surveillance results, all the Omicron variants that were detected from the wastewater surveillance were detected in the clinical specimens at the SNPHL. We compared the timeframe between the clinical specimen with the wastewater specimen for each of the omicron variants. We observed that the timeframe between the clinical specimens and wastewater was comparative and close. One of the major observations when comparing the data was the BF.7 variant. The UNLV team was able to detect the presence of BF.7 variant in May but the clinical specimen was not detected until August. This was due to the relatively low number of specimens received by the health district and its community partners, leading to a delay in the detection of the variants. The other observation was the re-introduction of BA.2 variant where the wastewater specimen detected BA.2 again in October and the clinical specimen in December. The trend observed from the wastewater was similar to our clinical specimens with BA.1 dominating by February 2022 and BA.2 begun circulating and displacing BA.1 as the dominant strain. We also observed that two of the variants BA.2.75 and BQ.1 were first observed from the wastewater and two weeks later, SNPHL observed the same variants from clinical specimens. In summary, six of the variants detected by SNPHL were detected roughly the same time as the wastewater specimens, three of the variants were detected early from the wastewater with two variants detected two weeks after the first discovery through wastewater surveillance. The wastewater surveillance data from Southern Nevada has proven to be a beneficial source of data. Together, wastewater surveillance

can complement public health surveillance to alert the community of new SARS-CoV-2 variants in Clark County, despite the low PCR testing by the community.

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Including Peptide Enrichment in a Mass Spectrometry-based Workflow for the Absolute Quantitation of SARS-CoV-2

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Introduction: SARS-CoV-2 particles contain proteins that are biomarkers of a COVID-19 infection that may be detected by bottom-up mass spectrometry (MS). Proteolytic digestion of proteins generates peptides, which can be separated by liquid chromatography (LC). A workflow had previously been developed that achieved low on-column detection limits for spiked nasal fluid samples in viral transport media (VTM). The addition of a peptide enrichment step, such as stable isotope standards and capture by anti-peptide antibodies (SISCAPA), could increase sample purity. This may reduce background, thereby improving detection limits and reducing LC run-times. Furthermore, SISCAPA could remove any need for sample clean-up and allow the concentration of samples, thereby increasing detection limits. **Methods:** A mass spectrometry-based SARS-CoV-2 absolute peptide quantification method was developed using a Thermo Scientific™ Vanquish™ MD LC system and a Thermo Scientific TSQ Altis™ MD MS. Recombinant SARS-CoV-2 proteins and stable isotope-labeled standards (SISs) were spiked into pooled nasal fluids, before being added to VTM. Samples were then precipitated, centrifuged, and enzymatically digested (Thermo Scientific SMART Digest™ Trypsin kit). The resulting peptides were enriched with peptide-specific SISCAPA antibodies and separated using a 2-minute LC gradient with a Hypersil GOLD™ C18 column (1.9 µm, 2.1 x 50 mm), coupled with a single reaction monitoring method. All assays were performed in triplicate and data was analyzed using Thermo Scientific TraceFinder™ LDT software. **Results:** Full workflow testing with four peptide-specific antibodies confirmed the antibodies specificity and ability to work in conjunction. Coupling a mass spectrometry-based method with peptide enrichment allowed robust data acquisition with a two minute LC run time. Absolute quantitation of targeted peptides was then performed by including the corresponding SIS for each peptide to mitigate measurement uncertainty, confirm the retention times, and correct for any possible matrix effects. LODs and LOQs were determined for each peptide, with all % RSD/CV 0.99. Clear chromatographic separation was observed for each nucleocapsid peptide with minimal variance in retention times observed (± 0.01 minutes). LODs and LOQs were determined to be between 0.25 and 2.5 femtomole on column for the three best performing peptides. **Conclusion:** SISCAPA allowed 4-fold concentration of samples and eliminated the need for any further sample clean-up. This greatly increased the detection limits when the amount of nucleocapsid protein on each nasopharyngeal swab is considered, meaning that the protein could be detected at lower concentrations or on swabs with less spiked nasal fluid present. The increased sample purity also facilitated the reduction of LC-MS run-times from four to two minutes.

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Molecular Detection of Respiratory Pathogens from Congregate Spaces Using Aerosol Samplers for Public Health Actions

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The COVID-19 pandemic has increased concerns regarding transmission of infectious respiratory pathogens, particularly in congregate spaces such as schools, transportation hubs, restaurants and shelters where these pathogens can spread rapidly. Monitoring for respiratory pathogens in the community can inform public health officials on how to best protect the public from disease outbreaks. Aerosol samplers actively filter air, collect respiratory pathogens that would otherwise be released in the space, allow for the detection of respiratory pathogens without the presence of symptoms or need for clinical sampling, and can be deployed for as little as two hours or up to several days. The City of Milwaukee Health Department Laboratory (MHDL), in conjunction with the MHD Emergency Preparedness Team, deployed four Thermo AerosolSense Samplers in a warming house in Milwaukee County, Wisconsin to monitor for the presence of SARS-CoV-2, influenza A/B and respiratory syncytial viruses (RSV). To detect pathogen presence, MHDL validated a commercially-available TaqMan real-time PCR assay that employs probe-based detection of SARS-CoV-2 (S and N genes), influenza A/B, and RSV. Commercially available kits such as these require individual laboratory validation, and here we describe a method to efficiently validate an assay optimized for a local public health laboratory. Using this method, we evaluated the limit of detection of the three targets, defined cut-off criteria for positive results, assured assay precision and assessed the effects of matrix inhibition. We compared the results of this assay with three other molecular assays used by MHDL to detect the presence of respiratory pathogens in clinical samples and during previous air sampling seasons. Air surveillance is an effective and low-cost strategy for pathogen surveillance in lieu of testing individuals, and this method can increase the speed at which outbreak mitigation can be initiated.

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Overcoming Variant Mutation-related Impacts on Viral Sequencing and Detection Methodologies

Y. Bei, K. Pinet, K. Vrtis, J. Borgaro, E. Alpaslan, M. Campbell, L. Sun, L. Apone, G. Patton, B. Langhorst, N. Nichols; New England Biolabs

In the last three years, we have experienced how disruptive a pandemic has been in every aspect of our lives. Prompt and accurate pathogen identification, by diagnostics and sequencing, has proven to be an effective tool for tracking and potentially curbing pathogen spread. Targeted detection and amplification of viral genomes depends on annealing oligonucleotides complementary to genomic DNA or cDNA. However, genomic mutations that occur during viral evolution may perturb annealing, which can result in incomplete sequence coverage of the genome and/or false negative diagnostic test results. Using SARS-CoV-2 as an example, we demonstrate how to assess, test, and optimize sequencing and detection methodologies to attenuate the negative impact of mutations on genome targeting efficiency. We developed a monitoring tool (primer-monitor.neb.com) to identify variant mutations that align with registered primer/probe sets. The effects of the variant mutations on sequencing and diagnostics

were evaluated using in vitro-transcribed (IVT) RNA as well as RNA extracted from clinical SARS-CoV-2 variant samples, including the heavily mutated Omicron variant. These results demonstrate how to maintain reliable targeted pathogen sequencing and how to evaluate detection methodologies as new variants emerge.

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Pacific Rim Consortium Quality Project

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The Enhanced Laboratory Emergency Network for Operations and Response (ELENOR) is a crisis communications tool that provides situational awareness on a common operating platform. ELENOR is a secure, web-based software designed to be used not only during an emergency, but also to enhance day-to-day communications, streamline collaboration, and improve overall situational awareness throughout the state of California. As ELENOR grows, it will interconnect the 28 county Public Health Laboratories with the California Department of Public Health (CDPH), California Office of Emergency Services, and other emergency preparedness organizations and services. The system is managed by a combination of user accounts, positions, and group assignments that allow each specific permissions. Its development has been driven by the users and through community collaboration. CDPH intends to build more daily processes, such as grant management and invoicing within the platform. ELENOR users can provide updates to their laboratory's staffing levels, equipment, testing capacity and capabilities, contact information, and more. Laboratories can utilize the continuity of operations planning (COOP) checklists for both emergency preparation and COOP activation and can notify other laboratories of significant events such as weather-related incidents or natural disasters. Specific positions can request resources, extra staff, and overflow testing from other laboratories. The ELENOR platform was adapted for the APHL Pacific Rim Consortium member laboratories for a quality improvement project. The intent of adapting ELENOR to the Pacific Rim Consortium laboratories is to create efficiency and improve information sharing. Efficiency will be tested through a tabletop exercise and roundtable, followed by an after-action review. The target date for this is March 2023. Evaluation of the data and efficiencies will be completed by May 2023.

Presenters: Katie Bolin (katiebolin@internationalrespondersystems.com); Holli Dowless Kozar, (holli.dowlesskozar@cdph.gov)

Preparing for the Next Surge: Building Resilient Inventory Management at New Jersey Public Health and Environmental Laboratories

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Effective inventory management practices include leveraging inventory management technologies, conducting regular inventory checks, setting periodic automatic replacement (PAR) levels, and

inventory forecasting. During the COVID-19 pandemic, The New Jersey Public Health and Environmental Laboratories (PHEL) joined the NJ Department of Health's existing emergency preparedness inventory management system, namely iCAM, in response to supply chain uncertainty and to build an integrated, robust inventory management system at the lab. We evaluated how iCAM is used and its impact on improving inventory management at PHEL and implemented inventory forecasting through advanced data analytics to increase our preparedness for future surge events. The iCAM system helps to enhance inventory visibility by informing the inventory quantity, location, expiration date as well as other product details. We also implemented point-of-use technology such as handheld devices to ensure a more accurate count of what is on hand and minimize human errors. So far, 30% of the lab has been onboarded to the system to manage their inventories; 18,175,770 individual items worth more than \$100 million have been processed through the system. Furthermore, we used data that we generated in the system to set PAR levels, identify and order commonly used items, and forecast inventory demands for select items through data analytics. Cycle counting was also incorporated into inventory management to confirm that physical inventory counts match inventory records in iCAM. In conclusion, iCAM helped to increase the visibility of inventories and improve inventory management at PHEL. With the integration of predictive analytics, we can make data-driven interventions earlier and avoid supply chain interruptions.

Presenter: Satyam Patel, satyam.patel@doh.nj.gov

The Laboratory Response Network in Action: 2022 Preparedness and Response Activities

R. Rahmat, J. Sutton, Association of Public Health Laboratories

The Laboratory Response Network (LRN) was established in 1999 as a collaborative effort between the US Centers for Disease Control and Prevention (CDC), Federal Bureau of Investigation (FBI), the Department of Defense (DoD) and the Association of Public Health Laboratories (APHL) and functions as a robust network of state and local public health, federal, military, and international laboratories that prepare for and respond to emerging public health threats. The LRN for Biological Threats Preparedness (LRN-B) supports our nation's public health laboratory system for biological threats and emerging infectious diseases (e.g., Ebola). In 2022, the LRN-B was leveraged to coordinate response to the international Mpox virus outbreak and simultaneously brought on testing capacity to prepare for Sudan Ebolavirus, an outbreak in Uganda that emerged in September. In addition, the LRN continued to implement operational improvements. The new LRN Secure Information Hub launched, a more user-friendly website for the LRN, and member laboratories focused on enhancing technology and data exchange capabilities by participating in multicenter evaluation studies, exploring whole genome sequencing for biological threat agents and implementing Electronic Laboratory Reporting (ELR) for more efficient and rapid exchange of high-quality data. Member LRN-B laboratories continue to build on their capabilities and capacity while working in tandem with CDC and other partners to prepare for and respond to biological threats and emerging infectious diseases. This poster will highlight the LRN in action and describe how LRN-B laboratories navigated

responses to simultaneous disease outbreaks in 2022 while also implementing strategies to prepare for future emerging threats.

Presenter: Rana Rahmat, rana.rahmat@aphl.org

The National Special Pathogen System of Care: How Partnerships with a Care Delivery Network Can Improve Care Coordination and Outcomes in High Consequence Infectious Disease Events

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Background: Recent challenges during high consequence infectious disease (HCID) outbreaks have created an urgency to transform healthcare preparedness and response. At the height of the COVID-19 pandemic, the Administration for Strategic Preparedness and Response (ASPR) called on the National Emerging Special Pathogens Training and Education Center (NETEC) to leverage their expertise to address these challenges. NETEC has since engaged over 100 experts across the healthcare continuum, including clinicians, government leaders, and professional associations, to design and begin to implement a National Special Pathogen System of Care (NSPS). This work culminated in a strategy that calls for the establishment of a Care Delivery Network (CDN) within the NSPS to improve access to laboratory testing and other essential resources. **Methods:** The NSPS is designed to integrate with existing infrastructure (e.g., regional health systems, laboratory networks, and forecasting centers) to strengthen national health care preparedness and response for HCID events. To deliver high-quality, patient and community-centered care to HCID patients in the US, the NSPS will leverage a coordinated and standardized healthcare network. The NSPS will transform the nation's HCID healthcare infrastructure to consistently and equitably provide care while protecting the workforce. **Results:** Following discussions among the various healthcare experts, a major gap identified during the COVID-19 pandemic was healthcare coordination with laboratories and laboratory networks. To address this issue, a CDN composed of four tiers with varying levels of capacity and capabilities to care for HCID patients was proposed. Suggestions were that this network be organized by regions, where each region will have a combination of facilities from each tier. Each facility in the CDN will thus be equipped for various HCID scenarios and know how best to collaborate with facilities in their region to ensure quality care is delivered. Strategic location of facilities in the CDN closer to public health laboratories for diagnostic testing will ensure that the network is equipped to identify HICIDs and deliver care informed by timely laboratory testing. **Conclusion:** Collaboration between existing networks and the NSPS will be vital to improving the nation's healthcare preparedness and response capability in future outbreaks. This poster will detail the NSPS operating model, the gaps the NSPS should address, how the NSPS is prioritizing equity and collaboration strategies with existing networks to form a CDN for HCID preparedness and response.

Presenter: Peter Iwen, piwen@unmc.edu

PARTNERSHIPS, ACADEMIC, RESEARCH AND INDUSTRY COLLABORATIONS, TRAINING AND OUTREACH

Five Lessons Learned in Developing Training Videos for High Containment Facilities

D. Boyles, J. Bickell, S. Shearrer, S. Altmann, L. Gardiner; MRIGlobal

Objectives: MRIGlobal's biosafety team traveled to a client's agricultural research facility to begin filming a series of scripted biosafety training videos. The goal of the trip was to assess and optimize the functionality of the current filming process. **Methods:** Prior to the visit, MRIGlobal wrote video scripts on topics including select agents, laboratory waste treatment, laboratory-acquired infections, and biosafety cabinets. Scripts were delivered to the client for feedback and approval. After approval, documents outlining specific spaces and props needed for each scene of each video were developed and submitted to the client in preparation for the filming trip. MRIGlobal then worked with a film crew to review scripts and create a filming schedule before departing for a 10-day filming trip. **Discussion:** During this visit, the team identified five areas unique to filming that required distinctive preparation compared to traditional training materials. We refer to these as the "five lessons learned." These lessons spanned the course of the development process from script writing to acting and post-production editing and can be summarized as: (1) adopt an appropriate writing style; (2) plan scenes as you write; (3) plan your resources; (4) provide oversight for actors; and (5) know your set ahead of time. These lessons are applicable to filming training videos in all settings but specifically help address some of the challenges of filming within biocontainment spaces. **Conclusions:** As a result of these lessons learned, a series of templates were developed that address these topics in advance to ensure that, once on site, filming can proceed without interruption. These tools created from the lessons learned can benefit anyone looking to develop training videos.

Presenter: Devin Boyles, dboyles158@gmail.com

Evaluation of Antiviral Properties of Amniotic Fluid-derived Extracellular Vesicles in Experimental Models of Influenza Virus Infection

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Influenza is one of the most frequently occurring viruses, with different strains circulating each year. Due to its prevalence and rapid mutation rate, it is imperative to research and develop novel antivirals that constrain influenza pathogenesis. Zofin™ is an amniotic fluid-derived therapeutic composed of naturally produced extracellular vesicles. Extracellular vesicles include both exosomes and microvesicles, which contain cargo, such as miRNA and proteins, that are used by host cells for intercellular communication and to regulate inflammation. This study explores the antiviral effects of Zofin™ using in-vitro and in-vivo models of influenza A virus (PR8; H1N1 subtype) infection. For *in vitro* experiments, human lung epithelial cells (A549) were infected at two different

multiplicities of infection (MOI of 1 [low] or a MOI of 10 [high]) and treated with either 5% or 25% of Zofin™. Cell culture supernatants were collected at 24 and 48 hours post-infection (hpi). For the in-vivo study, groups of Balb/c mice were infected, treated intranasally with Zofin™, and lung tissue homogenates were collected at four and seven days post-infection (dpi). Virus titers were determined by plaque assays for PR8-infected, Zofin™-treated cells/animals and compared to PBS-treated vehicle controls. Interestingly, we demonstrate that Zofin™-treated PR8-infected cell cultures have a significant reduction in virus titer at 24 hpi at both MOIs. At 48 hpi, a significant reduction was seen for the 25% Zofin treated infected cell cultures at both MOIs. Conversely, the antiviral effects seen in-vitro were not observed in-vivo at either 4 or 7 dpi. Ongoing studies are aimed at defining the mechanisms by which Zofin™ exerts antiviral effects in cell culture models and further research is needed to delineate any possible species-specific (human vs mouse) divergences in antiviral potential of Zofin™ observed in our experimental models.

Presenter: Camy Guenther, tzo9@cdc.gov

Experience Working on a Pilot Project with Public Health Laboratories and the CDC from a Fellow's Perspective

A. Lo, D. Mallal, A. Rossheim, S. Matzinger; Colorado Department of Public Health and Environment

Current national PulseNet surveillance protocols require culture and isolation to perform whole genome sequencing (WGS). Many clinical laboratories have transitioned to culture-independent diagnostic tests to identify enteric pathogens, increasing the burden on public health laboratories to perform culture and isolation in addition to WGS. To address this, US Centers for Disease Control and Prevention (CDC) proposed an alternative method to characterize enteric pathogens directly from stool samples via a highly multiplexed amplicon sequencing (HMAS) protocol. One food safety fellow was assigned to the Colorado Department of Public Health and Environment (CDPHE) to work on this pilot protocol during the 2022–2023 APHL Fellowship cycle. Overall, due to decreased cost, high throughput and likely ability to hit *Salmonella* HMAS targets (i.e., serotyping, antimicrobial resistance, virulence, core genome multilocus sequence typing), we expect stool HMAS to be a useful option to decrease manual efforts at the state public health laboratory for characterization of *Salmonella* specimens for national and local cluster detection and foodborne public health response. Currently, CDPHE is one of two state public health lab pilot sites tasked with performing side-by-side comparison of the JUNO HMAS protocol with the current isolate-based PulseNet WGS protocol on *Salmonella* isolates and the original stool from which the isolates were cultured. This will be accomplished by gathering three pieces of data for paired isolate and stool samples: Isolate-based PulseNet WGS, isolate-based HMAS and stool-based HMAS sequencing results. Under the supervision of mentors and other technicians, the fellow learned current PulseNet WGS protocols, summing in obtaining the PulseNet WGS lab and analysis certifications. Additionally, the fellow assisted in the development and implementation of the JUNO HMAS protocol on *Salmonella* isolates and stool samples. This included extraction from stool and bacterial isolates, WGS and HMAS sequencing library preparation, troubleshooting of proposed protocols and communication with CDC's culture-independent metagenomics subtyping group (CIMS) enterics group, other participating public health laboratories and

epidemiological partners. By participating in this pilot project, the fellow acquired real-time experience analyzing and responding to public health concerns, leading to an immediate impact on outbreak investigations and potentially informing future disease transmission modeling.

Presenter: Alexandria Lo, ali.lo@state.co.us

Integration of Oklahoma Public Health Laboratory Testing into the Nebraska Public Health Laboratory

A. Bartling, A. Paulino, A. Roden, S. McGill, E. McCutchen, P. Iwen; Nebraska Public Health Laboratory

Background: The challenge of moving the Oklahoma Public Health Laboratory (OKPHL) from Oklahoma City to Stillwater resulted in a decrease of qualified staff members to support biological testing that necessitated the need for interim outside support to meet the requirements of federal programs. This report describes the approaches used to integrate testing into the Nebraska Public Health Laboratory (NPHL) to meet the immediate needs for the OKPHL. **Methods:** A Memorandum-of-Understanding was generated with administrative personnel at OKPHL that described the processes for testing and billing for the PulseNet and LRN-B programs. To initiate testing at NPHL, new logs, accessioning files, and numbering schemes were created and implemented. Personnel at the Oklahoma laboratory were given access to NUIrt, the internal ordering and reporting software used at NPHL to place orders and view results. Additionally, an inventory was created to determine the need for supplies and laboratory space to meet the increased number of tests as well as rearranging testing to meet workload volumes for the staff. Finally, an after-hours pager was set up to provide support for biothreat and mpox testing. **Results:** There were 842 foodborne pathogen specimens collected between April 26, 2022 and December 31, 2022 submitted for culture. Of those, 690 isolates were available to NPHL for banking and whole genome sequencing. A total of 604 unique isolates were sequenced by Illumina DNA Prep on the MiSeq following PulseNet protocols. Additionally, 15 potential biothreat isolates along with 121 mpox specimens were submitted from the OKPHL for confirmation and diagnostic testing, respectively. During this time, NPHL also received 345 foodborne isolates for banking and sequencing, 22 potential biothreat isolates and 526 specimens to test for mpox from Nebraska hospitals and clinics. Moreover, the NPHL also verified a Sudan ebolavirus test at this time to provide regional testing for this pathogen to prepare for the potential of cases occurring in the US from the outbreak in Uganda. **Conclusion:** With a doubling for testing for PulseNet organisms, a 20% increase in testing of biothreat agents, testing to meet the demands of the mpox epidemic, and the potential need to screen for the Sudan ebolavirus, the NPHL was able to successfully integrate the additional OKPHL workload into the current testing demands in the laboratory and still fulfill the needs for the testing of Nebraska samples. This study showed that the availability of mechanisms in place to meet the ordering and resulting processes along with a clearly defined MOU for testing and billing allowed the NPHL to meet the challenge of this additional testing. Finally, current staffing arranged work schedules to meet this increased testing demand to support a partner laboratory during a time of need. Future studies are important to

define internal processes on how public health laboratories can support partner laboratories if the need arises.

