

LAB MATTERS

analysis|answers|action

Fall 2022 Issue 3



Strengthening the **Global Community** Through Laboratory Surveillance

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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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Welcomes, Salutations and Introductions

I am beyond honored and humbled by the opportunity to serve as APHL's president for the 2022-2023 term. I have been a member of the public health community for over two decades and I have seen so many changes over my career. But nothing had prepared me for the past three years.

When I accepted the president-elect position, we were just starting to catch our breath from two years of perpetual emergency response, both professional and personal. We are now able to actually appreciate what we are as a community, as individual laboratories and as individuals: we have accomplished so much through the COVID-19 pandemic that we can now take the time to determine what we need in order to sustain the work we have done and to keep moving forward.

As president, I intend to work with APHL, the membership and our partners to seek out those resources required to sustain what our laboratory system has developed so far and to enable us to keep building. Prior to the pandemic, our laboratories were regularly facing budget reductions, staffing cuts and high turnover, science ambivalence and data fragmentation. During the pandemic, we had the opportunity to build more infrastructure and partnerships, and showcase our resilience. So in a world post-pandemic, what does that infrastructure look like? Is it providing new equipment to laboratories who need it? Is it training and retaining our multi-talented workforce? Is it providing the public health community with pertinent data rapidly? It is all of that, and a lot more.

We are now in a moment where we can invest in our infrastructure so that in addition to being resilient, we also become robust. While I have always associated the word robust with "having or exhibiting strength," Merriam Webster also defines robust as "capable of performing without failure under a wide range of conditions."

This definition of robust is what I want for our public health laboratories moving forward.

In our fireside chat with Dr. Anne Schuchat at APHL 2022, we were asked about some thoughts and recommendations to respond to future threats. I emphasized the fact that we really need to establish the structure before the event happens is critical. The biggest thing we learned from COVID-19 is that having those relationships with internal and external partners pre-response means you can have the conversations you need to about data reporting and other response components. We need to keep our current public health structure in place, while continuing to forge new partnerships.

Even as we face a new public health emergency, I believe that we can keep moving forward together. We have all come so far over the past two years and not once did we back down from any of the challenges thrown our way. We can collectively maneuver the road ahead—regardless of obstacles or distance—because we can do anything as a community. Our community has recently welcomed over 100 new fellows into the APHL-CDC Public Health Laboratory Fellowship Program. We have received much-needed funds via the CARES Act and the American Rescue Plan for data modernization and infrastructure construction. We will keep pushing for sufficient, adaptable and permanent investments in data modernization, workforce development and physical infrastructure. I believe that with APHL's strategic focus and your determination, we will be able to sustain and build a truly robust public health laboratory system. ■

“We are now in a moment where we can invest in our infrastructure so that in addition to being resilient, we also become robust.”



*Daphne Ware, PhD
President, APHL*

Reimagining Public Health for the World We Live in Now...and the Future

At the beginning of August, I attended the International Conference on Emerging Infectious Diseases in Atlanta, GA. While there, I had the opportunity to not only reconnect with colleagues and peers in the public health community, but to talk about the future of public health laboratories post-pandemic.

APHL's overarching strategic goal for the next two years is to guide public health laboratories into the next normal after the pandemic. But it feels a little like *déjà vu*—we're back in early 2020: a new infectious disease sweeps the globe and we are forced to react “on the fly” to quickly changing parameters. Public health laboratories, once again, find themselves on the forefront of the response, at least initially.

As we saw—and continue to see with COVID-19—public health has a critical need for information. We have systems that encourage detailed fact-gathering to ensure the integrity of data that are collected. And that data is put to good use...eventually. When the first cases of monkeypox were discovered in Massachusetts, a test was already available through the **Laboratory Response Network** (LRN). As LRN laboratories stood up that testing, they also provided training to commercial laboratories on the CDC 510(k) test to assure that there was accuracy and consistency across both the five commercial labs chosen to test by CDC, and the LRN labs.