Presenter: Arlene Paulino, arlene.paulino@unmc.edu

Navigating Cross-sector Collaborations for Advanced Molecular Detection during the COVID-19 Pandemic: Applications, Challenges, Opportunities

M. Dorvil-Joanem, J. Narayana, J. Yackley; Tennessee Department of Health

Genomic sequencing has been crucial to the COVID-19 public health response. The Tennessee Department of Health (TDOH) established a dedicated COVID-19 Genomic Surveillance Team in 2022 to review ongoing sequencing efforts, develop robust collaborative infrastructures for SARS-CoV-2 sequencing, and improve the detection of circulating SARS-CoV-2 viruses and emerging variants in Tennessee. TDOH utilized targeted outreach and dedicated funding to establish a network of internal and external sequencing partners. Partnerships were built through brainstorming, coordination, and recurring check-in meetings. Frequent discussions with partners ensured that appropriate logistical details for SARS-CoV-2 sample acquisition (e.g., storage, handling, transportation) were in place. TDOH leveraged the expertise of bioinformaticians, epidemiologists, and public health lab scientists to rapidly develop high quality systems for capturing and sharing sequencing data. Initial challenges included sample acquisition and timely routing of specimens to sequencing facilities. Supply chain disruptions led to delays in securing necessary infrastructure for proper specimen collection and storage specific to advanced molecular detection (e.g., freezer, media). TDOH established a passive genomic surveillance system through which all sequencing facilities processing Tennessee resident samples report identified variants of concern, variants of interest or variants being monitored, as defined by CDC. Additionally, TDOH actively works with sequencing partners, including one large healthcare system, one research laboratory and the State Public Health Laboratory to sequester samples for sequencing. TDOH has received sequencing data for 65,008 specimens, approximately 3.6 % of all confirmed SARS-CoV-2 cases (1,785,513) in Tennessee through December 2022. Sequencing is also in progress for 15,000 samples from 2020 by a research laboratory to aid in historical understanding of variants. Next steps will aim to improve the representativeness of samples across Tennessee's 95 counties. The COVID-19 Genomic Surveillance Team fostered multi-sector sequencing partnerships. These partnerships led to the creation and strengthening of sequencing infrastructures, the development of informatics processes for secured data exchange both pre and post sequencing analysis, and the optimization of surveillance systems that can be leveraged for future applications (e.g., wastewater, Mpox, influenza). TDOH will work to strengthen and grow collaborations with regional, county, academic, and commercial partners to improve the depth and breadth of genomic surveillance in Tennessee.

Presenter: Magdalena Dorvil-Joanem, magdalena.dorvil-joanem@tn.gov

The Functional Role of Naturally Evolved Gene SNPs in Clinical Strains of *Pseudomonas aeruginosa*

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Pseudomonas aeruginosa is an opportunistic pathogen of significant public health concern because it is highly antibiotic resistant, ubiquitous and persistent in healthcare environments, leading to tens of thousands of hospital-acquired infections each year. Healthcare settings drive evolution of clinical *P. aeruginosa* strains due to the use of antibiotics and antimicrobials. Clinical strains that cause chronic infections often evolve deleterious inactivating mutations in *lasR*, which encodes a master transcriptional regulator of virulence genes. *LasR* is part of a hierarchical network of quorum sensing signaling that coordinates virulent behaviors like biofilm formation, a major source of contamination of indwelling medical devices. Similarly, these clinical strains also evolve single nucleotide polymorphisms (SNPs) in a quorum sensing enzyme, *rhII*. *RhII* is the synthase of N-butyryl homoserine lactone (C4HSL), which is the ligand for a second major transcription factor, *RhIR*. However, the functional advantage of maintaining these evolved SNPs in *rhII* is unclear. We chose three SNPs of interest to investigate the altered function of *RhII* variants in clinical strains and their role in virulence. We found that these *RhII* variants (G62S, D83E, P159S) partially compensate for deletion of *lasR* to restore virulence factor gene expression. Structural analysis of *RhII* indicated that these variants could synthesize a homoserine lactone molecule with a longer acyl chain. To test this hypothesis, we used mass spectrometry to analyze the metabolites secreted in the supernatants of strains harboring *RhII* variants. We found that these variants produce proportionally more C6HSL than C4HSL, implicating that a higher affinity interaction between *RhIR* and its ligand is advantageous in clinical strains of *P. aeruginosa*. Indeed, the structure of the *RhIR* ligand binding pocket revealed that ligand selection is driven by hydrophobic contacts with acyl tail groups, indicating that C6HSL is likely a higher-affinity ligand than C4HSL, the cognate ligand. Moving forward, we aim to understand the fitness advantage *RhII* variants confer when these strains are challenged with antibiotics. It is important to understand the nuances of quorum sensing mediated virulence expression in clinical strains of *P. aeruginosa* to improve the treatment of life-threatening infections, especially in the age of antibiotic resistance.

Presenter: Kayla Simanek, ksimanek@albany.edu

The Toxic Crusaders Program

M. Koltunov, H. Gutierrez, Z. Xie, S. Ko; California Department of Toxic Substances Control

The California Department of Toxic Substances Control (DTSC) seeks to promote scientific engagement, critical thinking skills and interest in STEM careers in high school students through the Toxic Crusaders Program, which provides a variety of hands-on experiments relating to environmental science and analytical chemistry. The Toxic Crusaders Program is coordinating with a high school in the Los Angeles Unified School District to work with 11th grade students on a STEM curriculum to simulate sampling, analysis and reporting of hazardous chemicals (e.g., poly- and per-fluoroalkyl substances (PFAS), hexavalent chromium, lead and cadmium) found at a hypothetical contaminated site and communicating these results to the public. The framework of Toxic Crusaders consists

of: 1) developing an interactive workbook with hands-on activities and relevant background information such as California laws and regulations on hazardous chemicals, sampling and lab procedures, worksheets and presentation templates; 2) providing additional resources, such as links to videos, suggested readings and other relevant media for teachers to introduce concepts to the students at the beginning of the curriculum; 3) hosting an initial lab visit to familiarize students with background information on the chemicals of interest, sampling and testing requirements, lab techniques and public engagement skills; 4) leading a series of virtual workshops to reinforce concepts during class hours; 5) hosting a second lab visit where students initiate their own investigation, sampling and testing of hazardous chemicals; and 6) encouraging students to present their results to their community. To measure the effectiveness of Toxic Crusaders, preliminary evaluation surveys were administered after the initial lab visit, which indicated a self-reported increase in student knowledge of environmental chemistry topics and terminology, as well as increased interest in DTSC careers. Surveys will be administered before and after the second lab visit to further measure student engagement with scientific processes, critical thinking, and careers in STEM. After the completion of the program, students will have experienced working as researchers, environmental scientists, communication specialists and other professions within DTSC. They will have acquired practical field and laboratory techniques and used state-of-the-art analytical instruments such as Inductively Coupled Plasma/Optical Emission Spectroscopy (ICP-OES) and Liquid Chromatography/Tandem Mass Spectrometry (LC-MS-MS). Students will develop a better understanding of environmental issues within their community. Ultimately, students will discover possible career paths and professions in science.

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Training the Next Generation of Laboratory Scientists during the COVID-19 Pandemic

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Laboratory testing is a high-volume medical activity in the United States, with over 13 billion tests being performed each year. The pandemic exposed the severe shortage of trained laboratory personnel needed to run a variety of tests in clinical laboratories. Thus, there is an urgent need for training programs to encourage students to consider careers in clinical laboratories. To this end, the District of Columbia Public Laboratory (DC PHL) has been dedicated in their efforts to provide the highest levels of education and professional development. During the COVID-19 global pandemic, there was a severe shortage of CLIA laboratories available for training college and university students. Hospitals were limited in their ability to host clinical rotations due to the potential dangers of the pandemic. However, clinical rotations are an essential part of obtaining a degree in Clinical Laboratory Science and becoming testing personnel in the laboratory. The DC PHL Microbiology Unit established a partnership with the Howard University Department of Clinical Laboratory Science to host the microbiology clinical practicum for their senior students. We collaborated and designed a program that exposed the students to modern instrumentation, cutting-edge scientific methods and high complexity testing.

Students were required to perform isolate identification using gram stains, ancillary testing and laboratory identification systems. The students completed trainings in the microbiology, molecular biology, immunology and virology departments. The program started in January 2022. Since then, we have completed rotations for eight students. Each practicum participant's understanding of the training information was evaluated before and after training was complete. Students were given pre-training exams to obtain a knowledge reference point and a post-training exam to demonstrate improvement. We also used this information to measure program efficacy. Students were required to pass a post-training test with a score of 90% or greater and to correctly identify five unknown isolates. The average pre-training exam result was 48.3%, and the average post-training exam result was 96.1%. The students showed 47.8% improvement in post-training exam scores. Each student remarked positively about their learning experience after the practicum was complete. Four of the eight students have graduated and work as medical laboratory scientists adding to the available work force. The other four students are scheduled to graduate in this spring and all plan to work in the field as well. We are currently evaluating the program and making improvements to provide the best training possible for the next generation of laboratory professionals.

Presenter: Reginald Blackwell, reginald.blackwell@dc.gov

POLIO, MPOX, SARS-COV-2, EXPANDED TARGETS NWSS, STIs

Automated HPV DNA Extraction and Detection from Cervical, Anal, and Throat Samples

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Human papillomavirus (HPV) is the most common sexually transmitted infection in the United States. The 14 high-risk HPV types cause 91% of anal and cervical cancers and 70% of genital and throat cancers annually. Traditionally, the Pap smear has been used to screen for early cellular changes that may progress to cervical cancer, but this method is prone to human error due to its visual result interpretation. The World Health Organization recently recommended DNA-based HPV testing as the first-choice screening method for cervical infection with high-risk HPV types, which can guide additional surveillance measures for early detection of cancer. However, the recommendations do not address testing of other affected areas like the anus, genitals, and throat. Here we describe methods developed for automated extraction of HPV DNA from cervical, anal, and throat samples and detection by multiplex qPCR. The use of automation in DNA extraction saves time while reducing the potential for errors in processing. DNA was extracted from throat samples collected in Universal Transport Medium®, or cervical and anal samples collected in SurePath™ and ThinPrep® preservative using the Maxwell® RSC Instrument, an automated benchtop nucleic acid purification platform or using a high-throughput extraction method developed for the KingFisher™ Flex System. The HPV DNA type present within each sample was successfully detected using a multiplex qPCR assay with primers and probes specific to high-risk HPV types 16, 18, and 45. This study demonstrates the development of straightforward and efficient workflows for HPV DNA extraction and qPCR detection

from cervical, anal, and throat samples using benchtop and high-throughput automation, helping to address the need for HPV DNA detection methods for multiple affected areas.

Presenter: Emma Fischer, emma.fischer@promega.com

Detection of Antibody Responses Against SARS-CoV-2 in Serosurvey Participants from Distinct Periods of the COVID-19 Pandemic – New York City, New York

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Background: Antibody responses after SARS-CoV-2 vaccination and infection target the viral spike (S) protein (anti-S antibodies). Neutralizing antibodies (nAb) capable of blocking infection develop in a subset of anti-S antibody responses and contribute to immunity. Antibodies against the SARS-CoV-2 nucleocapsid (N) protein (anti-N antibodies) only develop after viral exposure. The 2020 (June 2020–October 2020) and 2021 (April 2021–October 2021) New York City (NYC) COVID-19 serosurveys were conducted during two distinct pandemic periods, including pre- (2020) and post-vaccine availability (2021). This study characterized and compared three anti-SARS-CoV-2 antibody responses (anti-S, nAb, and anti-N) among participants during these periods. **Methods:** Blood samples were collected from 1,532 NYC residents during the 2020 (n = 497) and 2021 (n = 1,035) serosurveys. Serum was separated from blood and used to detect anti-S antibodies and anti-N antibodies using the LIAISON® SARS-CoV-2 S1/S2 IgG assay and the VITROS™ Immunodiagnostic Products Anti-SARS-CoV-2 Total N Antibody Test, respectively. Anti-S antibody positive serospecimens (n = 1,059) were tested for nAbs using the SARS-CoV-2 Surrogate Virus Neutralization Test (sVNT) Kit. **Results:** Anti-S and anti-N antibodies were each detected in 20% (100/497) of 2020 serosurvey participants. Anti-S seropositivity increased to 93% (959/1,035) in 2021 serosurvey participants. Anti-N seropositivity increased to 26% (273/1,035) in 2021 participants. nAbs were detected in specimens with detectable anti-S antibodies from both periods, 91% (91/100) and 98% (944/959), respectively. Average nAb concentrations from both serosurvey periods were 3,529 U/ml (95% CI: 2,867–4,191) and 23,523 U/ml (95% CI: 20,573–26,473), respectively. In the 2021 serosurvey, history of prior SARS-CoV-2 exposure (i.e., detectable anti-N antibodies) yielded approximately 5-fold higher nAb concentrations, compared with no history of prior viral exposure (54,947 U/ml [95% CI: 46,423–63,458] vs 11,272 U/ml [95% CI 9,579–12,695]). **Conclusion:** In the 2020 serosurvey, conducted before vaccine availability, one in five participants had detectable anti-S and anti-N antibodies. Anti-S positivity (93%) increased substantially in the 2021 serosurvey conducted after vaccines became widely available, whereas anti-N positivity remained stable. The high anti-S positivity is likely attributed to widespread vaccination in NYC during this period. Most participants with detectable anti-S antibodies produced functional nAbs against the SARS-CoV-2 S protein. Notably, nAb levels were higher in 2021 serosurvey participants with detectable anti-N antibodies, suggesting that prior exposure to SARS-CoV-2 may have provided better immunity in this cohort.

Presenter: Scott Hughes, shughes@health.nyc.gov

Evidence of Mpox Virus Infection from Non-Lesion Specimens Among Persons Without Characteristic Lesions or Rash—District of Columbia, August 2022

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Since May 2022, mpox infections have been confirmed in non-endemic countries worldwide. Classic signs and symptoms of mpox infection include a prodromal period of influenza-like symptoms that generally start within three weeks of exposure to the virus, with a rash appearing approximately 1–3 days after fever onset. During the 2022 multinational mpox outbreak, a significant number of patients reported rash as their first symptom, in the absence of a prodrome. Diagnosis is by real-time PCR, after rash onset, with lesion material being the most accurate specimen type for testing. Mpox virus has been detected in non-lesion specimens from persons experiencing signs and/or symptoms of mpox infection, but evidence supporting the utility of non-lesion specimens for detection of mpox infection is limited. Transmissibility data from non-lesion sites with detectable mpox virus are also lacking. Over a three-week period in August 2022, various specimen types were collected from persons without characteristic mpox rash presenting for their first dose of Jynneos vaccine at two DC Health clinics to identify previously undiagnosed mpox infection. Participants (n=543) provided blood, pharyngeal swab and rectal swab specimens; PCR was used to determine the presence of mpox virus in these specimens. PCR-positive specimens were cultured and mpox virus titers were determined for viable specimens. Serum specimens were tested for anti-orthopoxvirus IgG antibodies, with a subset tested for IgM antibodies. Of 543 participants providing ≥ 1 specimen, three individuals had a specimen with detectable mpox virus DNA by real-time PCR: one pharyngeal (1/522, 0.2%) and two rectal (2/164, 1.2%) specimens. Only the two rectal specimens were culture positive, with titers of 2.9×10^7 pfu/mL and 1.5×10^5 pfu/mL. The three participants with PCR-positive specimens were IgG negative; however, two of the three participants were IgM positive, indicating a recent infection. Two of the three participants with mpox-positive specimens reported having no symptoms, while the third participant reported only fever and headache with no other symptoms. These results demonstrate that individuals may have detectable mpox virus before the recognition of, or in the absence of, characteristic mpox lesions and may be capable of viral shedding, as evidenced by the high-titer values in the rectal specimens. While the mpox virus infectious dose for humans is unclear, animal study data suggest that the high titers like those observed from the two rectal specimens may be sufficient for human-human transmission. Although data on alternative non-lesion specimen types is informative, the low number of infections detected in the absence of rash suggests that screening efforts using these specimen types may have limited effectiveness in reducing transmission and controlling future outbreaks.

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Identification of Tecovirimat Resistance-associated Mutations in Human Mpox Virus, Los Angeles County

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Tecovirimat (also known as TPOXX or ST-246) is a drug available for treatment of mpox through the FDA's Expanded Access program. Previous studies identified more than 20 mutations in VP37, a conserved orthopoxvirus envelope protein required for extracellular virus particle generation, that are associated with tecovirimat resistance. In Los Angeles County, epidemiologic surveillance of mpox cases based on review of medical history including immune-compromising conditions such as HIV infection, severe disease including hospitalization, and disease that did not improve after tecovirimat treatment identified nine cases with suspected resistance, including two patients who succumbed to infection and expired. To further investigate the possibility of tecovirimat resistance in these cases, a laboratory surveillance network was established whereby clinical and commercial laboratories provided specimens for whole-genome sequencing of mpox virus (MPXV) to the Los Angeles County Public Health Laboratory (LACPHL). Specimens obtained from these individuals harbored a total of seven different tecovirimat resistance-associated mutations in VP37 (H238Q, N267D, A288P, A290V, D294V, A295E, and I372N), as well as six additional mutations previously unassociated with resistance (T220A/I, P243S, T245I, A265D, and T289A). Allele frequency analysis showed some patients displayed resistance mutation heterogeneity within single lesion specimens, and different mutations were identified among distinct lesion specimens collected from the same patient, suggesting these mutations were selected or acquired over the course of treatment and not present when infection occurred. Importantly, phenotypic testing confirmed tecovirimat resistance in a subset of specimens, demonstrating that these mutations indeed result in resistance. Taken together, these findings confirm a low barrier for tecovirimat resistance and underscore the need for additional therapeutics when treating mpox in certain patients. Clinical management needs to ensure optimization of drug delivery/dosing as well as immune function when treating with tecovirimat to prevent resistance. LACPHL is working to validate MPXV sequencing for identification of mutations associated with tecovirimat resistance to be used as a diagnostic tool.

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Lessons from Lesions: Trends in Specimen Submissions and Test Performance During the 2022 Mpox Outbreak in New York City

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Background: New York City (NYC) was an early epicenter in the 2022 mpox outbreak. The NYC Public Health Laboratory (NYC PHL), a Laboratory Response Network (LRN) lab, was initially NYC's sole testing facility. NYC PHL tested thousands of specimens using the United States Food and Drug Administration-cleared Non-Variola Orthopoxvirus (NVO) real-time PCR assay distributed by the LRN. We

evaluated patient demographics, lesion site frequency and assay performance by lesion site from specimens collected to characterize trends during the mpox response in NYC. **Method:** Swab specimens from suspected mpox lesions were submitted to NYC PHL May 19 –October 14, 2022 for testing with the LRN NVO real-time PCR assay. Patient demographic characteristics (age and zip code), mpox lesion sites, and test result (including real-time PCR threshold cycle [Ct] value) were queried from the Laboratory Information System. Assay-defined cut offs were used to determine positive (Ct < 37), equivocal (Ct = 37–40), inconclusive (no amplification of target or control) and negative (no signal within 40 cycles) specimens. Specimens were analyzed by age, location and lesion site. Test results were used to evaluate assay performance among different lesion types. **Results:** NYC PHL tested 2,260 specimens using the LRN NVO real-time PCR assay during the mpox outbreak. Of those, 1,917 (84.8%) were collected from NYC residents, and 1,643 (72.7%) were from patients aged 25–44 years. Specimens collected from genital lesions were the most frequently submitted lesion site (19.4%; 438). Overall, 1,349 (59.7%) specimens tested positive, 749 (33.2%) were negative, 141 (6.2%) were inconclusive and 21 (0.9%) were equivocal. Among 419 specimens tested from anal/rectal lesions 318 (75.9%) were positive, whereas among 162 specimens tested from leg lesions only 57 (35.2%) were positive. Specimens from hand lesions yielded inconclusive results in 31 (21.7%) specimens, approximately 3.5-fold higher than the average specimen inconclusive rate. **Conclusions:** Our data revealed differences in NVO PCR assay performance based on specimen site that can be used to guide lesion site prioritization. As a result, these data can help public health officials provide lesion site collection guidance that maximizes NVO assay performance to more efficiently detect mpox lesions. This is especially important when prioritizing collection from patients presenting with lesions from multiple anatomical sites.

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Mpox Virus Lineage Assignments and Phylogeny in New York City, 2022

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Since May 2022, mpox cases positive for sub-lineage mpox virus (MPXV) Clade IIb have emerged across several non-endemic countries. In New York City (NYC) alone, nearly 3,800 mpox cases have been reported as of December 2022. With the emergence of MPXV, some genomic clusters may have specific genomic or phenotypic characteristics that could impact diagnostic testing, treatment, vaccine efficacy, disease severity, or transmission. Genomic sequencing and phylogenetic analysis have previously been used to cluster viral genomes that likely share genetic characteristics. Naming the clusters can aid in discussions of infectious disease surveillance and outbreak investigations. For example, Pangolin has aided in surveillance efforts by allowing researchers and public health officials to communicate across the globe with a common nomenclature for SARS-CoV-2. Similarly, Nextclade is a tool that assigns viral lineages using the nearest neighbor from a reference tree. Here, we report on MPXV full-genome phylogenetic analysis and Nextclade lineages identified during the NYC mpox outbreak in 2022. Lesion specimens collected from patients in NYC and submitted to the NYC Public Health

Laboratory (NYC PHL) between May–December 2022, were tested using the CDC LRN Non-Variola Orthopoxvirus assay. Positive specimens with a Ct < 30 were sequenced using Illumina technology and analyzed at the NYC PHL. Phylogenetic analysis was conducted using IQTree. Using ggtree, a bioconductor R package, the phylogeny was labelled with Nextclade lineage assignments to examine clusters with inconsistent lineages and determine the proportion of monophyletic designations. All 1,052 sequences sequenced by NYC PHL were MPXV Clade IIb, which Nextclade assigned correctly as B.1 or its sublineages. Nextclade assigned 536 genomes as B.1, which were correctly placed as basal genomes of MPXV Clade IIb on the phylogenetic tree. The remaining 516 sequences were assigned predominantly as B.1.2, B.1.3, B.1.7, B.1.8 and B.1.12 sublineages and were clustered as distinct clades in the tree except for four genomes. Overall, genome phylogeny demonstrated that Nextclade lineage mis-assignment account for 0.4% of NYC PHL mpox sequences. In conclusion, genomes sequenced at the NYC PHL from the 2022 mpox outbreak were all MPXV Clade IIb and were assigned as B.1 or its sublineages. We find that Nextclade lineage assignment may serve as an appropriate tool for communicating genomic clusters for public health surveillance of mpox and will be critical to monitor the extent of its genome evolution in NYC.

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Multiplex RT-qPCR Assay of Detecting Influenza A Virus, Influenza B Virus, SARS-CoV-2, & Human Respiratory Syncytial Virus

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Respiratory viruses cause acute respiratory disease worldwide. Acute respiratory infections are the world's third leading cause of death. Before the COVID-19 outbreak, there had been 4 million deaths each year caused by acute respiratory infections. Monitoring respiratory virus occurrence from the environment such as wastewater could provide surveillance of respiratory virus infection agents in communities. We developed a multiplex RT-qPCR assay of detecting Influenza A, Influenza B, SARS-CoV-2, & Human Respiratory Syncytial Virus (RSV). The assay also provides a possibility for a quick determination of respiratory virus from clinical samples.

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Optimized Methods for Antimicrobial Resistance Testing

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Introduction: Antimicrobial resistance (AMR) detection and surveillance has become a high priority in both healthcare and environmental settings for the safety of patients and the general public. Samples of interest for AMR, such as stool and wastewater, are challenging substrates for nucleic acid extraction, and choice of extraction method will determine the success of downstream analysis. Extraction methods must be able to address high levels of inhibitory substances and achieve extraordinary sensitivity to provide the best possible sensitivity of downstream assays. Downstream analysis must also be tuned to maximize information content derived from these samples. We investigated entire workflows for antimicrobial resistance. **Methods:** Fecal and

wastewater samples were collected and DNA and RNA were isolated using extraction kits specialized for microbiome and inhibitory samples. Extraction efficiency was evaluated by conventional and digital PCR, as well as NGS. In addition, these samples were tested in a hybrid capture based NGS workflow for detection, sequencing, and characterization of AMR gene targets using a variety of sample types. Human stool samples were used as a comparison. **Results:** Significantly more diverse AMR markers were found in wastewater than in individual fecal samples. Fecal samples showed strong bias towards resistance markers against particular common antibiotics. Very high concordance was found between NGS read number and dPCR copy number, indicating the potential complementary value of these methods. **Conclusion:** These tools can provide insights into the development and dynamic of AMR, and could thus be a powerful method to combat antibiotic resistance. Identifying the most efficient and consistent workflows will support this effort. Learnings from these workflows can also be extended to further targets or application fields, such as adventitious agents or respiratory pathogens, improving understandings and outcomes for public health.

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Optimizing Workflow for Comprehensive Immunological Assessment in Response to Influenza Vaccination and Infection

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Influenza is a vaccine-preventable respiratory virus infection and is estimated to cause 20–60 thousand deaths in the United States. The annual economic burden due to influenza in the US alone is between 6.3–25.3 billion dollars. Although annual vaccination is recommended to reduce morbidity, mortality and economic impact, the vaccine effectiveness ranged from 10-60% in the United States from 2004–2021. Several host factors, including preexisting influenza immunity, age, weight, sex and immune status, can impact vaccine efficacy. In addition, despite being vaccinated, many individuals get infected with influenza and the reasons for these break-through infections are unclear. Here we describe the development and optimization of a workflow to assess the frequency of antigen specific B cells with high-dimensional flowcytometry, B-cell receptor usage, and B- and T- cell transcriptomic signatures in peripheral blood mononuclear cells and hemagglutinin (HA)-specific serological repertoire in plasma with liquid chromatography and mass spectrometry to identify the clonotypes of Influenza HA-specific antibodies. The workflow will be utilized to investigate the immunological mechanisms of breakthrough infections of influenza-infected subjects (break through infection group) compared to vaccinated and uninfected subjects (vaccinated control group).

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Simultaneous Detection of Respiratory Infectious Diseases using Immunoprecipitation and Liquid Chromatography-Tandem Mass Spectrometry

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We are now living in a new era with new normal after the COVID-19 pandemic. Recently, respiratory syncytial virus (RSV) has been another concern as it surges among children. In addition to influenza viruses, not only are their symptoms similar at early stages, but they are also both enveloped viruses with several common biological properties, often leading to challenges in accurate identification. Thus, there is a need to develop a faster and more specific analytical tool that can differentiate infectious diseases. This study describes a targeted approach for the simultaneous detection of different respiratory infectious diseases by targeting nucleoprotein (or nucleocapsid protein, NP) using immunoprecipitation (IP) and liquid chromatography-tandem mass spectrometry (LC-MS/MS) because NP is highly conserved and specific for infectious disease virus types among different viral components. Prior to IP, equal amounts of all biotinylated antibodies were pooled together as one antibody panel for this study. The biotinylated antibody panel was added to samples collected via nasopharyngeal swabs in viral transport media (VTM). The antigen-antibody complex in VTM was directly subjected to IP using streptavidin magnetic beads. The IP purified samples were then tryptic digested and analyzed by Thermo Scientific™ Vanquish™ MD HPLC system hyphenated to Thermo Scientific™ TSQ Altis MD mass spectrometer. Data acquisition, processing and reporting were performed using TraceFinder™ LDT software 1.0. Multiple viruses, SARS-CoV-2, influenza virus A and B types, RSV, and human coronavirus (HCoV-229E), were selected to show that this method can distinguish different disease viruses. The workflow was optimized from sample preparation to LC-MS analysis. The protein precipitation and post sample clean-up were eliminated. From IP procedure, two incubation steps for antigen-antibody complex formation and immobilization on the magnetic beads were reduced to 15 minutes each (originally one hour each). Trypsin digestion incubation time was optimized to 15 minutes (previously 90 minutes). Particularly, the reduction of trypsin digestion time was achieved owing to a generation of much cleaner sample matrix by IP. The entire process was finalized to less than one hour from four hours. LC-MS run time was also optimized to five minutes. A total of 12 peptides were successfully monitored (2-3 peptides per disease) by SRM. Calibration curve was generated with stable isotope-labeled standards (Thermo Scientific HeavyPeptide AQUA Ultimate). With criteria of % RSD 0.99, 0.05 to 1 fmol of peptides on LC column were determined as the LOQs for each peptide with retention time variation ± 0.01 minutes. The method significantly improved the sensitivity and turn-around time, compared to other digestion or peptide enrichment methods.

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Successful Identification and Consensus Assembly of Mpox Genome using Direct DNA Sequencing Approach

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Mpox virus is a member of the poxvirus family which can infect humans typically causing a skin rash and may cause death in rare cases. Historically, most mpox cases were confined to Africa, but in 2022, a significant number of cases have been reported in the US. As of Jan 4, 2023, there have been 29,913 cases in the US, of which 764 cases occurred in New Jersey. As a part of New Jersey mpox surveillance, the NJ PHEL has received Mpox specimens and performed confirmatory qPCR testing and whole genome sequencing employing amplicon-based sequencing method. In addition, as a part of the NJ PHEL sequencing capability expansion, we also performed exploratory no-PCR direct DNA sequencing using the Oxford Nanopore Technology (ONT) sequencing platform, taking advantage of its long-read sequencing capability. Genome sequencing was conducted according to institutional SOPs. Briefly, the extracted DNA was subjected to a standard qPCR test for Mpox detection and the determination of Ct values and then sequenced using both PCR-amplicon and direct-DNA sequencing methods. Six Mpox-positive specimens with a range of Ct values between 15 and 25 were selected for the comparison of amplicon-based and DNA-based sequencing methods. Generated raw reads were subjected to a series of bioinformatic analysis tools for comparison. Our results show that all six samples were successfully sequenced and assembled to >87% genome coverage from the amplicon-based approach on Illumina platform whereas two of the samples with Ct ~25 failed to generate sufficient mpox-specific reads in direct-DNA sequencing approach on ONT platform. Assembly length in direct DNA method showed a correlation between genome coverage and qPCR Ct values. A near complete genome (Mpox reference genome used is ~197kb) was assembled from the samples with Ct ~15 in direct-DNA sequencing. As expected, the PCR amplicon-based method produced more complete assemblies in all 6 samples whereas direct-DNA sequencing generated high coverage assemblies in only 2 samples with the lowest Ct values. A taxonomic identification of the reads using Kraken2 showed that only 0.33-0.47% of the trimmed reads were classified as Mpox (taxonomy id=10244). A clade and lineage assignment by Nextclade also showed good agreement between the amplicon- and direct-DNA-sequencing methods. Despite being more error prone, the direct DNA sequencing approach was able to successfully identify clade and the pathogen present. In summary, although further refinement in direct DNA sequencing is necessary, our exploratory work demonstrated that it is possible to assemble a target species using the native genomic DNA sequencing (without PCR) approach from a clinical specimen. This opens the possibility that this approach may be used for identification and assembly of potential pathogenic genome(s) presented in an unknown clinical sample using ONT platform's long-read capability.

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Validation of the bioMérieux EMAG® Nucleic Acid Extraction Instrument for Non-variola Orthopox Real-time PCR

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Mpox virus is a member of the genus Orthopoxvirus within the family Poxviridae. Infection with mpox presents clinically similar to other pox virus infections causing the formation of rash and pimple like lesions. Mpox is endemic to West and Central Africa and is a zoonotic disease. Rodents are believed to be the primary carrier or reservoir. May 2022 marked the beginning of a large number of positive cases of mpox clade II virus being reported in non-mpox endemic countries including the United States. On July 23, 2022 the magnitude of this outbreak was realized and the WHO declared mpox a public health emergency. The primary test used in the U.S. for mpox response is the CDC non-Variola Orthopoxvirus (NVO) real-time PCR assay. This CDC developed test has FDA 510(k) clearance and is used for the presumptive detection of non-Variola Orthopoxviruses, including Vaccinia, Cowpox, mpox, and Ectromelia viruses. Samples positive by the NVO test at state public health laboratories were sent to CDC for confirmation with a monkeypox-specific PCR test. In summer 2022 the FDA provided enforcement discretion on a number of NVO-related issues, including allowing additional extraction supplies and instruments. However, the bioMérieux EMAG® extraction instrument, the preferred extraction instrument at WSLH, was not included in this enforcement discretion. Benefits of the EMAG® include its familiarity within the virus team, sensitive extraction ability on all viruses tested at WSLH to date, and ease of use within existing testing workflows. Therefore, following FDA EUA guidance, we validated the EMAG® as a modification of the NVO assay, and to date we are the only laboratory in the country to perform a modification of an FDA-cleared assay for mpox. To validate the EMAG®, we evaluated the LOD, accuracy, specificity, precision, and reproducibility data of the NVO PCR test after EMAG® extraction. Performance characteristics showed that the EMAG is acceptable for use with the NVO PCR assay. The accuracy panel performed resulted in 100% concordance with known results and no carry-over of positive samples into negative samples. The limit of detection was calculated to be 39 copies/reaction. Taken together, the data show that the bioMérieux EMAG® nucleic acid extraction instrument has comparable performance characteristics to Qiagen spin columns, and the EMAG® is acceptable for extraction prior to NVO real-time PCR.

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Wastewater-based Epidemiology for Human Influenza A and B, Parainfluenza, Metapneumovirus, RSV, Seasonal Coronaviruses, Rhinovirus, Norovirus, Mpox, and SARS-CoV-2

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In order to provide more complete data, especially representing individuals who do not seek healthcare and may not be included in traditional surveillance systems, additional methods for understanding respiratory, enteric, and emerging, novel disease dynamics are needed. Enhanced data can be used to better inform

public health responses with an eye toward equity and to guide clinical decision making. Wastewater monitoring for infectious disease targets is a passive method for disease monitoring in a community. Here we show that concentrations of viral nucleic acids from a diverse suite of human viruses in wastewater solids correlate well with customary clinical data streams on disease occurrence. We measured concentrations of mpox, SARS-CoV-2 variants, norovirus GII, influenza A and B, RSV A and B, parainfluenza (1-4), rhinovirus, seasonal coronaviruses, and metapneumovirus nucleic acids in wastewater solids between three and seven times per week for extended periods of time (a year or more). We measure viral nucleic acids in wastewater solids rather than wastewater influent because viruses partition to wastewater solids. Their concentrations are approximately 1,000 to 10,000 times higher in solids than in influent on an equivalent mass basis. We detected nucleic acids from all tested viruses in wastewater solids. We compared viral nucleic-acid concentrations to customary measures of disease occurrence including laboratory-confirmed disease incidence rates and test positivity rates. Virus concentrations correlated significantly and positively with occurrence of associated viral diseases. This work suggests that respiratory, gastrointestinal, and novel, emerging viral diseases can be tracked in communities using wastewater monitoring regardless of individual health seeking behavior. Wastewater monitoring represents a powerful tool for informing public health responses including clinical decision making, allocation of resources, timing and locations of vaccine campaigns, and implementation of non-pharmaceutical interventions. Based on these findings, we have scaled measurements of many of these viral targets nationally through our project WastewaterSCAN; we currently provide measurements three times per week at over 130 wastewater treatment plants in twenty-five states. Data are available publicly within 48 hours of receiving a sample at the laboratory and displayed at: data.wastewaterscan.org.

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Wastewater-based Techniques: Optimizing Bench Protocols from Sample Concentration to dPCR in a Local Public Health Laboratory

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Wastewater analyses have been used for many years to surveil concentrations of chemicals, pharmaceuticals, biomarkers, and other substances that could reflect population behaviors. Wastewater-based epidemiology (WBE), however, is a more recent approach to using wastewater for public health. This approach has now been widely adopted, primarily in response to the SARS-CoV-2 pandemic, to surveil community disease burden, emerging pathogens, and leading indicators of community outbreaks. The CDC has provided guidance on wastewater method development through the National Wastewater Surveillance System. However, the onus to develop technical protocols has been left to individual laboratories, in part, because wastewater matrices are site specific. For public health laboratories at the local level, optimizing new techniques may be complicated by such restrictions as limited resources, constrained space, and fewer staff, when compared to larger-scale State, Federal, and commercial laboratories. Here, we describe how the City of Milwaukee Health Department Laboratory (MHD), a local Wisconsin public health laboratory, approached these obstacles when it began wastewater surveillance of SARS-

CoV-2 in August 2021. Wastewater samples provided to MHD for analysis undergo the following steps: viral particle concentration, nucleic acid extraction, and digital PCR for the quantification of viral load. These three processing steps must not only be dependable, but also balance the restraints imposed on a local public health laboratory. Therefore, each step was evaluated using four criteria: 1) consistency and accuracy of viral particle recovery, 2) feasibility and practicality in the context of the laboratory layout, 3) long-term cost efficiency, and 4) turnaround time. Using these criteria, MHD successfully compared several methods to determine the most reliable protocol that best optimized limited resources and available laboratory space, whilst creating a time-efficient process to allow meaningful WBE. Optimization of these steps for SARS-CoV-2 will allow MHD to expand analyses to additional targets such as influenza, mpox, antimicrobial resistance, and more.

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STRENGTHENING LABORATORY SYSTEMS AND STRATEGIC PLANNING

A Response Playbook for Early Detection and Population Surveillance of New SARS-CoV-2 Variants in a Regional Public Health Laboratory

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The Regional Innovative Public Health Laboratory (RIPHL) is a public-academic partnership between Chicago Department of Public Health (CDPH) and Rush University. It was established in March 2021 to serve as the molecular epidemiology laboratory for CDPH, providing flexible molecular detection, characterization, and genomic surveillance of pathogens of public health importance with the goal of timely and actionable data for public health response. Between April 3, 2021 and December 31, 2022, RIPHL received 11,557 SARS-CoV-2-positive remnant clinical respiratory specimens from 15 acute care hospitals in Chicago for molecular characterization. Of these, 8,886 (77%) specimens were successfully sequenced and yielded lineage assignments. Across the study period, RIPHL sequenced 3% of all incident SARS-CoV-2 infections reported to CDPH, and accounted for 62% of Chicago SARS-CoV-2 sequences in GISAID. Demographically, individuals who provided specimens for sequencing were broadly representative of all SARS-CoV-2 infections reported to CDPH in terms of sex, age, race/ethnicity, and ZIP code of residence. RIPHL has altered molecular methods in response to public health needs. When Omicron BA.1 was rapidly emerging, RIPHL developed and implemented a genotyping PCR to provide lineage proportion estimates at much faster turnaround compared to whole genome sequencing (WGS). TaqMan SARS-CoV-2 Mutation Panel Assays (ThermoFisher) targeting mutations K417N and L452R were used to distinguish BA.1 from the background of Delta variant. Genotyping PCR was able to call lineage in samples with low viral concentration more successfully than WGS, resulting in 1,657 (90.4%) lineage calls from genotyping PCR compared to 1,560 (85.1%) for WGS. Of 1,541 samples with both WGS and genotyping PCR data available, 1,539 (99.9%) concordant. Since late 2021, Chicago has experienced waves of successive Omicron sublineages. The relative speed with which sublineages

gained dominance was used as a proxy for the level of public health concern posed by particular sublineages. Logistic growth rates of emerging sublineages were calculated weekly: low risk variants had logistic growth rates 0.13. Only one sublineage had a log growth rate warranting a “high risk” designation (BA.1), 7 sublineages met medium risk criteria (BA.4, BA.5, BA.4.6, BA.2.75, BF.7, BQ.1, BQ.1.1). These metrics were considered alongside other epidemiological indicators to inform public health responses, like proof of vaccination requirements. The public-academic partnership at the heart of RIPHL has enabled innovative techniques to be quickly incorporated into public health responses, and continued innovation will be required to continue to respond to SARS-CoV-2 and other infectious threats.

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Barriers and Facilitators to Use of Quality Management System Tools and Resources for Next Generation Sequencing in Public Health Laboratories

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Introduction: Next generation sequencing (NGS) technologies have advanced public health practice. Public health laboratories (PHLs) are currently adapting NGS for various applications; however, there are several challenges PHLs face when implementing NGS. To address these, the US Centers for Disease Control and Prevention (CDC) and Association of Public Health Laboratories (APHL) launched the NGS Quality Initiative (QI) to develop an NGS-focused quality management system (QMS) that provides ready-to-implement guidance documents, customizable standard operating procedures and other tools that support implementing sequencing workflows. To assess barriers and facilitators to use of these products, CDC used NGS QI website data to identify a sample of nine PHLs who are current or intended users of the NGS QMS products for key informant interviews. **Methods:** CDC used Adobe Analytics to track the number of page views, downloads, unique visitors and locations of unique visitors to the NGS QI website. We used visitors' zip codes and addresses of PHLs as a proxy to identify users of the NGS QMS products and then got input from CDC and APHL NGS experts to ensure a representative sample of PHLs with diverse NGS experience and geographic location. CDC piloted the interview guide with three CDC staff with laboratory and NGS expertise to ensure applicability and utility of the questions. In early 2023, CDC will interview nine individuals from the identified PHLs about facilitators and barriers to using NGS QMS products (e.g., as a reference or incorporated into practice) and additional support needed on NGS workflows. Interview transcripts will be coded and analyzed using MAXQDA and the analysis will be informed by the Consolidated Framework for Implementation Research and Diffusion of Innovations theory. **Results:** Lessons learned from the pilot test of the instrument with CDC laboratories were used to inform changes to the interview guide for PHLs. Each PHL has a unique experience using NGS QMS products, including whether and to what extent they use them and for what purposes. We will present key themes on facilitators, barriers, needs and suggestions for improvement from the interviews with PHLs. **Conclusion:** In addition to informing NGS QI activities and QMS product development, these findings will provide valuable insights into how state, local and territorial PHLs

adopt guidance and resources from scientific organizations into their quality practices, the challenges they face, and the resources they need to support implementation of such guidance. These data have a few limitations: 1) many staff are involved in the NGS workflow but we will only be able to interview one person from each PHL, which relies on that individual to collect information from others on their experience with NGS QMS products; and 2) the experiences of these PHLs may not generalize to the barriers and facilitators experienced by other PHLs.

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Deficiency Dilemma - A Systematic Approach for an Audit Response

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CLIA-certified laboratories must undergo regular biennial surveys to meet regulatory requirements, but also to identify areas for operational and quality systems improvements. A successful, comprehensive deficiency response, especially within a limited time frame, requires strong leadership, teamwork, and multiple resources. In 2021, the North Carolina State Laboratory of Public Health (NCSLPH) successfully responded to deficiencies cited in a routine recertification survey. Deficiencies, which required a response within ten days of the audit report, were primarily related to pre-analytical systems and related operations. During the survey, NCSLPH management met daily to discuss audit progress and potential deficiencies. At the exit meeting with the auditors, the NCSLPH took extensive notes and asked questions to ensure complete understanding of the CLIA team's findings. This proactive approach enabled the NCSLPH to immediately begin an audit response plan before issuance of a final report. The NCSLPH Laboratory Director assembled a multi-disciplinary team to review audit findings, define needed resources, and identify specific team members to develop a system-wide corrective action plan. Team members included: executive leadership, quality assurance, and lab managers. Resources included: project management support to assist with organization and timeline, staff reallocation to complete needed studies, consumables for needed studies, records retrieval support, IT for website updates, and laboratory outreach to develop training materials and engage submitters. A multi-faceted approach to address pre-analytical issues included an analysis of previously reported results, review of specimen acceptability criteria based upon kit inserts or validation data, and identification of specimen stability studies needed to revise acceptability criteria for clinical assays. Existing state supported courier operations were adjusted to provide specimen transport to NCSLPH to ensure specimen acceptability. As a result of the audit response, all relevant documents containing specimen acceptability criteria (laboratory guide to services, website, method SOPs, etc.) were revised and aligned to ensure consistent messaging. The laboratory information management system (LIMS) was reconfigured to run daily reports to verify specimens tested met acceptability criteria prior to resulting. Retraining of laboratory staff and outreach to all submitters (webinars and training guides) to relay specimen acceptability criteria and new processes for submission of specimens to NCSLPH was documented. In an extremely short timeframe, the NCSLPH instituted significant changes to specimen submission procedures

to ensure all pre-analytical processes were compliant with regulatory requirements.