Created in 1999 to respond to biological and chemical threats and other public health emergencies, the LRN includes state and local public health, veterinary, military and international laboratories. The LRN was built to be ahead of the curve, but there have been so many curves since 1999! Two decades ago there was no communication of health information (or disinformation) by social media, partisan politics remained in the background of the overall public health mission, and the

effects of climate change and movement of humans into animal ecosystems was not as widespread. Our world has accelerated at a pace that was unheard of 20+ years ago, and it continues to do so.

But clinicians requesting testing do not want to go through what is seen as bureaucratic governmental public health agencies—filling out multiple questionnaires—they want to test and give quick results to their concerned patients. The monkeypox test that LRNs have relied on for decades is a labor-intensive manual extraction test, costing more time inside and outside of the domestic laboratory system—and yet monkeypox has been endemic internationally for a very long time. So why didn't we take the time to modernize this test? Because there was not a pressing need for it in our country.

Public health laboratories should be the place to modernize and streamline laboratory testing and processes: they are connected through CDC to assist with pathogen identification and characterization, are able to provide broad testing at early stages in disease diagnostics, and have built-in redundancies to avoid a single point of failure. As a community, we need to address the tension between the gatekeeping function and the urgent need for broad testing in these situations. Our laboratorians would like nothing more than to make their systems more accessible and flexible. And in a world where most everything is “on demand,” why should laboratory testing be any different? The question is how to be faster and more efficient, but to also gather all the information needed at the start of a new outbreak.

We need to re-imagine our laboratory systems to encompass multiple players and partners in the public, private and healthcare sectors, and the academic community. Not only do we need to do it



*Scott Becker, MS
Chief Executive Officer, APHL*

“[I]n a world where most everything is “on demand,” why should laboratory testing be any different?”

now, we need to think more globally about what surveillance is required in other countries to provide an early warning. We never know when the next pathogen—old or new—will present itself, and as we know all too well, disease knows no borders. We must be prepared to do everything in our power to engage and support our communities both inside and outside of the laboratory. No one group can do this alone. It really will take all the partners in our public health community to ensure a healthier world through quality laboratory systems. ■





Strengthening the Global Community Through Laboratory Surveillance

By Jill Sakai, PhD, writer

In May, a small cluster of monkeypox cases was identified in the United Kingdom. Global public health agencies acted quickly, monitoring the outbreak from its earliest days and gearing up for possible wider testing needs.

Emerging amid the spread of yet another variant of **SARS-CoV-2**, the monkeypox outbreak feels a bit like a test of how well public health agencies have learned the lessons of the COVID-19 pandemic. The past few years have made

clear that global collaboration and communication are critical for identifying and managing public health risks, and that a strong system of public health laboratories—in the US and abroad—is central to these surveillance efforts.

That global perspective is a critical foundation for successful public health efforts domestically.

“We have to care about what’s going on outside our borders. Monkeypox didn’t originate here, yet here we are,” said Lucy Maryogo-Robinson, MPH, APHL director of Global Health. “Surveillance is really the best tool we have.”

Well into the third year of the COVID-19 pandemic and staring down an uncertain autumn, the timing is right to build on the momentum of support for public health laboratories, to further strengthen laboratory systems and their place in ongoing global surveillance efforts.

“I think that we are at a turning point now,” said Sébastien Cognat, PharmD, MSc, head of the Public Health Laboratory Strengthening unit of the **World Health Organization** (WHO). “We need to think of how to make sure that all the investments, the momentum, the recognition of the role of laboratories is sustained, ... that laboratories are not seen only as a tool to support patient care or as a tool to support surveillance, but truly as a key element.” At the same time, there is an opportunity to harness that progress to integrate efforts across multiple health sectors toward a more unified, One Health approach that is ready and able to detect future threats.