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Effective Solutions for Maintaining Specimen Integrity during the Pre-analytical Testing Phase

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Ensuring appropriate specimen storage conditions and timely delivery is essential for meeting the acceptance criteria during the pre-analytical phase of testing. The Public Health and Environmental Laboratories (PHEL) plays an important role in maintaining the integrity of specimens by identifying and resolving issues impacting specimen integrity during transportation. Last year, we observed an increased number of delivery delays, furthermore, there was no mechanism in place to track the specimen storage temperatures while in transit. To identify factors that were causing the delays and identify solutions for reducing delivery times, we conducted individual interviews and online surveys with our state-contracted courier and client facilities. The survey results indicated that finding parking spaces and long, complicated navigation through the hospitals were two important reasons for delays. Additionally, specimens were often not readily available for pickup at some facilities. Our courier and clients also had differing responses on pickup duration, which may be due to a lack of understanding of the logistical challenges faced by the courier during the pickup process. Following process changes, we observed that the solutions we implemented at the select facilities contributed to reducing pickup durations by up to 50%. During this study, we also tracked specimen storage temperatures by using temperature data loggers. By using these devices, we standardized and improved procedures for transporting specimens to meet the acceptance criteria. In conclusion, strengthening partnerships with our courier and clients is vital for improving pre-analytical processes and the quality of testing. Therefore, it is important that we engage with them regularly and identify issues and collaboratively develop solutions. Best practices and lessons learned from this study be incorporated into our workflow to promote good specimen handling and transportation practices.

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Improving Public Health Laboratory Testing Processes in Iowa

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The COVID-19 pandemic forced the State Hygienic Laboratory at the University of Iowa (SHL) to alter testing processes to meet the dramatically increasing test volume. Laboratory staff worked 20/7 for several months before reverting back to a single shift, 6-day work week. SHL hired additional staff to meet the data entry, verification, and accessioning and testing workload. Simultaneously, SHL instituted the Test Iowa program using a digital format on behalf of the Iowa Department of Public Health and the Iowa Governor's Office to increase testing outreach statewide. To date, the two

testing programs have reported over 1.6 million PCR results. SHL's conventional testing required increased staffing for data entry and verification, accessioning and testing, which lead to long turnaround times (TAT). SHL made sequential improvements by implementing a COVID-19 electronic test request form, providing web portal access for expedited result retrieval, and modifications to the accessioning process to improve testing efficiency. Although these improvements contributed to decreasing laboratory TAT, Test Iowa's program still proved to be more efficient than SHL's conventional testing. We hope to demonstrate the efficiency of using a digital format similar to that of the Test Iowa program to improve patient care and laboratory testing efficiency.

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LIS Automation of a Point of Care Field Deployable Laboratory

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During the pandemic, one of the greatest assets to limiting spread and notifying positive cases was the use of pop-up point of care (POC) testing events. These pop-up events were a great way to perform rapid BinaxNOW tests or other POC tests. A member of the public would register, take the test, and receive a result. At the end of the day, the entire day's results would be delivered to the lab for resulting into the LIS- Orchard Point of Care. This entire process was manual and, in most cases, written on paper. There were many issues related to this process; data integrity, result to patient on a small piece of carbon paper, and delay in reporting for possible outbreak situations to name a few. We took this problem and saw an opportunity to use technology to automate this entire process. The State of New Jersey was attending POC events with a Field Deployable Laboratory (FDL), where a Cepheid GeneXpert PCR instrument was installed. We outfitted the FDL with a dual band wireless router with dual sim cards with 5G network speeds. This allows the FDL to maintain internet connectivity wherever it would travel throughout the state and is the key to automating the POC process. With this network connectivity, we were able to create a mobile laboratory that has access to our cloud-based LIS. Network connectivity was only part of attaining the automation process desired. To accomplish full automation, we installed a small microcomputer in the van that acts as an inbound/outbound HL7 server that communicates with the instrument in the FDL to receive orders and send results back to the LIS. With this server in place, we fully automated the entire POC process within our LIS. At registration, a staff member will input the order into our ETOR system, while sample collection is occurring, this order is delivered from our LIS and received on the server in the FDL, and a label prints out. Simultaneously, the order is sent to the instrument to be run. Once the instrument's run is completed, the result is automatically sent back up to our LIS to wait for approval. While other tests are being run, the scientist can approve the tests as they are sent back up to the LIS. At approval, the results are delivered electronically based on the Provider, HL7 messages are delivered accordingly, and finally an official lab report is printed inside the FDL to hand to the patient. At last, a manual process converted to an electronic one; resolving data integrity issues with handwritten

paper, faster reporting to all parties involved and a more seamless and friendly flow for all involved.

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When and How a Public Health Laboratory Should Discontinue a Test

M. Pentella, State Hygienic Laboratory at the University of Iowa

All public health laboratories face the question of whether to keep or delete a test from their test menu from time to time. Changes in pathogen or biomarker detection technologies, trends in emerging disease, availability (or unavailability) and cost of certain reagents or supplies are only some of the drivers behind such decisions. Unlike private sector laboratories where the business considerations drive these decisions, public health laboratories face more complex dilemmas. Questions of test discontinuation are sometimes easily answered based on public health needs in the jurisdiction, but there are numerous other considerations to be accounted for including: disease prevalence, test availability from other sources, staff capacity, grant commitments, and legislative code requirements. Each jurisdiction is different, and the decision may differ by jurisdictional needs. To assist with the critical thinking that must accompany the decision, a workgroup was formed with representatives from Kentucky, Connecticut, Michigan, Delaware, New York, Iowa, and APHL to develop a tool kit and critical thinking guidance to assist laboratory management with these important decisions. The intent of the tool kit is to guide the user through the questions while presenting options to consider. Included in the document is an introductory narrative that discusses the different scenarios that might be faced by the laboratory in making the decision to delete a test from the test menu. This tool kit is intended to be a useful to make critical test deletion menu decisions and help to standardize the approach laboratory managers take to make these decisions.

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Year 1 and Year 2 Laboratory Flexible Funding Model (LFFM) Activities from the NYSAGM Food Laboratory

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The New York State Department of Agriculture (NYSAGM), Division of Food Laboratory was approved to conduct nine technical projects from the Microbiology (Human and Animal Food), Chemistry (Human and Animal Food), and Special Projects Tracks. This poster will provide a summary of the activities from different tracks. During Year 1, the laboratory tested 504 Microbiology samples (Human and Animal Food) and 422 Chemistry samples (Human and Animal Food). In year 2, the surveillance consisted of 384 Microbiology samples (Human Food) and 367 Chemistry samples (Human Food). Our participation in the Surveillance sampling tracks has increased the volume and diversity of the surveillance samples received by the laboratory. Additionally, communication with our State Regulatory Program (SRP) was improved due to the increased interaction necessary to accomplish LFFM special requirements. Operating a working laboratory during the COVID-19 pandemic proved challenging, especially considering the pandemic's impact on the global supply chain. Supply shortages and expanded pet food testing led the laboratory to update our qPCR protocols. Initial

issues caused a search for replacements, which improved our detection capabilities and the robustness of screening for *Listeria monocytogenes* and *Salmonella*. Another adaptation experienced by the laboratory was the virtual external ISO 17025 assessment in 2021. The laboratory also participated in a number of LFFM special projects. The Dead-End-Ultrafiltration (DEUF) project took much longer than expected due to COVID-19 pandemic-related delays impacting the delivery of reagents and instrumentation. The filter-based method allows for the testing of agricultural water, but the sampling and testing procedures' uniqueness made the laboratory pause the capability implementation. The SRP Produce Safety activities also influenced the laboratory's hesitation to participate in the Year 3 capacity development. Participation in the Multi-laboratory validation projects has allowed staff to cross-train in new methodologies and collaborative projects outside of regularly assigned tasks. The NYSAGM is engaged in using the LFFM activities to promote growth and improvements within our agency. The interaction with FDA also enabled open communication with NYSAGM (laboratory and SRP) on challenges not addressed routinely. We look forward to contributing to the identification of potential issues and providing recommendations for this multi-component cooperative agreement.

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SURVEILLANCE, OUTBREAKS AND EMERGING INFECTIONS

Antimicrobial Susceptibility Surveillance of Invasive *Candida* Species

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Invasive *Candida* infections have been increasing over the last two decades. These tend to affect blood, heart, brain, eyes and other parts of the body and are among the most common fungal infections in healthcare settings. Previously, *Candida albicans* was the major pathogen associated with candidiasis, but over the past few decades, many non-*albicans* species such as *C. auris* and *C. glabrata* have become more common. *C. auris* is of particular concern as, an emerging pathogen, it spreads rapidly, causes a wide spectrum of infections and, most alarmingly, it is rapidly becoming resistant to all the available antifungal agents. Thus, surveillance of fungal infections as well as the antibiotic sensitivity profile (AST) of these pathogens has become increasingly important. We report here a study of the AST profiles of >100 clinical *Candida* isolates from patients within the Washington DC hospitals between the years 2019 and 2022. All the isolates will be grown on agar plates, suspended in broth and subject to AST testing via the Sensititre system. We will use the YeastOne YO9AST plate with nine antifungals, including Amphotericin B and Micafungin. Preliminary studies indicate that majority of the strains isolated from patients with fungal infections are either *C. glabrata* or *C. auris*, with the latter being more common over the last two years than in 2019. Furthermore, strains of *C. glabrata* isolated over the four-year period show high resistance to fluconazole, micafungin and anidulafungin (to date, Clinical Laboratory Standards Institute does not have performance standards for *C. auris*). Through this study, we hope

to understand any trends in the resistance profile of these fungal pathogens over the last few years and identify the gaps within surveillance testing within the DC region. This will eventually help us to develop better programs to control *Candida* infections and decrease the rate of antifungal resistance.

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AWaRe Classification: Increasing use of Access Antibiotics as a Stewardship Tool. Assessing its Effectiveness in Decreasing Resistance Patterns – A Retrospective Study

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Background: Antibiotic resistance is contributing to the burden on healthcare infrastructure worldwide. As an antibiotic stewardship initiative to combat antibiotic resistance, the World Health Organization (WHO) introduced the Access, Watch, and Reserve (AWaRe) classification for antibiotics. This classification is based on the spectrum and potential of antibiotic classes to induce resistance. In 2021, WHO recommended increasing the use of the Access group of antibiotics and set a target for at least 60% of the total antibiotics consumed. The present study evaluates the effectiveness of this recommendation. **Methods:** Using the WHONET/Backlink software, antibiotic resistance trends of specimens submitted to the Washington State Public Health Laboratory (WA PHL) were analyzed for the past ten years. For each bacterial species, the antibiotic resistance rate was expressed as the percentage of resistant isolates to all isolates from the same specimen type. Individual submitters were contacted to provide data for antibiotics consumed by the healthcare facility. The trends of antibiotic consumption were analyzed using the WHO Anatomical Therapeutic Classification/ Defined Daily Dose (ATC/ DDD) methodology and then categorized according to their AWaRe group. **Results:** Both antibiotic resistance and consumption patterns will be matched to examine the possible effect of increasing Access antibiotic consumption on antibiotic resistance in a retrospective way. **Conclusion:** Special consideration for the WHO AWaRe classification when designing stewardship programs for antibiotic use would be recommended based on the outcome of this work.

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***Bordetella pertussis* Exhibits Genomic Diversity within Patients and Laboratory Culture**

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Whole genome sequencing (WGS) showed that although *Bordetella pertussis* (BP) exhibits little genetic variation, genomes from clinical isolates frequently differ in gene order through rearrangement between insertion sequences (ISs). To better understand rates of genome rearrangement and single nucleotide polymorphism (SNP) in BP, genomic diversity within patients was examined at select sites within the Enhanced Pertussis Surveillance/Emerging Infections Program Network (EPS). Between 2016 and 2020,

five US states submitted sets (2–7 isolates each) of BP isolates, following diagnostic culture confirmation, to the US Centers for Disease Control and Prevention for characterization by pulsed-field gel electrophoresis (PFGE), WGS and vaccine antigen production screening. Analysis of 128 sets revealed only rare SNP variation, while 26 sets included evidence of genomic variations such as rearrangement or duplication within a single patient specimen. We further investigated the frequency and stability of one type of observed genomic variation during laboratory culture. Five replicate cultures of one pair of isolates (J766, J767) selected from a set exhibiting duplication variation were maintained for eight serial passages on Regan-Lowe Charcoal Agar media. Genomic DNA was extracted from passages for shotgun sequencing on an Illumina MiSeq. WGS revealed the stable maintenance of gene duplication in culture, both for mutations present at passage 1 (J767) and for those becoming dominant at intermediate passages (J766). All five replicates of J766 developed the same duplicated genomic region at identical intermediate passages. In summary, these data suggest that BP can not only exhibit genomic diversity within a single patient specimen but can also acquire or accumulate genomic variations during serial laboratory passages. However, this study cannot rule out the possibility that the emerged variation was present within the patient or isolate at a very low frequency prior to the passage experiment. These findings underscore the importance of minimizing the laboratory manipulation of clinical isolates used for infectious disease surveillance during genomic characterization.

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Characterization of US Urogenital and Extragenital Meningococcal Isolates Collected through the Enhanced Gonococcal Isolate Surveillance Project

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Neisseria meningitidis (Nm) is a Gram-negative diplococcus that colonizes the nasopharynx and can cause invasive meningococcal disease (IMD), including bacterial meningitis and septicemia. Nm is closely related to *Neisseria gonorrhoeae*, a common cause of genitourinary infections, and has been infrequently reported to cause urethritis that is clinically indistinguishable from gonococcal urethritis. Some Nm urethritis-associated strains form a distinct phylogenetic clade known as “U.S. Nm nongroupable (NmNG) urethritis clade.” These isolates have acquired gonococcal genes (aniA and norB) that allow for improved survival in anaerobic environments. A possible mode of Nm urethritis transmission is hypothesized to be oral sex from persons colonized with typically commensal Nm strains in the upper respiratory tract. We characterized urogenital (urethral, endocervical) and extragenital (rectal, pharyngeal) meningococcal isolates collected from 12 sexually transmitted disease (STD) clinics participating in an enhanced Gonococcal Isolate Surveillance Project (eGISP). Suspected Nm isolates collected during 2017–2019 were tested by real-time PCR, slide agglutination and/or whole genome sequencing (WGS) to determine species, serogroups and genomic diversity. Isolates were cultured from urethral swabs (from symptomatic males), cervical swabs (from symptomatic females or females with suspected exposure to *N. gonorrhoeae*) and pharyngeal and rectal swabs from asymptomatic and symptomatic persons with

reported sexual exposures. Among 1,652 samples from suspected meningococcal cases, 1,593 (96.5%) bacterial isolates were recovered and 1,472 (92.4%) were confirmed positive for Nm. Of the 1,472 Nm isolates, the majority were pharyngeal (n=1342, 91.2%) followed by urethral (n=104, 7.1%), rectal (n=25, 1.7%) and endocervical (n=1, 0.06%). In total, 66.7% of isolates were NG of which 88.4% were pharyngeal, 9.5% urethral and 2.3% rectal. More than 60% of urethral isolates, 8% of rectal and 1.7% pharyngeal isolates were from the US NmNG urethritis clade. Seventy-one percent of urethral NmNG and 2.3% of pharyngeal NmNG isolates were clonal complex 11. A few individuals (n=81) were repeat carriers, defined as same Nm strain collected at the same anatomic site at different visits, 79/81 were from pharyngeal samples. Others (n=17) were mixed carriers, defined as having strains with different CCs (n=7) or same strain (n=10) collected at different anatomic sites at the same visit. Overall Nm was detected at both urogenital and extragenital anatomic sites. This systematic surveillance of meningococcal urethritis detected a mixture of CC11 urethritis clade isolates and non-CC11 isolates. Mixed anatomical site carriers carrying oral and genital isolates with the same genetic lineage supports the hypothesis of urogenital transmission and repeat oral carriers inform the possible duration of carriage.

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Comparison of the Accuracy of Antimicrobial Susceptibility Testing (AST) for Carbapenem-resistant Enterobacteriaceae using the Biomic V3

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Antimicrobial resistance is one of the leading public health concerns globally, occurring with the overuse and/or misuse of antibiotics. The group of carbapenem-resistant organisms (CRO), are one of the major contributors in the spread of resistance. CRO are strains of bacteria that are resistant to the antibiotic class, carbapenems, used to treat gram negative bacterial infections. CROs have become a global endemic and very hard to treat, often with outbreaks in healthcare settings causing high mortality rates. Many microbiology laboratories, both clinical and non-clinical, utilize antimicrobial susceptibility test (AST) to identify the antibiotics that are effective against the strain of bacteria as well as the minimum inhibitor concentration (MIC). AST analysis involves use of the Kirby-Bauer method to measure the zones of inhibition when bacteria is exposed to a specific antibiotic as well as use of the E-test to determine the MIC. Both the Kirby-Bauer and E-test are usually read manually by technologist. The Biomic V3 (Giles Scientific USA, Santa Barbara, CA) is an open system that utilizes digital imaging to automate the reading and CLSI/EUCAST interpretation of microbiology test and susceptibilities. Utilization of automated methods enable laboratories to standardize laboratory process with can ultimately improve laboratory efficiency and the quality of results. We evaluated the use of the Biomic V3 to determine the AST profiles for 50 Antimicrobial Resistance laboratory Network (ARLN) CRO isolates provided by the CDC. We compared manual reading of Kirby Bauer and E-test profiles with the automated Biomic reading of the zones of inhibition and Etest MICs. In this study, we demonstrate that

there was very minimal error between the two methodologies and the readings were comparable. Thus, use of automated readings for AST would be a valuable time saving method to for both, accuracy and standardization.

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Development of *Candida auris* Surveillance RT-PCR Test in a Prison Hospital Setting

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Since its detection in 2009 in Japan, *Candida auris* has emerged as an often multidrug-resistant fungal pathogen associated with global outbreaks. Most notably, invasive *C. auris* nosocomial infections can cause high-mortality rates in healthcare facilities. *C. auris* can persist on environmental surfaces and spread from asymptomatic colonized patients to immunocompromised patients who are more susceptible to infection. The US Centers for Disease Control and Prevention put forth guidelines to screen for *C. auris* within the high-risk patient population. Following this guidance, our facility implemented a systematic *C. auris* screening protocol for high risk patients in November 2021 using the routine fungal culture method. A breach in this protocol was discovered in July 2022, when a patient admitted to our healthcare system's adjoining acute-care prison hospital was identified with *C. auris* fungemia. Due to this discovery, mass screening of all patients within the prison hospital was conducted and additional patients were found to be colonized with *C. auris*. However, the Infection Control team found that the collection to results turnaround time (TAT) of *C. auris* surveillance cultures was extended, impacting patient care during the outbreak. To shorten the TAT and improve patient care, we developed and validated an RT-PCR laboratory-developed test (LDT) on the Hologic Open Access Fusion platform for the detection of *C. auris* DNA from environmental and patient surveillance specimens. During the assay validation, our LDT detected 9/9 *C. auris* culture-positive samples. Additionally, *C. auris* DNA was detected in 9/139 *C. auris* culture-negative samples, thus indicating that the RT-PCR test has increased sensitivity compared to the traditional culture method. In addition, implementing this LDT reduced the TAT from 72 to 24.8 hours. With the adoption of this assay, our infection control professionals have been able to identify and isolate *C. auris* colonized patients more efficiently. Additionally, rooms previously occupied by a *C. auris* -positive patient can be made available for a new patient sooner after successful decontamination. However, one critical limitation of this molecular technique is the inability to determine whether the detected *C. auris* DNA originates from live or dead yeast. Therefore, further cleaning and/or the reflex of *C. auris* culture is needed after a positive molecular test. With the collaboration of our laboratory and institutional Infection Control team, we have demonstrated that multidisciplinary efforts were effective in improving the TAT and sensitivity of the *C. auris* surveillance testing process within our healthcare system.

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Development of Real Time PCR Assays for Detecting Tick-borne Pathogens in Support of a Statewide Tick Surveillance Program

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According to the US Centers for Disease Control and Prevention (CDC), New Jersey has tallied ~10% of the country's case load of tick-borne disease (TBD) cases for the last 30 years. In 2019 there were 48,494 cases of five different TBD* recorded in the US by the CDC, while the New Jersey Department of Health (NJDOH) recorded 4,319 cases – again 10% of the country's burden of TBD. To address this public health problem, in 2021, NJDOH initiated a pilot tick surveillance program with funding from the CDC. The objectives were to: (1) enlist the expertise of the NJ county mosquito control agencies for tick surveillance, (2) training additional staff to supplement tick collections throughout the state and (3) develop assays at the state health lab for testing ticks for various pathogens. In NJ there are five species of tick that have the potential to transmit pathogens. They are: the lone star tick (*Amblyomma americanum*), the newly discovered Gulf Coast tick (*Amblyomma maculatum*), the American dog tick (*Dermacentor variabilis*), the Asian longhorned tick (*Heamaphysalis longicornis*) and the blacklegged tick (*Ixodes scapularis*). Each species of tick transmits different pathogens. Therefore we had to develop RT-PCR assays that would detect a pathogen or pathogens from each tick species. Here we describe the development of our real time polymerase chain reaction (RT-PCR) assays in support of a state-wide tick surveillance program.

*Lyme disease, Powassan virus infection, Ehrlichiosis, Babesiosis, Anaplasmosis and Spotted fever rickettsiosis.

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Distribution of Carbapenem-Resistance Mechanisms among Enterobacteriales, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii* in Texas, 2017-2022

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Introduction: Antimicrobial resistance genes, such as carbapenemase, can be located on mobile genetic elements, making for easy transfer between bacteria. Carbapenemases are grouped into three classes: class A (e.g., blaKPC), class B (e.g., blaIMP, blaNDM, blaVIM), and class D (e.g., blaOXA). As a member of the CDC Antimicrobial Resistance Lab Network (ARLN) since 2017, the Texas Department of State Health Services (DSHS) has performed antimicrobial resistance (AR) testing for carbapenem-resistant Enterobacteriales (CRE), carbapenem-resistant *Pseudomonas aeruginosa* (CRPA), and carbapenem-resistant *Acinetobacter baumannii* (CRAB). Here, we describe carbapenemase gene frequency and distribution in the eleven Texas public health regions from 2017 to 2022 in order to build robust public health measures. **Results:** Using RT-PCR assays, the DSHS Laboratory tested 4,924 CRE; 2,433 CRPA; and 2,308 CRAB specimens. Specifically, the CRE specimens harbored class A (24.9%), class B (4.6%), and class D (1.3%). We detected class A (1.1%) and class B (8.9%), but no class D (0%), in the CRPA specimens. Finally, the CRAB specimens were positive for class B (1.1%) and class D (67.4%), but not class A (0%). Interestingly, 0.36% of specimens were positive for dual resistance mechanisms. As expected, the

frequency of each class, and subclasses, varied by public health regions. Public health region 10, which includes El Paso, had the highest frequency of class A. Depending on the subclass, regions 1 (blaVIM), 7 (blaIMP), and 10 (blaNDM) have the highest frequencies of class B. These include major cities such as Lubbock, Austin, and El Paso, respectively. Likewise, the highest frequencies of class D depended on the subclass also. These included public health regions 3, 5, 10, and 11 for subclasses blaOXA-24/40-like, blaOXA-23-like, blaOXA-48-like, and blaOXA-58-like, respectively. Of note, subclass blaOXA-58-like was only detected in region 11.

Conclusion: Based on the samples collected in Texas between 2017 and 2022, there was a considerable geographic distribution in carbapenem resistance mechanisms. The detection of dual resistance mechanisms against carbapenem is of great concern for treatment and prevention of infection. Understanding the frequency distribution of these mechanisms helps with the development of public health containment strategies. Further, these data have contributed substantially towards detecting AR organisms.

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Dynamic Molecular Surveillance of SARS-CoV-2 at the Regional Innovative Public Health Laboratory in Chicago, IL

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The Regional Innovative Public Health Laboratory (RIPHL) is a public-academic partnership between Chicago Department of Public Health (CDPH) and Rush University. It was established in March 2021 to serve as the molecular epidemiology laboratory for CDPH, providing flexible molecular detection, characterization and genomic surveillance of pathogens of public health importance with the goal of timely and actionable data for public health response. Between April 3, 2021 and December 31, 2022, RIPHL received 11,557 SARS-CoV-2-positive remnant clinical respiratory specimens from 15 acute care hospitals in Chicago for molecular characterization. Of these, 8,886 (77%) specimens were successfully sequenced and yielded lineage assignments. Across the study period, RIPHL sequenced 3% of all incident SARS-CoV-2 infections reported to CDPH and accounted for 62% of Chicago SARS-CoV-2 sequences in GISAID. Demographically, individuals who provided specimens for sequencing were broadly representative of all SARS-CoV-2 infections reported to CDPH in terms of sex, age, race/ethnicity and ZIP code of residence. RIPHL has altered molecular methods in response to public health needs. When Omicron BA.1 was rapidly emerging, RIPHL developed and implemented a genotyping PCR to provide lineage proportion estimates at much faster turnaround compared to whole genome sequencing (WGS). TaqMan SARS-CoV-2 Mutation Panel Assays (ThermoFisher) targeting mutations K417N and L452R were used to distinguish BA.1 from the background of Delta variant. Genotyping PCR was able to call lineage in samples with low viral concentration more successfully than WGS, resulting in 1,657 (90.4%) lineage calls from genotyping PCR compared to 1,560 (85.1%) for WGS. Of 1,541 samples with both WGS and genotyping PCR data available, 1,539 (99.9%) concordant. Since late 2021, Chicago has experienced waves of successive Omicron sublineages. The relative speed with which sublineages gained dominance was used as a proxy for the level of public health concern posed by particular sublineages. Logistic growth rates of emerging sublineages were calculated weekly: low risk

variants had logistic growth rates 0.13. Only one sublineage had a log growth rate warranting a “high risk” designation (BA.1), 7 sublineages met medium risk criteria (BA.4, BA.5, BA.4.6, BA.2.75, BF.7, BQ.1, BQ.1.1). These metrics were considered alongside other epidemiological indicators to inform public health responses, like proof of vaccination requirements. The public-academic partnership at the heart of RIPHL has enabled innovative techniques to be quickly incorporated into public health responses, and continued innovation will be required to continue to respond to SARS-CoV-2 and other infectious threats.

Presenter: Hannah Barbian, hannah.j.barblian@rush.edu

Evolution of Echinocandin Resistance in Massive Southern Nevada Outbreak

L. Massic, D. Siao, A. Gorzalski, D. Hess, M. Pandori; Nevada State Public Health Laboratory

As of 2019, *Candida auris*, an inherently drug resistant fungus, is one of five pathogens under the US Center for Disease Control (CDC) antimicrobial resistance (AMR) urgent threat category. Since the identification of *C. auris* in 2009, the pathogen has caused ongoing outbreaks in healthcare settings in over thirty countries with a mortality rate ranging from 33-60% among the immunocompromised population. Echinocandins are the drug class of choice for treating *C. auris* candidemia. Resistance to echinocandins has been shown primarily to occur due to mutations in a “hotspot” region of the FKS1 gene. Other genomic sites have been implicated. Southern Nevada is currently experiencing the fastest-growing outbreak of *C. auris* to reach 100 cases. Eighteen months later, this outbreak has surpassed 1,000 cases, affecting over 30 health care facilities. The Nevada State Public Health Laboratory has aggressively sequenced the genomes of isolates associated with the outbreak, with over 800 isolates assessed by WGS. Analysis has shown this to be two genomically distinct simultaneous outbreaks, consisting of both Clade I and Clade III organism. This aggressive genomic surveillance has allowed a visualization of genomic changes over time, including a detailed assessment of mutations associated with minimum inhibitory concentrations (MIC) to echinocandins. Herein, we show data assessing potential correlations of specific mutations in the FKS1 gene with susceptibility (MIC) to echinocandins. In at least two instances, we visualize genomically and phenotypically, the evolution of resistance within individual patients, during treatment. These data demonstrate that genomic data applied to outbreak surveillance can provide not only phylogenetic intelligence, but also highly informative data with regard to antibiotic stewardship and susceptibility.

Presenter: Lauryn Massic, lmassic@unr.edu

Genomic Epidemiological Characterization of SARS-CoV-2 Reinfection Cases, Colorado August 2020 – February 2022

M. Hetherington-Rauth, A. Bell, A. Rossheim, L. Bankers and S. Matzinger; Colorado Department of Public Health and Environment

Background: Despite global SARS-CoV-2 whole genome sequencing (WGS) efforts, few studies investigating SARS-CoV-2 reinfection cases have used WGS to characterize both primary and subsequent infections. The paucity of studies is likely due to two challenges: 1) the viral load associated with reinfection cases is often lower

relative to primary infections, which results in poor sequence quality or a complete inability to sequence the reinfection specimen; and 2) only one specimen from either the primary infection or reinfection is available for sequencing but not both (i.e., paired viral specimens are not available), making a paired whole genome sequence comparison not possible. **Methods:** We investigated 27,985 SARS-CoV-2 reinfection cases occurring in Colorado from August 2020 to February 2022 that were reported to the Colorado Department of Public Health and Environment (CDPHE). We defined a reinfection case as a positive antigen or RT-PCR diagnostic test occurring at least 90 days after the primary infection. We collected descriptive data on age, gender, vaccination status at time of reinfection and whether the patient showed symptoms, was hospitalized, resided in a congregate living setting or whose case could be traced to an outbreak. We also performed whole genome sequencing on all specimens available at the State Laboratory (n = 11,141 specimens), resulting in high quality (>90% of the genome represented) paired consensus sequences for 344 cases. We performed lineage characterization on all sequences and calculated the pairwise single nucleotide variant (SNV) difference between each pair of sequences. **Results:** Among the 344 cases with paired sequencing data, the average number of days between infections was 368 (range: 93–635 days). The majority of reinfection cases were caused by the Omicron variant (n = 304 cases, 88.4%), while the majority of primary infections were caused by non-variants of concern (non-VOCs) (n = 303 cases, 88.1%). The average number of pairwise SNV between primary and reinfection sequences was 59.9 (range: 0–84). The highest number of average pairwise SNVs occurred in the S gene (23.2), followed by the ORF1ab region (19.9) and the N gene (5.68). **Significance:** With 344 paired primary and reinfection sequences, this study represents the largest genomic dataset of SARS-CoV-2 reinfections to date. Genomic epi characterization of SARS-CoV-2 reinfection cases can add real world data to the understanding of SARS-CoV-2 viral evolution and immune evasion. Additional studies utilizing this dataset to look at specific mutations and their prevalence compared to vaccine breakthrough-only cases may help to better understand both waning immunity and vaccine breakthrough as a result of SARS-CoV-2 infection or currently available vaccines.

Presenter: Molly Hetherington-Rauth, molly.hetheringtonrauth@state.co.us

Genomic Epidemiological Investigation of a Multi-year Carbapenem-resistant *Pseudomonas aeruginosa* (CRPA) Outbreak at a Colorado Healthcare Facility

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Background: Carbapenem-resistant *Pseudomonas aeruginosa* (CRPA) is a serious antibiotic resistance threat to public health. We used epidemiological and genomic methods to investigate CRPA patient samples from a bone marrow transplant (BMT) unit spanning the years of 2019 to 2022 to investigate whether these infections constitute one or multiple outbreak clusters. **Methods:** Our investigation focused on facility-wide and unit-specific case counts to initially identify 24 cases from 2019 to 2021. Medical record reviews combined with two on-site infection control assessments were conducted to determine epidemiological links. For our genomics investigation, we identified all CRPA samples that

had been submitted by the facility to the CDPHE laboratory through routine surveillance and successfully performed whole genome sequencing on 32 samples spanning all four years (eight samples from 2019, four from 2020, 16 from 2021, four from 2022). Using the WGS data, we generated de novo assemblies for each sample and performed WGS-based serotyping, multi-locus sequence typing (MLST) and phylogenetic analyses. **Results:** Cases were median age 61.8 (range 41-76), 48% female, and 96% either had a hematologic malignancy and/or underwent BMT. All cases were housed on the same two units. On-site observations were notable for the close proximity of sinks to clean supplies and medication preparation areas. WGS-based serotyping analysis demonstrated that all 32 samples from the facility were the same serotype (O5). Twenty-seven of the 32 samples had the same MLST (ST235) and were highly related with a mean pairwise distance of 0.00011 (99.989% sequence identity among the ST235 samples). To determine whether the facility samples cluster together phylogenetically, which would be suggestive of a single outbreak, we compared the facility samples to 23 publicly available serotype O5 CRPA genomes (5 ST235) that were not epidemiologically associated with the facility. This analysis shows that while the 27 ST235 facility samples share a common ancestor with the five publicly available ST235 sequences (mean pairwise distance of 0.00021, 99.979% sequence identity among all ST235s), they form a distinct cluster and are more closely related to each other than to any other sample analyzed by WGS. **Conclusions:** Supported by epidemiological evidence, our WGS analyses confirmed that these samples represent a single CRPA outbreak spanning multiple years in an immunosuppressed patient population. The prolonged nature of the outbreak, the ability of CRPA to form biofilms within plumbing systems, and findings from infection control assessments suggested that the source of infections was possibly an environmental reservoir. In conclusion, by integrating genomic and epidemiological data, we were better able to understand and inform hypotheses regarding transmission of an important pathogen of public health concern.

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Human Adenovirus Surveillance and the Impact of the COVID-19 Pandemic on Participation – United States, January 2017–August 2022

F. Abdirizak, A. Winn, H. Scobie, X. Lu, E. Vega, O. Almendares, H. Kirking, B. Silk; US Centers for Disease Control and Prevention

More than 100 human adenovirus (HAdV) genotypes exist, and their clinical and epidemiologic characteristics vary. Many HAdVs are commonly associated with respiratory infections and outbreaks of respiratory illness. The National Respiratory and Enteric Virus Surveillance System (NREVSS) collects untyped weekly aggregate tests and detections of HAdVs. The National Adenovirus Type Reporting System (NATRS) collects HAdV typing results accompanied by clinical and demographic data. We describe trends in HAdV data reported to NREVSS (tests, detections, percent positivity) and NATRS (detections, types) from January 2017 through August 2022, including seasonality, type circulation and the impact of the COVID-19 pandemic on surveillance reporting and HAdV epidemiology. From January 2017–August 2022, 3,558,316 respiratory tests were reported to NREVSS; 113,218 (3.1%) were HAdV positive (annual range: 2.1%–3.9%). From 2017–2019, the annual number of respiratory HAdV detections and percent positivity remained relatively consistent in NREVSS (range: 14,720–21,005;

2.1% to 3.9%). Additionally, the annual number of respiratory HAdV detections and percent positivity reported from 2021–2022 (ranged: 20,775–24,393; 3.0–3.9%) were comparable to previous years, however, there was a statistically significant decrease in detections and percent positivity in 2020 ($n=11,702$; 2.1%) ($p \leq 0.01$). Weekly HAdV positivity peaked bi-annually in the summer (May–July) and winter (November–January) from 2017–2019 and 2022, but seasonality was disrupted by the COVID-19 pandemic during 2020 and early 2021. Weekly HAdV positivity did not vary by region from 2017–2019; but HAdV positivity was higher in the South and Midwest from 2020–2022. In NATRS, the annual number of HAdV typing results reported increased from 389 (2017) to 547 (2019). During the COVID-19 pandemic, annual number of results in NATRS declined to 58 (2020), 101 (2021) and 148 (2022). From 2017–2019, the most common HAdV types reported were types 4 (22.9%), 3 (22.0%) and 7 (17.3%). Between 2020–2021, HAdV types 2 (38.2%) and 1 (25.5%) were most common. In 2022, an increase was noted for type 41 (21.6%), which was likely attributed to a national investigation of children with acute hepatitis of unknown etiology. Types 4 (19.6%), 2 (17.6%) and 5 (17.6%) were also frequently reported in 2022. HAdVs circulation continued during the COVID-19 pandemic, with observed decreases in 2020 and impacts to seasonality. Reporting of HAdV types to NATRS decreased during the pandemic. Expanded HAdV typing and reporting could allow for better understanding of factors associated with seasonal variation, geographic patterns and clinical syndromes of this respiratory virus; understanding these factors could inform the development of control measures, diagnostics tools, antivirals and vaccines.

Presenter: Fatima Abdirizak, fabdirizak@gmail.com

Implementing FungiNet in the Antimicrobial Resistance Laboratory Network

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Introduction: Pathogen genomic sequencing is a powerful tool that has transformed the public health landscape—providing the resolution to enhance investigation of disease clusters and outbreaks, characterize transmission dynamics, identify introductions of new strains and monitor concerning strains. With fungal diseases becoming a growing concern, there is a need to establish a community of practice that bolsters fungal sequencing capacity and accelerates data generation for public health action. Since 2021, the US Centers for Disease Control and Prevention (CDC) has been working with domestic and global partners to stand up FungiNet, a network that aims to lift global capacity for conducting genomic surveillance and genomic epidemiology of fungal diseases and to equip the public health workforce with tools and resources to rapidly generate, analyze, share and act on genomic data. Domestically, FungiNet is being launched through the Antimicrobial Resistance Laboratory Network (AR Lab Network). Here, we highlight the process for implementing FungiNet in the AR Lab Network, with early stages focusing on *Candida auris*. **Methods:** First, a pilot was launched in collaboration with CDC, the Association of Public Health Laboratories (APHL) and two AR Lab Network regional laboratories to test and establish approaches for *C. auris* whole genome sequencing (WGS), which later informed broader guidance and standard operating procedures. Second, MycoSNP,

a CDC-developed bioinformatics workflow for fungal pathogens, was made open-source and adapted to Nextflow software. Third, a Research Electronic Data Capture (REDCap) database was established for reporting genomic-related data to CDC. Fourth, a series of monthly workshops were organized to support the implementation of laboratory, bioinformatics and data sharing processes to both CDC and National Center for Biotechnology Information (NCBI) database. Finally, CDC conducted analyses in parallel with partners to ensure comparability of sequencing results. **Results and Conclusions:** Today, 19 AR Lab Network public health partners have committed to building *C. auris* sequencing capacity through FungiNet activities. As a part of these efforts, participating public health laboratories have generated and made publicly available sequence data for more than 900 *C. auris* isolates. Further expanding this capacity will strengthen preparedness and response to *C. auris* and other emerging fungal threats.

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Implementing Measles Testing at the New Jersey Public Health Molecular Virology Lab

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Measles virus (MeV) is a morbillivirus which is the causative agent of measles, a highly contagious respiratory virus that causes serious illness in children. Although the United States has maintained elimination status of the measles virus since 2000, meaning there are no longer endemic cases of measles in the US, cases can still be imported by people traveling outside of the country and cause an outbreak. The World Health Organization (WHO) listed measles as an imminent threat to public health in 2022 due to measles outbreaks and cases worldwide and growing vaccine hesitancy. It is important that public health labs and hospitals can rapidly and reliably test suspected cases of measles. Currently the New Jersey Public Health and Environmental Laboratories (NJ PHEL) does not offer confirmatory testing via RT-PCR in-house, and suspect cases must be sent to the Wadsworth Center in New York for confirmation, creating a turn-around time of several days at best. With the recent increase in vaccine hesitancy in the United States it is important that the state of New Jersey is prepared to rapidly and accurately test measles cases in the event of an outbreak. NJ is validating and implementing a series of singleplex real time RT-PCR assays using the CDC method and automated Qiagen EZ1 extraction platform that is able to identify MeV in a clinical sample and differentiate between wild type measles (MeWT) and vaccine-associated measles (MeVA). This diagnostic test will allow NJ PHEL to confirm a measles diagnosis in suspect cases and differentiate between the two strains of the virus during an outbreak. This poster will detail the accuracy, precision and limit of detection demonstrated in the assay in New Jersey. To prepare for the constant threat of measles outbreaks in the United States, NJ PHEL will be able to have a rapid, robust and repeatable clinical assay for suspected measles samples with a quick turnaround time to produce results.

Presenter: Claire Bilyeu, cbilyeu2018@gmail.com

Insights on the Role of the Conserved Mycobacterial Transcription Factor WhiB6 in Regulating ESX Secretion Systems

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WhiB6 is a conserved transcription factor in mycobacteria. In *Mycobacterium marinum*, WhiB6 regulates the expression of a virulence-associated secretion apparatus called ESX-1, but little is known of its role in other mycobacteria and its mechanism of action. We have begun to characterize WhiB6 in the model mycobacterium, *Mycobacterium smegmatis*. In particular, we are interested in understanding its function in the ESX-1-dependent process of distributive conjugal transfer (DCT), a unique form of horizontal gene transfer that occurs between genetically distinct donor and recipient strains of *M. smegmatis*. Our lab has previously shown that a second secretion system, ESX-4, is also required for DCT and that ESX-4 is directly activated on donor-recipient contact by the extracytoplasmic transcription factor SigM in the recipient. We now find that recipients carrying a deletion of the whiB6 gene fail to induce ESX-4 upon donor contact, thus preventing conjugation and indicating that WhiB6 regulates both ESX-1 and ESX-4. We hypothesize a DCT signaling network model wherein WhiB6 connects ESX-1 with ESX-4 through SigM. This poster will explore the effects of WhiB6 on DCT and its role in intercellular communication in mycobacterial conjugation.

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Laboratory Response to the Emergence of Mpox Virus in North Carolina

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Mpox is a zoonotic disease recently characterized by fever, swollen lymph nodes, and an itchy or painful rash that typically is located near the genitals, or on the hands, feet, chest, face, or mouth of patients infected with mpox virus. This virus is a member of the orthopoxvirus genus and can be detected in dry swab specimens collected from skin lesions using a non-variola orthopoxvirus (NVO) Real Time PCR assay that was distributed to members of the Laboratory Response Network. This assay was previously verified by the North Carolina State Laboratory of Public Health Laboratory (NCSLPH) to ensure preparedness for the emergence of orthopoxviruses in North Carolina and was rapidly deployed to detect mpox virus in North Carolina citizens. As part of the emergency response, healthcare providers were instructed to collect lesion material from 2 or more locations using 2 dry swabs simultaneously to scrub each selected lesion from patients meeting the established or revised (as the outbreak progressed) case definition for mpox. On June 22, 2022, the NCSLPH documented the first confirmed mpox case in NC. This finding was consistent with an unprecedented number of mpox cases and clusters that were reported globally in non-endemic regions. Clinical submissions for mpox testing included duplicate dry swabs from one to six anatomical locations per patient. The site of specimen collection was recorded for ~93% of specimens tested. Specimens collected

from locations near the genitals had the highest positivity rate (~41%), followed by hands (~38%), face (~33%), mouth (~32%), chest/abdomen (~23%), and feet (~20%). Specimens from all other sources, including those that were unspecified, had a positivity rate of ~24%. When 2 or more lesions from the same individual were tested, 97% of individuals had consistent results for all collections, either detecting (33%) or not detecting (67%) NVO DNA. Discrepant results with multiple collections, some having NVO DNA and others not having NVO DNA were seen in 3% of individuals. These individuals were considered to be positive for mpox virus. From May through December of 2022, the NCSLPH tested 841 dry swab specimens derived from 450 patients to detect NVO DNA in 142 individuals (32% positivity rate). Forty-two duplicate specimens derived from infected individuals were sent to the CDC for further characterization and all 42 were confirmed to be the West African clade (Clade II) of mpox virus. Overall, specimens collected from locations near the genitals were more likely to be NVO positive, while specimens from the feet were least likely to be positive. These data suggest that collection from near genital sites would increase the likelihood of mpox virus detection. In addition, our data supports that collection from two anatomical sites, with one being located near the genitals would have detected all cases that were submitted for testing.