Centering Surveillance

Even as more testing is now shifting out of the laboratory to clinic and at-home testing, public health laboratories still have a key role to play. Ongoing COVID-19 monitoring—as well as experiences with Ebola and, increasingly, monkeypox—shows that laboratory data can be a useful real-time tool for surveillance.

Many laboratory systems are now leveraging the substantial pandemic-driven investments in infrastructure and capacity to bolster ongoing surveillance efforts. In Africa, for example, when COVID-19 was declared a public health emergency of international concern, only two national laboratories on the continent had validated testing protocols for SARS-CoV-2—in South Africa and Senegal. Over the following months and years, **Africa CDC** spearheaded an effort to build laboratory capacity across the continent.

The team trained laboratorians from more than 40 countries and coordinated partnerships with governments, companies and national and international organizations to mobilize support and supplies. They provided laboratories with guidelines, technical assistance and quality assessment.

At the same time, Africa CDC built capacity for next-generation sequencing (NGS), mobilizing funding and manufacturers to enable molecular surveillance at a network of laboratories across the continent. In 2019, just seven national public health institutes had sequencing capabilities; now 37 countries can do in-country sequencing and the rest can access sequencing support through regional hubs.

In addition to representing an enormous step for the region, this network lays key groundwork for future preparedness efforts, said Yenew Kebede Tebeje, MD, MPH, MSc, head of the Division of Laboratory Systems and acting head for the **Division of Surveillance and Disease Intelligence at Africa CDC**.

“The next step for us will be how to sustain this program, which means applying these technologies beyond COVID-19.”

Similarly, laboratorians in Southeast Asia are thinking about how significant investments in NGS infrastructure and expertise can now be used to support genomic surveillance for multiple pathogens and disease threats, including acute febrile illness, antimicrobial resistance and priority zoonotic diseases such as influenza, rabies, nipah virus and more. Disease-agnostic approaches are an important strategy to improve efficiencies and integrate a One Health focus that spans sectors to simultaneously support human, animal, food and environmental health.

“Right now, we have really good opportunities to develop infrastructure [and] human resources that will last to help with other diseases,” said Ong-orn Prasarnphanich, DVM, MPH, DACVPM, chief of **US Centers for Disease Control and Prevention** (CDC) **Thailand’s One Health Branch**. One example in the region is wastewater-based SARS-CoV-2 surveillance, which is being expanded beyond humans to monitor the environment in Thailand, wildlife in Vietnam, and both bats and the animal-human interface in Indonesia.



A scientist loads samples for testing in Kenya’s Kakuma Refugee Camp. APHL collaborated with CDC and the International Rescue Committee to bring molecular testing for SARS-CoV-2 to the camp’s laboratory (see story on p. 18)

An Environmental View

Wastewater-based surveillance is an important focus in Africa as well, part of the [Global Fund's project STELLAR](#) to scale up testing for long-term strengthening of laboratory systems. Environmental surveillance offers a powerful opportunity to span disease pillars, said Toni Whistler, PhD, a US CDC detailee to the Global Fund. Up to now, she noted, most programs have had a singular focus: HIV, for example, or tuberculosis, or malaria.

"One thing this pandemic has shown us over and over and over again is that, from a laboratory systems point of view, that really doesn't work when you want preparedness."

Environmental surveillance programs are already used to monitor polio and other agents in parts of Africa. "Wastewater is a valuable, complex sample that has a host of molecular and NGS applications, including polio, monkeypox, antimicrobial resistance markers, pan-respiratory disease agents and emerging infectious diseases," said Noah Hull, PhD, MPH, APHL laboratory technical manager for Global Health. "It is also independent of healthcare-seeking behaviors. This is really where NGS and wastewater-based surveillance can intersect and provide valuable—and actionable—public health intelligence."

Newer initiatives in South Africa and Botswana (as well as in the US) have shown the utility of wastewater for COVID-19 monitoring as well.