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Machine Learning-based Typing of *Staphylococcus* species by the Fourier-Transform Infrared (FT-IR) Spectroscopy with IR Biotyper for Clinical Surveillance

T. Stephens, B. Adams, H. Naikare; Tifton Veterinary Diagnostic & Investigational Laboratory

Staphylococci are a group of microorganisms occurring widely in nature and commonly found on the skin, mouth, nose, and gastrointestinal tract of dogs. The *Staphylococcus* genus includes over 30 species correlated with both animal and human pathogens. Typing of these bacterial pathogens in clinical hospitals relies on traditional methods that are expensive, time-consuming, and often limited to referral laboratories. IR Biotyper (Bruker Daltonik, Germany) is a new alternative physico-chemical method based on measurement of vibration of a molecule excited by IR radiation at a specific wavelength range. The IR Biotyper utilizes FTIR spectroscopy to discriminate bacterial isolates at different taxonomic levels (genus, species, serogroup/type, and strain level) by analyzing their total composition (proteins, fatty acids, carbohydrates, nucleic acids and lipopolysaccharides) on the cellular surface of the microbial cells. In this study, we evaluated the ability of the FTIR spectroscopy to discriminate amongst *Staphylococci* isolates derived from canine skin samples. With the IR Biotyper, strain typing and data analyses could be accomplished in less than three hours. The use of this technique by clinical microbiology laboratories could help to assist in rapid source tracking, clinical surveillance and microbial surveillance tracking.

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Maximizing Efficiency in Arboviral Mosquito Surveillance Through Multiplex Polymerase Chain Reaction and Sample Pooling

D. Bolton, R. Lovell; New Hampshire Public Health Laboratories

Introduction: Jamestown Canyon virus (JCV) is spread to people through the bite of an infected mosquito, and is an emerging disease in New Hampshire (NH). Nineteen human cases have been detected in NH since 2013. The New Hampshire Public Health Laboratories (NH PHL) recognized the importance of adding JCV to the existing mosquito surveillance program, but needed to work within the constraints of limited funding for reagents and personnel. One of the biggest challenges presented by the COVID-19 pandemic has been allocating resources for testing other than SARS-CoV-2. Historically, the mosquito surveillance program in NH included testing mosquito batches for Eastern Equine Encephalitis virus (EEE) and West Nile virus (WNV) by duplex PCR. The NH PHL sought to optimize a multiplex PCR to include all three targets: EEE, JCV, and WNV. Additionally, a pooling strategy was utilized to maximize efficiency in testing for the extraction and PCR steps of the process. **Method(s):** The NH PHL performs real-time reverse transcriptase polymerase chain reaction (rRT-PCR) for mosquito surveillance using the CDC developed TaqMan method. Mosquito batches are processed using a Retsch Mixer Mill; nucleic acid is extracted using the KingFisher Flex instrument; and PCR testing is performed using the QuantaBio qScript™ XLT One-Step RT-qPCR ToughMix®, Low ROX™ enzyme kit on the ABI 7500 Fast (or Fast DX) instruments. PCR primer/probe concentrations were optimized for the three targets in the multiplex. Next, verification testing was performed to determine accuracy and precision, using a panel of RNA samples. Once the verification work was complete, the NH PHL established a pooling strategy that allowed for an increase in testing capacity from 93 mosquito batches to 372 batches per PCR plate. **Results:** The NH PHL's verification demonstrated 100% accuracy for the multiplex PCR. Accuracy was determined by whether the expected results (detected or not detected) were obtained with all targets. Evaluation of precision data showed the average absolute difference in cycle threshold (Ct) values for each target was less than one when comparing the multiplex with previous methods. Pooling did not significantly affect sensitivity in detecting EEE, JCV, and WNV. **Discussion:** Verification of a multiplex PCR and pooling maximized efficiency and enabled the NH PHL to enhance mosquito surveillance with limited resources. The results of this work helped to establish routine testing for JCV without significant additional cost or time.

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Method Optimization and Validation of Per- and Polyfluoroalkyl Substances in Human Serum Using On-line Solid Phase Extraction Ultra Pressure Liquid Chromatograph Tandem Mass Spectroscopy

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Per- and polyfluoroalkyl Substances (PFAS) are a large group of manufactured chemicals that are persistent and potentially hazardous. PFAS is tested in human serum with high performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS), typically after solid phase extraction (SPE). Although it can

be challenging, resource intense and time consuming, SPE is deemed necessary in this instance. In our lab, SPE is performed online system. A major limitation of this approach is that sample preparation occurs sequentially before sample injection, therefore limiting throughput. The main goal of this project is to improve the sample throughput of the SPE system by offline automation, where samples are prepared concurrently using a 96 well plate format using a Janus automated liquid handler. The proposed off-line SPE has been successfully deployed by others on various platforms. Sample pre-treatment was deemed the most crucial in eliminating analyte recoveries. The SPE will be conducted on weak anion extraction (WAX) stationary phase. The automated procedure includes sample pre-treatment: Combine 50 μ l, 150 μ l Acetonitrile and internal standard, load the supernatant to the SPE plate after conditioning and washing before sample elution. The new automated offline SPE sample preparation method is expected to enable the lab to prepare PFAS samples from 48 aliquot of 50 μ l human serum in less than two hours for analysis of the 18 PFAS. Meanwhile, the accuracy of the further LC-MS/MS analysis must be within $\pm 30\%$ of nominal value or 3 times of STD value.

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Next Generation Sequencing at Texas Department of State Health Services

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The Texas DSHS Laboratory has been successfully performing next generation sequencing (NGS) since 2015. DSHS routinely sequences clinical and non-clinical enteric bacterial pathogens as part of the US Centers for Disease Control and Prevention (CDC) and the US Food and Drug Administration (FDA) PulseNet and GenomeTrakr programs to track outbreaks related to human disease, in addition to other microorganisms including *Cyclospora* and SARS-CoV-2. DSHS uses Illumina DNA Prep kits and Illumina sequencing platforms for performing Whole Genome Sequencing (WGS) for bacterial pathogens and amplicon-based sequencing for *Cyclospora* and SARS-CoV-2 samples. DSHS has been using BioNumerics 7.6 for enteric bacterial WGS data analysis and in-house Secre pipeline to analyze SARS-CoV-2 sequence data. As of January 2023, 18,587 clinical foodborne bacterial pathogens including 15,252 *Salmonella*, 2,477 *STEC E. coli*, 249 *Listeria*, 137 *Vibrio*, 234 *Shigella*, and 238 *Campylobacter* isolates have been sequenced. A total of 2,450 non-clinical environmental and food isolates were sequenced as well, including 1,222 *Salmonella*, 606 *STEC E. coli*, 241 *Listeria*, and 381 *Campylobacter*. DSHS also sequenced 750 *Cyclospora* and 3,583 *Neisseria gonorrhoeae* samples. Since June 2020, DSHS sequenced 18,520 clinical SARS-CoV-2 samples for variant identification and tracking. DSHS also participated in an FDA SARS-CoV-2 wastewater sequencing pilot project in 2022 and are continuing to sequence wastewater from nursing homes and correctional facilities. Two new NGS tests, WGS for Mpox and carbapenem-resistant bacteria, were implemented in December 2022. SHS is in the process of implementing WGS for *Mycobacterium tuberculosis* and *Candida auris* in 2023, and newborn screening (NBS) in 2024. In order to act as a sequencing core laboratory for the state, DSHS is expanding testing capacity with automation and robust sequencers to meet that need. NGS for

infectious diseases are critical for our epidemiologists to investigate outbreaks, ensuring precise patient care and improving the public health and well-being of Texans.

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NIH/RADx Collaboration for Pandemic Surveillance using the TaqMan SARS-CoV-2 Mutation Research Panel

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Surveillance testing demonstrated its importance in driving public health decisions during the COVID-19 pandemic. Whole genome sequencing (WGS) served as the primary method for surveillance to monitor spread of known variants and detect new variants. However, WGS is expensive and can take weeks before sequence data is available in public repositories. We describe an alternate, low-cost genotyping-based approach that enables broad-based surveillance and identifies potential new variants using the TaqMan SARS-CoV-2 Mutation Research Panel. As part of the NIH Rapid Acceleration of Diagnostics (RADx) initiative, we developed methodology for selecting a small number of highly specific mutations for variant identification. Genotyping results from a panel of these mutations were used to identify variants associated with each positive sample at a pace roughly 10 days earlier than sequencing when put in practice. Variant identification by genotyping was compared with sequencing to verify accuracy. Furthermore, we demonstrate that these panels are capable of detecting SARS-CoV-2 variant co-infection and recombination that are frequently assigned by sequencing to incorrect subtypes, and we show that these panels can be used to analyze low-input mixed samples at levels found in wastewater.

Presenter: Kathleen Hayashibara, kathleen.hayashibara@thermofisher.com

Onsite SARS-CoV-2 Sequencing Procedures Following Ongoing Workplace CLIA Surveillance

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Viral pathogen transmission within workplace environments has negatively impacted employee health and business continuity throughout modern history. The SARS-CoV-2 pandemic is an extreme example of this issue. Common workplace responses to the SARS-CoV-2 pandemic have been to promote social distancing, mask usage, and vaccinations. However, the efficacy of these safety precautions within a work environment are rarely confirmed through viral-sequencing-based contact tracing. Here we report on a proactive workplace SARS-CoV-2 surveillance system established at a private biotech company in MA with 500-1,000 local employees and contractors (New England Biolabs, NEB). NEB's viral surveillance strategy tracks SARS-CoV-2 positivity rates amongst the employee population through RT-LAMP based testing and then identifies variant genomes present through targeted sequencing. Central to this viral surveillance strategy was the establishment of an on-site CLIA certified lab to perform rapid-turnaround testing of

saliva samples for SARS-CoV-2 via a direct RT-LAMP assay one to three days-a-week. In addition to monitoring the presence/absence of SARS-CoV-2 across the employee population, NEB participates in a non-CLIA variant identification program. On a weekly or bi-weekly basis, RNA from all positive samples is extracted and sequenced. The sequencing approach here, derived from the ARTIC network protocol, utilizes NEB reagents and variant avoidant VarSkip Short primers. This approach is compatible with multiple sequencing platforms, which provides additional sample processing flexibility based on how quickly the sequencing results are needed. Patient results are reported to state health labs as required, and sequence data is anonymized before submission to NCBI SRA, NCBI Genbank, and GISAID. Furthermore, the testing and sequencing data are used to inform workplace safety measures and continually monitor for potential work-place transmission events. Ultimately, workplace SARS-CoV-2 viral surveillance has the dual benefit of promoting employee safety and minimizing disruptions to business operations. Current and future efforts for workplace viral surveillance efforts can also be expanded to include additional respiratory viruses, such as influenza and RSV.

Presenter: Betsy Young, eyoung@neb.com

Outbreak of Carbapenem-producing *Pseudomonas aeruginosa* at an Ophthalmology Clinic, Los Angeles County

S. Bharula, K. Yong, J. Mephors, A. Clarke, J. Garrigues, P. Hemarajata, Z. Rubin, N. Green; Los Angeles County Public Health Laboratory

In May 2022, our department identified transmission of VIM+ *Pseudomonas aeruginosa* in an ophthalmology setting from Public Health reporting of resistant *Pseudomonas*. Four patients were involved. All were hospitalized and either had lost sight permanently or suffered severely diminished vision in the infected eye(s). Investigation of the four cases found that all patients were seen at the same ophthalmology clinic. The clinic performed exams using tonometers with reusable tips and provided non-prescription dry eye drops to patients. A retrospective review of reported VIM+ *Pseudomonas aeruginosa* from clinical laboratories and hospitals from our jurisdiction did not identify additional cases. Isolates from corneal scrapings were sent to the public health laboratory for whole genome sequencing. Genomic analysis of the 4 VIM+ *Pseudomonas* isolates showed that all shared the same MLST (type 1203), had predicted serogroup O2, and harbored the blaVIM-80 and blaGES-9 combination of carbapenem resistance genes. There were only 1-6 SNP differences among the patient *Pseudomonas* isolates suggesting relatedness. In addition to carbapenem resistance, genes were identified conferring resistance to other antibiotic classes including aminoglycosides, cephalosporins, macrolides, chloroamphenicol, fosfomycin, tetracyclines, and sulfonamides. A site visit with environmental sampling by Public Health was conducted at the ophthalmology clinic. Environmental microbiology testing at CDC revealed the presence of *P. aeruginosa* in a bathroom sink sample and in samples obtained from a tonometer cleaning system. Isolate sequencing in our laboratory showed that none of the three recovered environmental *Pseudomonas* isolates genetically matched patient isolates. Although infection control gaps were identified at the clinic including improper disinfection of the tonometer cleaning system, no exact mechanism of transmission was identified. In January 2023, an Epi-X alert was issued. CDC is

now investigating this as a multi-state outbreak now involving more than 50 patients in 11 states for VIM+ *P. aeruginosa* with the same MLST type, serotype, and unique resistance genes combination as initially identified in our laboratory. Artificial tear eye drops are being investigated as possible common source of infection.

Presenter: Nicole Green, nicgreen@ph.lacounty.gov

Potential Transmission Dynamics of blaKPC in an Ongoing Outbreak Response Involving Multiple Bacterial Genera

J. Dale, B. Craft, L. Tournet, S. O'Malley, A. Beaudoin, P. Snippes; Minnesota Department of Health

Increased antimicrobial use, insufficient infection prevention and control, and larger than normal burdens on the healthcare system during the COVID-19 pandemic contributed to significant increases in antimicrobial resistant (AR) organisms. Surveillance to detect AR threats declined early in the pandemic amid focus on the COVID-19 response, but both reactive and proactive surveillance efforts are again increasing. The Minnesota Department of Health (MDH) initiated point prevalence surveys (PPS) in March 2022 at long-term care facility A (LTCF-A) upon identification of a clinical *Klebsiella oxytoca* isolate harboring the carbapenemase gene blaKPC. During March–December 2022, a total of 160 residents were screened in multiple rounds of colonization PPS and 13 tested positive for blaKPC using the GeneXpert Cepheid Carba-R assay. Culture-based methods successfully isolated a total of 15 organisms harboring blaKPC from 10/13 residents; 2 additional organisms were obtained from clinical cultures. The predominant blaKPC-positive species identified was *K. oxytoca* (7). Six additional species of bacteria harboring blaKPC were detected including *Escherichia coli* (2), *Enterobacter cloacae* complex (1), *Citrobacter amalonaticus* complex (1), *Citrobacter freundii* complex (1), *Klebsiella pneumoniae* (2), and *Raoultella ornithinolytica* (1). Whole genome sequencing results suggest three separate introductions of *K. oxytoca* based on single nucleotide polymorphism (SNP) analysis demonstrating two distinct clusters (Cluster 1: 14-27 SNPs; Cluster 2: 2-5 SNPs) separated by approximately four months based on specimen collection dates. The 2 *E. coli* were isolated from different residents approximately 3 months apart and are 30 SNPs different, suggesting relatedness. Two residents had multiple blaKPC organisms isolated from a single rectal swab; *K. oxytoca* and *E. cloacae* complex from patient 1, and *K. oxytoca*, *C. freundii* complex, and *R. ornithinolytica* from patient 2. All 17 organisms harbored the blaKPC-4 gene variant, but short-read sequencing is insufficient to determine whether a common extrachromosomal element harboring blaKPC exists across multiple bacterial genera. It is therefore unclear whether the 6 non-*K. oxytoca* organisms were introduced into the facility harboring the carbapenemase gene or if they acquired blaKPC-4 through horizontal gene transfer (HGT) in LTCF-A. Long-read sequencing methods and simplified bioinformatics workflows would inform elucidation of the transmission dynamics within patients 1 and 2, and in LTCF-A. Understanding the impact of HGT in outbreak scenarios would aid epidemiological response and prevention. The aim of future work at the MDH Public Health Laboratory is to develop sequencing and bioinformatics workflows that enable predictive assessment of HGT events to aid in outbreak response.

Presenter: Jennifer Dale, jennifer.dale@state.mn.us

Prediction and Characterization of *Proteae* Tribe Isolates Carrying Class D carbapenemase genes – Antimicrobial Resistance Laboratory Network, 2017-2021

G. Orazi, A.G. Kent, K. Berhe, C. Longa, N. Wilmott, A. Bhatnagar, A. Laufer, A. C. Brown and S. Sabour, US Centers for Disease Control and Prevention

Through their ability to hydrolyze carbapenems, Ambler class D beta-lactamases endanger the clinical efficacy of these antibiotics. blaOXA-23-like, blaOXA-24/40-like, and blaOXA-58-like are predominantly found in *Acinetobacter* spp.; however, these genes have also been reported in Enterobacterales. At least ten reports from outside the United States describe their detection in *Proteus mirabilis*. These isolates were susceptible to carbapenems and third generation cephalosporins, but resistant to penicillins. Using this unique antimicrobial susceptibility testing (AST) profile, we investigated whether *Proteae* tribe (*Proteus*, *Providencia*, and *Morganella*) isolates tested in CDC's Antimicrobial Resistance Laboratory Network harbored these *Acinetobacter*-associated class D genes. We also describe the frequency of Enterobacterales-associated blaOXA-48-like among *Proteae* tribe isolates using PCR testing data. We analyzed AST and PCR data on 4,943 *Proteae* tribe isolates collected through the Network from 2017–2021. Whole-genome sequencing (WGS) samples from over 10,000 Enterobacterales isolates collected through surveillance, outbreak, reference, and the Network were analyzed using CDC's in-house bioinformatics pipeline (QuAISAR-H). All data were generated by public health laboratories or CDC. AST profiles of individual *P. mirabilis* isolates carrying blaOXA-23-like or blaOXA-58-like and current Clinical and Laboratory Standards Institute M100 breakpoints were used to identify isolates in CDC's collection that met the above AST criteria. Among 4,943 *Proteae* tribe isolates, seven (three *P. mirabilis*, three *P. stuartii*, and one *P. rettgeri*) were PCR positive for blaOXA-48-like. WGS data were available for one of these isolates, a *P. mirabilis* found to carry the blaOXA-48-like variant, blaOXA-181, on a 13-kb plasmid-associated contig. This isolate was susceptible to meropenem, ceftazidime, and cefepime. Although no other class D genes were detected among *Proteae* tribe isolates with available WGS data, two other Enterobacterales, one *Klebsiella* and one *E. coli*, were found to harbor *Acinetobacter*-associated class D genes. These WGS data were biased towards carbapenem-resistant isolates. One hundred additional isolates from the Network met the described AST criteria for potentially harboring other class D genes and are undergoing supplementary testing to identify and characterize any with a class D gene present. Using a large collection of contemporary isolates, we found that blaOXA-48-like are circulating in *Proteae* tribe members infrequently. The finding of blaOXA-181 on a 13-kb plasmid highlights the potential for rapid spread of class D genes. The presence of AST phenotypes that are potentially predictive of the presence of other class D genes indicates a need for targeted surveillance to better understand their frequency and molecular epidemiology.

Presenter: Giulia Orazi, tzn7@cdc.gov

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

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Background: *Mycoplasma genitalium* (MG), while not a nationally reportable or notifiable disease, is highly prevalent compared to other sexually transmitted infections (STI), e.g., Chlamydia, Gonorrhea, and Trichomonas. A previous national meta-analysis of MG by Lisa Manhart, PhD, and colleagues in 2020 reported a prevalence of sexually active women (19.80%) and men (16.50%) between the ages of 15 and 24. From these data, it is expected that prevalence in the population of individuals tested at the Dallas County Health and Human Services (DCHHS) Sexual Health Clinic (SHC) would represent high-risk for MG, and therefore warrant monitoring. Considering that persistent MG infection has been associated with adverse reproductive outcomes, determining prevalence is imperative to implement testing and direct treatment. It is the goal of this study to provide supportive evidence of MG prevalence in the DCHHS SHC population to justify a monitoring program. **Aims/Purpose:** To conduct a prevalence study, determining the burden of and risk factors for MG among the high-risk cohort of DCHHS SHC patients. **Methods:** Collect ten (10) reserve urine specimens submitted for Gonorrhea, Chlamydia, and Trichomonas testing at the DCHHS SHC every day until 400 specimens are collected. To obtain clinical and demographic data, a retrospective review will be conducted using Greenway Laboratory Information System (LIS). All specimens will be de-identified and tested for MG utilizing the Aptima MG NAAT Assay on the Hologic Panther System. Results will be analyzed for prevalence and adjusted ratios for sex, age, race, ethnicity, co-infection and symptom status using logistic regression via STATA. **Results:** This cohort, comprised of female (53.75%) and male (46.25%) groups, has the following disease prevalence for each STI: Chlamydia (11.00%), Gonorrhea (6.75%) and Trichomonas (4.50%). Prevalence of co-infection is 3.50%, with the most common co-infection being Chlamydia and Gonorrhea (2.75%). The prevalence of all STIs is the highest in those 25–34 years of age (47.19%), and second highest in those 15–24 years of age (34.83%). **Conclusions:** In the reviewed literature, MG prevalence is similar to Chlamydia, often exceeding Gonorrhea (Getman, et al., 2016). If this trend follows in Dallas County, then MG is expected to be as prevalent as Chlamydia (11%)—constituting a public health concern. Risk factors for MG include young age and coinfection with other STIs (Manhart, et al., 2020). This trend is currently being observed in the data to date. Young age, specifically between ages of 25 and 34, have the highest prevalence of STIs. Though coinfection is currently observed to be low (3.50%), it is anticipated to rise with the addition of MG results. The results of this study will aid in providing a reference range for subsequent verification studies and onboarding of the Aptima MG Assay for patient testing.

Presenter: Margaret Gearhart, gearhartm33@outlook.com

qPCR Assay for Identification of OXA-235 Variants Associated with Carbapenem-resistance in Enterobacteriaceae

S. Cossette, C. Connelly; Streck

Background: Carbapenem-hydrolyzing class D (CHDL) enzymes, including members of the oxacillinase (OXA) family are resistant to antibiotics of last resort for potentially fatal infections. Organisms harboring these genes can acquire them through mobile genetic elements, including Carbapenem-resistant *Acinetobacter* spp., classified as an urgent threat by the CDC in 2019. Identification of new gene subfamilies, such as OXA-235 in Enterobacteriaceae, continues to increase the threat of carbapenem resistance (CR). As new AMR genes emerge, methods for AMR surveillance are imperative for outbreak prevention and antimicrobial stewardship. Therefore, this study describes the design of a qPCR-based assay that detects OXA-235 variants, expanding detection of OXA gene variants commonly detected in commercial PCR assays to include these medically relevant resistance mechanisms. **Methods:** Carbapenem-resistance OXA-235 subfamilies were identified through the β -Lactamase DataBase (BLDB). Sequence alignments for the OXA-235 variants were downloaded from the NIH Pathogen Detection Reference Gene Catalog and analyzed in Unipro's UGENE software to facilitate primer design. Oligos were optimized by qPCR using a synthetic target sequence and then multiplexed with an internal control (IC) targeting a conserved region in Gram-negative bacteria to discriminate false negative results. **Results:** The OXA-235 subfamily, associated with CRE and carbapenem resistant *Acinetobacter* spp., consists of 9 *A. schindleri* variants including OXA-235, OXA-236, and OXA-237. In silico analysis confirmed the primers and probe designed for OXA-235 variants could discriminate OXA-235 genes from the OXA-134-like subfamily. Additionally, this model predicts 100% sensitivity and specificity for the identified OXA-235 variants. A 5-point serial dilution of the target sequence was analyzed by qPCR and demonstrates the assay is 100% efficient with a correlation coefficient of 0.999. **Conclusions:** As AMR spreads, it's crucial to collaborate with the public health sector in monitoring emerging resistance to ensure tests are updated to detect these critical mechanisms. Assays that are not updated can lead to suboptimal rapid diagnostics and antimicrobial misuse, further contributing to the primary causes behind the spread of AMR. The development of the qPCR assay described above is aimed at supporting this need and the continued identification of AMR mechanisms associated with CR Enterobacteriaceae.

Presenter: Stephanie Cossette, scossette@streck.com

Rapid Identification and Response of a Salmonella Typhimurium Outbreak Traced to Raw Alfalfa Sprouts, Nebraska, 2022

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Background: On December 19, 2022, Douglas County Health Department (DCHD) identified a cluster of *Salmonella* cases reporting a common food item that contained raw alfalfa sprouts at a local restaurant. Nebraska Department of Health and Human Services (NDHHS) was notified about the cluster immediately and informed the Nebraska Public Health Laboratory (NPHL) to prioritize genetic analysis for isolates of interest. This report describes a

collaborative effort among state and national partners to rapidly identify a salmonellosis outbreak. **Methods:** DNA was extracted from *Salmonella* isolates using the Qiagen EZ1 Advanced XL instrument with resulting concentration determined using a Qubit fluorometer. Whole genome sequencing (WGS) was performed using the Illumina DNA Prep Kit and run on a MiSeq 500 cycle v2 kit. Sequence results were assessed for quality using Illumina Sequence Analysis Viewer and BioNumerics Version 7.6. Sequence comparisons were made using NDtree and BioNumerics software. Consequently, sequences were shared with the US Centers for Disease Control and Prevention (CDC) for comparison with other state WGS data to identify the extent of the outbreak. **Results:** Sequencing results were available on December 23, 2022. Due to technical difficulties with BioNumerics at the time of the outbreak, alternative comparison tools were used to define similarities among *Salmonella* strains. The outbreak strain was identified as *Salmonella* Typhimurium. Fastq files were shared with the CDC via ftp on December 23, 2022, who then prioritized the Nebraska outbreak isolate's analysis and conducted a national comparison search. Eight Nebraska isolates and one Oklahoma isolate (tested at the NPHL) were indistinguishable at 0-1 allele difference by cgMLST. Subsequently, the CDC declared a multistate outbreak of *Salmonella* Typhimurium following potential contamination of raw alfalfa sprouts on December 27, 2022. **Conclusion:** The collaboration among DCHD, NDHHS, NPHL and the CDC resulted in quick identification and rapid response for early detection of a *Salmonella* Typhimurium outbreak traced to raw alfalfa sprouts. Despite issues analyzing the sequence data due to technical software problems, alternative means were utilized to determine genetic relatedness of isolates of interest. The combination of strong epidemiologic data, laboratory data and environmental traceback information successfully recognized a potential contamination of raw alfalfa sprouts leading to a voluntary recall of the affected products on December 29, 2022.

Presenter: Steve McGill, stmccgill@unmc.edu

Syphilis and HIV Take a Road Trip!

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New Jersey's Field Deployable Laboratory (FDL) unit was developed in response to the COVID-19 pandemic, to perform testing, aid in outbreaks, as well as perform surveillance testing throughout the state. As COVID-19 deployments slowed, NJ PHEL explored use-cases for mobile testing and decided, in combination with our Division of STD/HIV/TB, to develop a workflow for rapid, on-site confirmatory testing of Syphilis and HIV. To facilitate on-site testing, PHEL identified instrumentation that could perform a majority of testing on-site using minimally invasive procedures (e.g., fingerstick). The Chembio's DPP HIV-Syphilis instrument, which does combination antibody testing for HIV and treponemal antibody testing for syphilis, was chosen as the initial screening test. PHEL was then able to develop a testing algorithm for syphilis, incorporating RPR nontreponemal testing, and HIV 1 & 2 differentiation utilizing the Geenius platform if the initial specimen tested positive. In certain cases, involving discrepancies, confirmatory samples may need to be taken back to PHEL for further confirmation testing, but preliminary results would still be issued to patients at the point of care site. In this poster, we detail the

processes used to perform risk assessment, SOP writing, correlation and validations of the various testing methodologies, and the necessary steps to integrate such testing into our LIMS point-of-care module, as well as pilot deployment data. This program has shown that not only are these mobile laboratories useful in the COVID-19 response but are also an asset to other departments within the State of New Jersey. The FDLs are opening the road to more testing avenues for vulnerable populations.

Presenter: Lisa Kimball, lisa.kimball@doh.nj.gov

TheiaEuk: Bioinformatics Workflow for Species-Agnostic Characterization of Fungal Pathogens

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Clinical incidence of antifungal-resistant fungal infection has increased dramatically in recent years leading to the CDC's Antimicrobial Resistance Laboratory Networks (ARLN) adding drug-resistant *Candida auris*, other drug-resistant *Candida* species, and azole-resistant *Aspergillus fumigatus* to the AR Threats report in 2019. These pathogens are capable of colonizing various body cavities as well as causing life-threatening bloodstream infections. There does not exist a comprehensive laboratory assay for the characterization of fungal pathogens or the detection of antifungal resistance biomarkers. Here we introduce TheiaEuk_PE, a cloud-native bioinformatics workflow which accepts paired end next generation sequencing data as an input, performs de novo genome assembly, taxon identification, clade-typing for *C. auris*, and for several fungal species it will report any mutations found in genes known to contain mutations that confer resistance to any antifungals. The Shovill genome assembly package is used to perform de novo assembly with SKESA set as the default assembler. This workflow addresses the issue of taxon identification using GAMBIT, with a custom fungal database containing over 5000 unique genomes representing 245 species. Snippy is used to call variants relative to a reference genome, and the variant call format file is queried for genes known to contain mutations associated with antifungal resistance. This workflow is open source and available on the [Theiagen Genomic github page](#). The workflow was designed using dockerized components and written in the workflow description language to facilitate deployment on the cloud-based bioinformatics platform Terra.bio.

Presenter: Frank Ambrosio, frank.ambrosio@theiagen.com

Validation and Implementation of Sanger Sequencing for Cryptosporidium Subtyping in the State of Texas

J. Cen, M. Sciulli, R. Tuladhar, C. Wang; Texas Department of State Health Services

Introduction: The protozoan parasite *Cryptosporidium* is an important cause of diarrheal disease (cryptosporidiosis) in humans and animals. Between 2011-2020, an average of 597 cryptosporidiosis cases per year were reported in Texas, with 2019 alone having 12 outbreaks resulting in 1,244 reported cases. Due to the sexual lifecycle, large-scale genetic recombination may occur within a host, potentially confounding epidemiological analysis. Previous studies indicate that different *Cryptosporidium* species and subtypes have varying clinical manifestations and

severity of virulence. Therefore, it is important to speciate and genotype *Cryptosporidium* at the subtype level. Sanger sequencing analyses of gp60 and 18S rRNA are widely used in *Cryptosporidium* subtyping. Texas validated a modified CDC procedure for the subtyping of *Cryptosporidium* from fecal specimens by using nested polymerase chain reaction (PCR) detection and Sanger sequencing.

Method: The CDC provided a panel of fecal samples for this validation study. Following DNA extraction using Fast DNA SPIN Kit for Soil, nested PCRs were performed for either gp60 or 18S rRNA using GoTaq Hot Start Master Mix. Utilizing the BigDye Terminator v3.1 Cycle Sequencing kit, Sanger sequencing was employed for subtype identification, and subsequent sequences were analyzed with Geneious Prime software. Identified species and/or subtypes were reported to the CDC and compared with their analyses. To optimize workflow efficiency, modifications were made to the current CDC protocol. These include the use of 1) a commercial mastermix for nested PCR, 2) a quick gel running system, 3) PCR and sequencing reaction optimization, 4) new PCR and sequencing clean up kits, and 5) a 96-well plate format to facilitate future large scale, high throughput applications. **Results and Conclusion:** The panel provided by CDC was successfully validated with the above-mentioned modifications. Upon comparing results, there was 100% agreement with the CDC on accuracy, precision, sensitivity, and specificity. The successful implementation of Sanger sequencing for *Cryptosporidium* subtyping in Texas allows for rapid and accurate identification of these infections as well as contamination sources. Further efforts have been made on recruitment and implementation of *Cryptosporidium* samples for sequencing by using the DSHS listserv, laboratory webpage, and recruitment letter.

Presenter: Joyce Cen, joyce.cen@dshts.texas.gov

Validation and Implementation of Antimicrobial Susceptibility Testing to Monitor Trends in Antimicrobial Resistant *Neisseria gonorrhoeae* in the State of Texas

N. Niyah, N. Gray, T. Baldwin, C. Wang, C. Schroeder; Texas Department of State Health Services

Introduction: *Neisseria gonorrhoeae* (GC) is the etiological agent of gonorrhea, a worldwide prevalent sexually transmitted infection. Individuals with untreated GC infections can develop pelvic inflammatory disease, prostatitis and infertility. Antimicrobial resistance (AR) within GC has risen sharply, creating an urgent public health concern. Surveillance of AR gonorrhea is a means to understanding the spread of AR and determining antimicrobial susceptibility patterns in a given population. Currently, only one Texas county performs routine GC antimicrobial susceptibility testing (AST). The implementation of GC AST at the DSHS Austin Laboratory will be a valuable tool in tracking AR GC prevalence across the state.

Results: Using 50 GC specimens from the Antimicrobial Resistance Isolate Bank and the Gonococcal Isolate Surveillance Project (CDC & FDA), we report successful validation of the Biomerieux ETEST® Gradient Diffusion Method for four antibiotics: azithromycin, cefixime, ceftriaxone, and ciprofloxacin. The overall accuracy, precision, sensitivity, and specificity of the ETEST® were found to be 98%, 100%, 100% and 97.2%, respectively. Further, three commercial specimen collection and transport kits were validated for organism recovery. These were: 1) Copan TransPorter Amies gel collection kit without Charcoal, 2) Copan E-swab™ Regular-Sized nylon flocked swab kit with liquid Amies, and 3) Copan E-swab™ Minitip nylon flocked swab kit with liquid Amies. GC was successfully

recovered from each kit following 24 hr incubation at ambient temperature. Here, the overall accuracy, precision, sensitivity, and specificity was 100% for all kits.

Implementation: The DSHS Laboratory instituted several steps for GC AST implementation:

1. LIMS system updated for specimen accession and result reporting.
2. GC Specimen Collection and Shipping Guidance Document for inclusion with specimen shipping containers.
3. Healthcare provider education about GC AST at the laboratory using webinar series, ListServ notification, and the Texas AR Laboratory website.
4. GC Whole Genome Sequencing validation for non-susceptible or resistant specimens.
5. A GC AST specimen recruitment letter sent to existing and potential submitters.

The successful implementation of GC AST at the DSHS Laboratory will allow for the rapid and accurate identification of drug resistant infections, especially ceftriaxone-resistant GC in Texas, so as to inform effective antimicrobial therapies. Additionally, susceptibility testing will contribute to quickly tracking and mitigating outbreaks.

Presenter: Naomi Niyah, Naomi.Niyah@dshtexas.gov

Viral Trends: A SARS-CoV-2 Timeline with Houston Texas

R. Penn, A. Lara, P. Brown, Y. Lai, M. Yerramilli, D. Corral; Houston Health Department

Since its first report in late November to early December 2019, the SARS-CoV-2 virus rapidly spread worldwide. Due to its highly mutagenic and infectious nature, tools were necessary to properly contribute to national SARS-CoV-2 surveillance efforts. Among those developments, whole genome sequencing (WGS) rose as an effective method to gather the genomic data necessary to understand the infectious trends and genetic diversity which emerged during the COVID-19 pandemic. Within Houston, the fourth most populated city in the United States, and neighboring counties, the Houston Health Department (HHD) Laboratory generated genomic data from 13,000 specimens using an amplicon-based whole genome sequencing approach on Illumina sequencing platforms. Between 2020–2022, the HHD analyzed SARS-CoV-2 variants collected from over 50 unique zip codes across Harris and neighboring counties. Within this data, we highlight an emergent timeline of major variants that spread over the city and propose geographical trends of infection. We also compare clinical sequencing data to data from sequencing wastewater samples, and national trend data from the same timeframe. Additionally, we highlight demographic trends associated with major SARS-CoV-2 waves (alpha, delta, omicron) with respect to the age and socioeconomic status of the sampled Houston population. Present results demonstrate the viability of a real-time WGS methodology to understand the everchanging landscape of SARS-CoV-2 genetic diversity within a population and inform public health for ongoing and future disease outbreaks.

Presenters: Adolfo Lara (adolfo.lara@houston.tx.gov) and Ryker Penn (ryker.penn@houston.tx.gov)

Wastewater Surveillance for *Candida auris* Utilizing Digital PCR

K. Richbow Dozier, C. Carlisle, A. Janik; Delaware Public Health Laboratory

A fungal species known as *Candida auris* is a multi-drug resistant yeast that is slated to become a serious global health threat. After being first identified in 2009, within a few years it has become an emerging pathogen in hospitals. *C. auris* occurs more commonly in individuals who are immunocompromised, have frequent stays or long-term residency in a health care setting or utilize invasive medical devices (i.e., breathing tubes, feeding tubes and central venous catheters). Infections caused by *C. auris* include but are not limited to: bloodstream, wound, respiratory and urine. *C. auris* is capable of colonizing on the skin prior to an infection occurring and can be transmitted from patient to patient. With the yeast's high resistance to antifungal medications commonly used to treat *Candida* infections, it is sometimes impossible to treat. As *C. auris* continues to spread globally with more than 2,000 clinical cases appearing in the United States, healthcare settings are beginning to experience outbreaks among their patients even with enhanced infection control. Upon the introduction of the National Wastewater Surveillance System (NWSS), wastewater surveillance is increasingly being utilized to monitor communities for presence of infection resulting from viruses and other pathogens. The Delaware Public Health Laboratory delegated a project to an APHL fellow to develop and implement a molecular method to detect *C. auris* in wastewater utilizing digital PCR for quantitative presence of the fungus. As part of the NWSS, untreated wastewater will be collected from several treatment facilities within the state of Delaware. The wastewater samples will be concentrated in the laboratory using the InnovaPrep Concentrating Pipette Select. Concentrated samples will then undergo DNA extraction using the Qiagen QIAcube Connect and DNA quantification using the Qiagen QIAcuity. Next generation sequencing will be conducted on extracted wastewater samples using the Illumina MiSeq and QIAxcel Connect. After successfully validating the data, this method can become an opportunity for healthcare settings to join wastewater surveillance testing to control the spread of *C. auris* and prevent potential outbreaks from occurring in the future.

Presenter: Kailene Richbow Dozier, kaidozier@gmail.com

West Nile Virus Surveillance in Ohio

S. Anders, L. Garrett, E. Leasure; Ohio Department of Health

West Nile Virus (WNV) is the leading cause of mosquito-borne disease in the continental United States. WNV is most commonly spread to people from an infected mosquito. *Culex* mosquitoes are the primary vector of transmission. WNV is a zoonotic disease that can cause mild symptoms, such as fever (1 in 5 people infected) to severe illness such as encephalitis or meningitis (1 in 150 people infected). There is no vaccine to prevent infection or medicines to help treat symptoms. WNV surveillance is important to detect seasonal epidemics and to perform prevention and control strategies. In 2022, 51 counties across Ohio participated in WNV surveillance by collecting mosquitoes and sending them to the Ohio Department of Health Laboratory (ODHL). The entomology laboratorians examined the mosquitoes under a microscope to determine species and sex. *Culex* species and female mosquitoes were selected to be tested, as male mosquitoes do not feed on

blood. The selected mosquitoes were pooled based on county and placed into sample racks. Each sample rack may contain up to 92 pools of 1-50 mosquitoes. The filled racks were then sent to the virology laboratory to be tested. Samples were homogenized with the QIAGEN Tissue Lyser II and BA-1 diluent (QIAGEN), centrifuged at 6,000 x g for 13 minutes at 4°, and prepared for RNA extraction using the RNeasy 96 QIAcube HT kit (QIAGEN). Extractions took place on the QIAGEN QIAcube HT robotic workstation which allows for 3-4 runs (around 300 mosquito pools) per day. RT-PCR was performed on the ABI 7500 Fast Dx instrument using CDC primers and probes for WNV. Previously confirmed WNV positive RNA samples were used as the positive control and PCR grade H₂O was used as the negative control. The final qualitative results were reported to respective counties. If there was an increase in expected WNV positive mosquitoes, spraying would be conducted in those areas to eliminate flying mosquitoes. From 2016-2022 (excluding 2020 due to no testing from COVID-19) a strong positive correlation ($R=0.9002$) was observed between the number of clinical human cases of WNV and positivity rate in tested pools of mosquitoes in Ohio with a P-value of $.0372 < .05$. This indicates that increased incidence levels of WNV in mosquitoes can lead to an anticipated increase in clinical cases. In conclusion, the QIAcube HT offers a high throughput method for WNV testing of around 300 mosquito pools per day over the manual method, leading to a more representative sample size. The data obtained in this study demonstrates that the use of efficient surveillance methods can help to improve mitigation strategies, resulting in an overall improvement to public health.

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WASTEWATER SURVEILLANCE OF INFECTIOUS DISEASES

Changes in the Correlation between Clinical Cases and Levels of SARS-CoV-2 RNA in Colorado Wastewater

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The utility and interpretation of wastewater signal as a predictor of community health can be confounded by factors that vary between locations and over time, such as wastewater system complexity, human behavior and viral evolution. We explored the relationship between SARS-CoV-2 concentration in wastewater and caseload for 23 locations in Colorado over a 22-month period, including multiple waves and the implementation of vaccines. Location-specific models showed strong correlations for all facilities, independent of normalization, with the best correlations seen for facilities with populations > 10,000. Change point analysis identified events leading to decreases in numbers of clinical cases reported per SARS-CoV-2 genome copies detected in wastewater. The first change points occurred in several, but not all facilities, over a six-month period during which vaccination became widespread and the Alpha variant arose. Another more dramatic increase occurred synchronously within a narrow one-month period for all facilities and coincided with extensive distribution of at-home test kits and the rise of the Omicron variant. Models accounting for these shifts in

pandemic phase showed significantly improved correlations, yet the original models likely provide a better estimate of actual caseload. In future pandemics, the effects of phase changes should be considered in modeling efforts.

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Genomic Surveillance of SARS-CoV-2: Comparing Data from Sequencing Clinical and Wastewater Specimens

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Genomic surveillance of COVID-19 has largely relied on whole genome sequencing of clinical respiratory specimens that test positive for SARS-CoV-2 by PCR. Many clinical specimens need to be sequenced to generate sufficient genomic data to approximate trends, and the availability of PCR-positive respiratory samples varies with epidemic conditions, between communities with differing healthcare access, and with changing testing behaviors. Sequencing wastewater might mitigate some limitations; a single sample represents a large population and is agnostic to testing behaviors or access to care. However, the concordance between clinical and wastewater genomic data is not yet well characterized. We aimed to compare the date that new variants were detected and the growth rates between clinical and wastewater surveillance strategies to assess utility of a multi-prong genomic surveillance strategy. The Chicago Department of Public Health (CDPH) coordinates sequencing of clinical respiratory specimens and wastewater samples—approximately 15 clinical remnant specimens from patients testing positive for SARS-CoV-2 at 10 Chicago hospitals and four samples collected from three wastewater treatment plants processing wastewater from the Chicago area were submitted for sequencing each week between April 2022 and December 2022. Lineage proportions were calculated: for clinical samples, this is a simple percentage. For wastewater, each sample was weighted according to the population served. We compared the date of first detection and the growth trajectories of two Omicron sublineages between clinical and wastewater samples; BA.5 (which reached dominance during the study period) and BQ.1 (which did not). During April 2022 to December 2022, 489 wastewater samples and 3,073 clinical respiratory specimens were sequenced. BA.5 was detected two weeks earlier in wastewater than in clinical samples, while BQ.1 was detected one week earlier in clinical samples than in wastewater. There was good concordance in growth trajectories across surveillance methods: in both clinical and wastewater samples, BA.5 reached 50% in the week ending July 2, 2022, and—as of December 3, 2022—was found at 44% and 24% in wastewater and clinical samples respectively. Analysis of other sublineages highlight lineage-specific differences in detection timing and growth characteristics. In Chicago, genomic sequence data from wastewater and clinical samples were largely concordant. Continued evolution of SARS-CoV-2 is inevitable, and genomic surveillance will remain critically important in the next phase of the pandemic, even as testing preferences continue to shift away from PCR testing in favor of antigen tests. Wastewater surveillance can be a useful supplement to genomic surveillance of respiratory specimens, and combining data sources may provide opportunities for earlier detection of emerging lineages.

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Going With the Flow: Implementing a COVID-19 Wastewater Surveillance Program in a Small State

C. Benton, State of New Hampshire Public Health Laboratories

The State of New Hampshire Public Health Laboratory (NH PHL) implemented wastewater testing for COVID-19 to act as an early outbreak warning system for the state's 1.5 million residents. Implementation of this testing required overcoming several challenges, such as identifying laboratory space, personnel, and equipment. Cross training of staff, utilizing shared lab space, and redirecting laboratory funds as well as partnering with the University of New Hampshire enabled NH PHL to efficiently bring on this testing. As UNH had an established wastewater surveillance program on their campus, NH PHL was able to share protocols, and received guidance for implementation. Wastewater surveillance for COVID-19 began on October 1, 2022, and currently consists of 14 sewage treatment plants throughout the state submitting weekly samples for testing. The submissions from each facility are effluent collected over a 24 hour period. Once received at the lab, the samples are processed and extracted using the KingFisher Apex platform, and digital PCR for COVID-19 detection is performed using the Qiagen QiaCuity. The data that is collected is then used to populate a publicly facing COVID-19 dashboard, allowing the general public to view trends in COVID-19 wastewater by facility. Currently, New Hampshire is expanding this testing to include WGS of these samples for variant determination, and is also working to add testing for additional pathogens.

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Lessons Learned from Two Years of SARS-CoV-2 Wastewater Surveillance: Comparison of Viral Concentration, Extraction and Quantification Methods

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Wastewater monitoring of SARS-CoV-2 throughout the COVID-19 pandemic has documented the utility of the wastewater-based epidemiology (WBE) approach as a cost-effective, fast and relatively unbiased method for assessing trends in community-wide viral concentrations. Just two years since the inception of many of the SARS-CoV-2 WBE programs there are now over 3,800 sites across 70 countries and over 150 public facing dashboards devoted to WBE surveillance. The pressing need for wastewater-specific viral methods motivated the rapid development of a large number of protocols and tools and that effort continues to date. Thus far, the WBE community gained a deeper understanding of the advantages and limitations of some of the protocols. Still, even though new guidelines have been written both by the CDC and the National Academy of Sciences, the information on which to judge the comparative performance of the methods for concentration, extraction and quantification of SARS-CoV-2 from wastewater remains limited. Despite the rapid evolution of WBE methods and an impressive body of literature emerging from those efforts, much remains to be evaluated and standardized. Our work attempts to systematically compare the most popular WBE methods in a step-by-step approach, including comparisons of HA electronegative filtration to Nanotrap® Microbiome A particles and polyethylene glycol (PEG) precipitation, as well as the direct capture using

Promega's PureYield binding column. We also evaluated different extraction methods and compared side-by-side qPCR with digital PCR platform. Overall, our data suggest that multiple concentration methods can perform adequately, though some stark differences can be observed with specific wastewater matrices. Among the methods tested, the automated paramagnetic bead-based extraction yielded better viral recoveries than the manual on-column extraction. In addition, the viral target quantification with traditional qPCR was found to perform adequately when careful quality control was applied, but the digital PCR was more sensitive and slightly less variable. In particular, the combination of Nanotrap® concentration with automated bead-based extraction and digital PCR quantification was chosen as the most practical and best performing WBE method.

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SARS-CoV-2 Wastewater Workflow Comparison for Upscale or Downscale Testing in the National Capital Region (NCR) Composed of the District of Columbia and Southern Maryland

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Clinical monitoring of the COVID-19 pandemic has been essential, but unintentionally taxing on the public's willingness to undergo nasopharyngeal swabbing or administer at-home self-tests. Population-level surveillance can be performed to supplement individual clinical tests. Infected persons shed SARS-CoV-2 in their feces, urine, sweat, and respiratory fluids. Research by CDC and WHO have demonstrated a correlation between SARS-CoV-2 found in wastewater and the SARS-CoV-2 infection rate found in clinical testing. By surveilling raw wastewater within the National Capital Region (NCR), the District of Columbia Public Health Laboratory (DC PHL) is able to surveil all eight wards of the District of Columbia, various K-12 schools during the academic year and parts of southern Maryland. For processing and analysis of wastewater samples, we first concentrate the raw wastewater, extract viral RNA, quantify SARS-CoV-2 abundance using digital PCR, sequence variant strains using the Illumina MiSeq, then transfer data to local and federal partners. The DC PHL utilizes one of two methods for the initial concentration step: the Innova Prep Concentrating Pipette or the Ceres Nanotrap Technology. The Innova Prep method concentrates one wastewater sample at a time in less than five minutes by capturing viral particles in the pores of an ultra-filtrating pipette tip. Whereas Ceres Nanotrap is a high throughput system that can concentrate 24 wastewater samples in 40 minutes and achieves viral capture through high affinity rapid binding. The purpose of this study is to compare these two methods based on utilization time, throughput, cost and how suitable the products are for downstream applications, including sequencing and digital PCR using the QIAcuity Digital PCR Instrument. This study will provide data to aid public health laboratories in optimizing workflows for analyzing wastewater samples for SARS-CoV-2, but also for other pathogens found in wastewater such as mpox, Candida auris and various other respiratory diseases.

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Surveillance of SARS-CoV-2 in Wastewater of Ohio: Consolidation of SARS-CoV-2 Testing from Academic, Commercial and Government Labs to Ohio Department of Health Laboratory (ODHL)

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Wastewater (WW)-based epidemiology is an effective tool for early detection of SARS-CoV-2 in communities before it can be clinically detected. In May 2020, "Ohio Coronavirus Wastewater Monitoring Network" was started at the Governor's request to provide communities information on SARS-CoV-2 in WW to proactively prevent outbreaks. Routine WW monitoring of SARS-CoV-2 in Ohio started in July 2020. Seventy-seven WW treatment facilities participated in the network and 8 academic, commercial and government labs performed the SARS-CoV-2 testing via different methodologies (liquid supernatant or solid fraction of WW; RT-PCR, digital PCR, and digital droplet PCR). For an optimal comparison of WW data, a standard method of SARS-CoV-2 testing was needed. Hence, all WW testing sites were planned to be transferred to Ohio Department of Health Laboratory (ODHL). By July 2022, 72 WW sites (few sites dropped) were being tested at ODHL. The GT-Molecular surveillance guide was used for virus filtration, concentration (Innovaprep Concentrating Pipette; ICP), RNA extraction and SARS-CoV-2 quantification (digital PCR amplification of N2 region of the SARS-CoV-2 nucleocapsid gene). Bovine Coronavirus (BCoV) was used as a matrix recovery control. A pilot study was performed at ODHL from October 2021 to December 2021 for testing of SARS-CoV-2 from 4 WW sites (APHL 2022 poster #86). In our pilot study, the ICP concentration method showed a significant correlation ($r = 0.86$; $P = 20$ to $r = 0.65$, $P = 30$). In conclusion, ODHL successfully consolidated the WW testing sites using Ceres concentration method to monitor COVID cases in Ohio.

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Wastewater Surveillance of SARS-CoV-2 in South Carolina

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Introduction: Wastewater surveillance studies are used to track the real-time presence of SARS-CoV-2, the virus that causes COVID-19. This surveillance is important because it serves as a more expedient surveillance system in comparison to tracking of positive COVID-19 results. People infected with SARS-CoV-2 can shed the virus in their feces, even if they don't have symptoms; thus, the virus can then be detected in wastewater and capture viral presence of those who are both asymptomatic and symptomatic. This leads to quicker warnings of COVID-19 spread in communities and health regulations and statistics can be shared quicker and more precisely. Testing for SARS-CoV-2 in wastewater has several technical challenges. One issue is determining the most effective method of wastewater extraction which is crucial to maintaining accurate surveillance (CDC, 2022). Currently, pasteurizing wastewater samples to reduce the chances of viral transmission while testing, while maintaining useful results are an area of interest. Pasteurizing wastewater samples will allow us to perform the extraction process without using the biosafety level 3 (BSL3) guidelines. The purpose of this research is to determine if pasteurizing wastewater samples prior to

extraction yields comparable results to non-pasteurized samples.

Methods: Wastewater samples received from two South Carolina counties, Georgetown and York County, with the highest observed concentrations of COVID-19, were selected for pasteurization testing. 40mL of each sample were aliquoted for pasteurization as well as non-pasteurization. Pasteurization tests were done twice, 1 hour pasteurized samples compared to non-pasteurized samples and 30-minute pasteurized samples compared to non-pasteurized samples in a 65 °C water bath. After extracting the pasteurized and non-pasteurized samples, quantification was conducted by digital PCR. The results from this procedure will determine if the pasteurization process will be validated by the South Carolina Department of Health and Environmental Control laboratory.

Results: For both Georgetown and York County, pasteurization methods have not been proven to yield comparable results to non-pasteurized wastewater samples for Sars-CoV-2 surveillance.

Conclusion: Thus far, the pasteurization results from the one-hour pasteurization method have been proven to be ineffective. The use of pasteurization on wastewater samples are unnecessary if there is significant viral loss for testing. For both counties, there is roughly a 4.5 decrease in antibody detection level of Sars-CoV-2 of the pasteurized samples compared to the non-pasteurized samples. The results for the 30-minute pasteurization method are still pending, there is hope that with a reduction of pasteurization time there will be less viral loss for wastewater samples.

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Wastewater Surveillance Reports and Analysis of SARS-CoV-2 Concentrations and Variants in Hawai'i

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Wastewater surveillance has grown worldwide since the beginning of the COVID-19 pandemic to become a valuable layer for monitoring community levels of SARS-CoV-2. Monitoring wastewater is especially vital to the State of Hawai'i to understand disease patterns and reduce mortalities, especially in the Native Hawaiian and Pacific Islander communities that consisted of 22% of COVID-19 cases and deaths. Beginning in Spring 2022, the Hawai'i State Department of Health (HDOH) joined the National Wastewater Surveillance System (NWSS) and enrolled 15 wastewater treatment facilities across the state to quantify SARS-CoV-2 in sewage. To monitor potential surges, identify lineages spreading in the community, and assist in public health responses, the HDOH publishes reports on the data provided by NWSS on the SARS-CoV-2 concentrations and variants present in wastewater. Surveillance has demonstrated that wastewater SARS-CoV-2 concentrations in sewage strongly correlates with COVID-19 case counts in Hawai'i. To use as an early warning indicator, concentrations are plotted over time and fit to a local regression to easily observe changes in trends that could indicate surges. Results have demonstrated that upward or downward trends have often reflected synonymous changes in COVID-19 case counts. As of January 2023, over 400 SARS-CoV-2 variants have been detected in Hawai'i wastewater since surveillance began with detection of variants in sewage often occurring prior to clinical detection. Notably, XBB.1.5 was identified in Honolulu County wastewater prior to detection in clinical samples. Overall, creating public reports of wastewater surveillance data for

the state of Hawai'i has assisted in interpretation of this data for public health officials and the community to better understand the prevalence and dynamics of SARS-CoV-2 in Hawai'i.

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WORKFORCE DEVELOPMENT AND RETENTION

A Prevention Plan for Laboratory-Acquired Burnout Modeled on the BMBL

M. Akre, B. Van; J. Michael Consulting

The first report of a laboratory-acquired infection (LAI) was in 1885. Unfortunately not uncommon, the laboratory community developed engineering and administrative controls and personal protective equipment to minimize risk. Here we propose using the principles outlined in the *Biosafety in Microbiological and Biomedical Laboratories* (BMBL) to mitigate the risks of burnout. Laboratory-acquired burnout (LAB) prevention will be described using the same methods that were successful in allowing today's laboratories to safely work with pathogenic agents.

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Data Modernization and Internships in the New Jersey Public Health and Environmental Laboratories 2019-2022

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Per- and polyfluoroalkyl Substances (PFAS) are a large group of manufactured chemicals that are persistent and potentially hazardous. PFAS is tested in human serum with high performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS), typically after solid phase extraction (SPE). Although it can be challenging, resource intense and time consuming, SPE is deemed necessary in this instance. In our lab, SPE is performed online system. A major limitation of this approach is that sample preparation occurs sequentially before sample injection, therefore limiting throughput. The main goal of this project is to improve the sample throughput of the SPE system by offline automation, where samples are prepared concurrently using a 96 well plate format using a Janus automated liquid handler. The proposed off-line SPE has been successfully deployed by others on various platforms. Sample pre-treatment was deemed the most crucial in eliminating analyte recoveries. The SPE will be conducted on weak anion extraction (WAX) stationary phase. The automated procedure includes sample pre-treatment: Combine 50 µl, 150 µl Acetonitrile and internal standard, load the supernatant to the SPE plate after conditioning and washing before sample elution. The new automated offline SPE sample preparation method is expected to enable the lab to prepare PFAS samples from 48 aliquot of 50 µl human serum in less than two hours for analysis of the 18 PFAS.

Meanwhile, the accuracy of the further LC-MS/MS analysis must be within $\pm 30\%$ of nominal value or 3 times of STD value.

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Development of Unit-Specific, Electronic Training Plans for Onboarding Laboratory Staff

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At the beginning of 2020, the Colorado State Lab at the Colorado Department of Public Health and Environment (CDPHE) was designing training plans to track the onboarding of new staff. When the COVID-19 pandemic hit, the State Lab prioritized responding to cases and protecting Coloradans. Faced with hiring more than 200 new staff in an all-hands-on-deck situation, a paper training plan in a generic, lab-wide format was implemented. Since then, feedback from staff, as well as some instances of nonconforming work related to improper training, have highlighted the weaknesses of the training plan: (1) the paper format creates barriers to efficient documentation and record retention; (2) the template is not easily customizable to individual units or staff members; and (3) the plan lacks clarity on what is required of a staff member before they can perform testing activities on customer samples. Working with management staff from each unit at the laboratory, the Quality Assurance Coordinator and Quality Assurance Fellow at the State Lab are now developing new training plans. The new plans are in an electronic format to provide accessibility to all staff—whether working remote, on-site or hybrid—and streamline the process of accessing, documenting and retaining training records. In addition, the training plans are individualized to each unit to contain all relevant quality management system (QMS) documents, as well as a list of all standard operating procedures (SOPs) used by the unit. Finally, the new plans employ a color-coded system for tracking completion of all training activities, including initial competency assessments, to provide clarity on which testing activities each staff member is authorized and competent to perform. The development of the new training plans is still in progress, but all feedback from management staff to date has been positive, including multiple comments on the marked improvement that the new format will provide. The State Lab is carefully designing training plans—an essential component of the quality management system—so that the onboarding of new staff is accessible, thorough and complete.

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Impact of the COVID-19 Pandemic on Workforce Development Preferences

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In a survey of 416 public health laboratorians with five or less years of experience in the field, respondents reported that online courses are the preferred form of professional development. APHL's Recruitment and Retention Strategic Implementation Group (R&R SIG) administered a survey to assess how the pandemic changed workforce development and job recruitment preferences. In a 2018 pre-pandemic Training Needs Assessment, classroom learning, seminars, in-person group activities, workshops and webinars were

the preferred learning methods. The pandemic has changed the methods in which professionals receive training. Now the top five professional development resources are online courses, mentorship, webinars/lunch-and-learns, professional conferences and target training programs. New career laboratorians are 61% millennial and 65% female, which also could contribute to the push for online professional development. As public health continues to grow and change, more online professional development resources would benefit the early career professionals.

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Knowledge Management Resources for Public Health Laboratories

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Knowledge management is “the process of capturing, developing, sharing, and effectively using organizational knowledge.” Knowledge enhances daily work, informs decisions, and increases efficiency. APHL encourages members to share knowledge, ranging from survey data, training, lessons learned through experience, technical assistance, and consultation. The APHL Knowledge Management Committee (KMC) captures a wealth of this knowledge and information generated and leverages that knowledge to develop resources to improve laboratory operations and management. This includes data repositories, toolkits, reports, and dashboards. For example, the Member Resource Center (MRC) is a document library for members to share and readily access practices and protocols in fields of study such as infectious diseases, environmental health, and emergency preparedness and response. The Public Health Laboratory System Database (PHLSD) provides a comprehensive inventory of laboratory testing and equipment capabilities as well as laboratory profiles for LIMS, document control software, and accreditations. This database supports training and knowledge sharing on new methods, instrumentation or software, surge support, and continuity of operations planning. Toolkits, including the Knowledge Retention Toolkit, provide a collection of resources and guidance to address topics within laboratory operations, workforce, and outreach. Surveys capture timely information that is used to generate model practices from the field or to develop infographics and dashboards to inform decision-making. These results have a particular emphasis on workforce recruitment, engagement, and retention. This poster will cover a full array of resources supported by the KMC and how they can be utilized within the public health laboratory.

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Structuring a Curriculum for Public Health Laboratory Fellowships in New Jersey

S. Mikorski and T. Kirn, New Jersey Department of Health Public Health and Environmental Laboratories

Between 2005 and 2019, the New Jersey Public Health and Environmental Laboratories (PHEL) hosted 8 APHL and CDC fellows. Two of these fellows are now serving as Program Managers within our laboratory and one as Director of another public health laboratory. In 2019, PHEL established our own fellowships with Epidemiology and Laboratory Capacity (ELC) COVID funding for Molecular Microbiology and Informatics and are also participating as a host laboratory for CDC and APHL fellows. As of November 2022, NJ PHEL numbers second only to CDC with 15 fellows onsite. Having fellows engaged in all aspects of laboratory operations has enabled a sea change for PHEL. Aside from providing fellows opportunities to develop competence in bench skills, knowledge and experience with regulatory compliance, confidence in leading technical efforts and presenting findings and to collaborate with colleagues in epidemiology and other state agencies, fellowships offer many direct benefits to our laboratory, to the larger laboratory community and the public. Staff gain confidence and experience by teaching and learning from fellows. PHEL increases the number and quality of tests in our molecular menu, gains a pool of trained candidates for employment as technical leaders and visibility in the academic community leading to collaborative opportunities. The residents of the state of New Jersey gain through improved laboratory services. PHEL decided to design the Molecular Microbiology Fellowship program similarly to health professions programs structured with didactic and experiential learning. Having long term experience with administering project-based internships, we added self-directed “contracted” projects to create a triad of learning: Experiential, Scientific and Business pyramid. We developed rotation schedules with learning objectives, identified Quality Assurance and Scientific Projects with Program Managers as mentors, and created online learning courses with a regulatory and business focus to add to existing mandated training. The goals for this program are to work with clinical and academic partners to apply for a post-graduate CPEP accreditation for Medical and Public Health Microbiology and to extend this workforce development model to other programs within the laboratory.

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The Future Workforce of the Environmental Laboratory

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The primary goal of environmental laboratories is to protect public health and the environment. In addition to the widely recognized regulatory responsibilities of these laboratories, there is limited understanding of the fundamental purpose of this testing. The testing performed is diverse and complex, requiring a variety of technical skills. Laboratories must employ technicians and scientists that are able to prepare for sample collection events, perform field sampling and/or analysis, process and analyze samples in the laboratory, and perform quality assurance/quality control (QA/

QC) procedures to validate data and ensure its quality. This work requires highly skilled individuals for its various roles, and new scientists quickly find if they are meant for a career on the bench and/or field. It is imperative that the industry fosters a culture of constant quality improvement and workforce development so that there are qualified individuals committed to fulfilling the requirements of federal public health and environmental testing programs, including the formation of partnerships between utilities and public health entities. When laboratories are undervalued, not only are these programs put at risk, but the purpose behind them are put at risk as well. This problem is only magnified by high turnover rates and hiring challenges, which leaves many laboratories in a constant cycle of training new scientists, only to see them leave soon after. Similar to other industries, the environmental laboratory workforce is aging out and needs to have creative solutions to recruit and retain effective staff. There are immediate industry needs for chemists in radiochemistry and highly-skilled chemists to conduct analyses for PFAS and other contaminants of emerging concern. Education is key for effective workforce development, and may occur through public outreach, internships, fellowships and citizen science. The industry needs young men and women that understand the role of the laboratory, are passionate about the public health and environmental protection and are willing to spend a career in public service. This poster will serve as a follow-up to the poster presented at APHL Annual Conference 2022 (The Vital Role of the Environmental Laboratory as Public Health Laboratories), by exploring the needs of the industry and how a strong workforce can be developed. It is based on an article written for Journal AWWA by members of the APHL Environmental Laboratory Science Committee, the AWWA Water Quality Laboratory Committee and the WEF Laboratory Practices Committee.

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topic areas depicted on the Framework have been developed into eLearning courses, particularly the Basic Level Core areas, also called the “Gen Eds”, while other content/topic areas are awaiting development. Many of these Gen Eds, in fact, have broad applicability, and can be used across many laboratory programs, not just human and animal food testing.

2. **Assessment.** Individuals can assess themselves against the Standard, while supervisors can also assess their staff against the Standard.
3. **Evaluation.** Existing courses and training programs/materials can be evaluated against the competencies and behavioral anchors, for inclusion within the Standard.
4. **Career Path Development.** Laboratory professionals can essentially “map out” their career advancement.
5. **Staffing Practices.** Laboratory managers and directors can use the Standard for hiring, onboarding, and for creating job descriptions.
6. **Open-Source Catalog.** Existing training materials and related resources can be arranged and categorized according to the Curriculum Framework.

Application of the State Food and Feed Laboratory National Curriculum Standard is critical to a national integrated food safety system, as outlined in FSMA. The Standard can be used for all state food and feed laboratory personnel, thereby assuring a competent laboratory workforce doing comparable work across all levels of jurisdiction. Additionally, use of the Standard will assure a continuous pipeline of competent hires at all levels, from beginning laboratory analysts to laboratory directors.

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Using the State Food and Feed Laboratory National Curriculum Standard

C. Weiss, S. Brace, C. Kaml, G. Wojtala; International Food Protection Training Institute

IFPTI and APHL, in collaboration with FDA, AFDO, and AAFCO have been building the State Food and Feed Laboratory National Curriculum Standard for more than ten years. The Standard is represented on the poster via the State Food and Feed Laboratory Curriculum Framework (LCF), a career-spanning, color-coded schematic of the human and animal food laboratory profession. The Standard is being built by experts representing state and local public health and agricultural laboratories, and federal agencies. The LCF spans five professional levels and depicts the content/topic areas in which laboratory professionals must possess competencies (knowledge, skills, and abilities) to successfully perform their jobs. Content/topic areas are either Core (apply to all laboratory professionals), Program-Specific (apply to laboratory professionals specializing in chemistry or microbiology) or apply to Specialized Laboratories. Each content/topic area of the LCF is built out as a set of competencies, with corresponding behavioral anchors, which act as guidance for competency assessment. There has been significant development of the National Standard, and it is now time to discuss how the Standard can be used within the laboratory environment. Practical applications of the Standard include, but are not limited to:

1. **Training Design and Development.** Many of the content/



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