"Through use of [waste]water-based surveillance systems, countries or public health institutions were able to identify potential new hotspots for COVID-19 before actual cases were realized," said Osborn Otieno, STELLAR project manager and advisor for the Global Fund's Integrated Laboratory Systems Strengthening and Health Security team. That makes the approach "a very critical tool as an early warning indicator before an outbreak or a spike can be reported within any community setting."

Earlier this year, thanks to support from the Global Fund, APHL started wastewater-based surveillance pilots for SARS-CoV-2 in Kenya, Uganda,

Ethiopia and Mozambique. The APHL team has conducted in-country needs assessments and is providing technical assistance to develop detailed protocols for implementation, drawing on protocols that have been used successfully in the US and working with the Ministries of Health and National Institutes of Health to adapt them to local contexts. Sampling and molecular analysis were scheduled to roll out in late summer, alongside efforts to develop local dashboards that would allow national public health institutes to access data in real time.

The project partners work with each country to model the implementation through a bottom-up process. It takes time, Otieno said, "but the beauty of it is that the countries have been able to come up with a scientific protocol that is country-owned and jointly developed through stakeholder participation at the country level."

That ownership is key for the sustainability of these projects. It is also critical when thinking about data management, because considerations and sensitivities around data and information vary from country to country.

"Some countries are open, while others have a lot of rules and regulations on how to manage public health information," Otieno said. In Kenya, with input from stakeholders across multiple ministries, they have been able to develop a laboratory information system interface to automate data collection and transmit it to a national repository to guide decision making, while ensuring data integrity and de-identification.

Importantly, these "environmental surveillance pilots are being implemented at a time where there's a massive decline in testing," Whistler said. In this context, environmental surveillance for enteric pathogens provides a critical window into the course of the pandemic, including tracking the emergence of new variants.

"It's the first time, really, that we've had tools available to us that would allow low-middle income countries or countries with lesser surveillance capacity to ... have a very useful tool at their fingertips for surveillance," she said.

“You cannot spend much time building your capacity during wartime. During war, you go with whatever tools that you have. We need to do a lot of investment during normal time.”

Yenew Kebede Tebeje, MD, MPH, MSc

A Laboratory Ecosystem

To be truly successful, such laboratory strengthening efforts need to occur within the framework of a broader laboratory ecosystem that can streamline disease-agnostic financing, procurement, workforce development and, ultimately, delivery of services.

"The last mile, as we say, is extremely difficult," Cognat said. "If you have done all the steps from research, development, delivery [and] regulations, but then your diagnostics are in laboratories that are not available for most of the population"—due to geographic, financial or information barriers, for example—"the efforts and investments made upstream are totally useless."

Currently, various aspects of that ecosystem fall under purview of a wide range of agencies, ministries and departments. In this fragmented environment, laboratory systems need to have strong leaders who can convey the importance and value of laboratory services to non-experts, including policymakers, Cognat said. Too often, "laboratories are seen as a cost, not as an investment," he noted.

"We need to change this mindset. We need to be able to articulate a new vision, we need to be able to convince decision makers."

This is where the [Global Laboratory Leadership Programme](#) (GLLP) comes in, to foster a new generation of laboratory leaders who can articulate the value of laboratories and advocate for laboratory needs. This program, developed in partnership by the APHL, US CDC, [European Centre for Disease Prevention and Control](#), [Food and Agriculture](#)

Organization of the United Nations, World Organization for Animal Health and WHO, includes a comprehensive framework of core competencies for building resilient, responsive laboratory systems and is now in the pilot phase.

One focus is to make the GLLP a true One Health program, engaging individuals from multiple sectors to work on joint projects that address the needs and strengths of each sector toward a better overall outcome. The goal is not just to know each other, but to know how to work together, Cognat said.

Spanning Silos

Such partnerships are at the heart of many COVID-19 success stories, as the scope, scale and urgency of the pandemic pushed a wide range of groups toward a shared focus. Powerful—and sometimes unexpected—collaborations emerged during the pandemic.

“We did not think that we can really get so many stakeholders together before,” Prasarnphanich said. But forging those connections is critical. Kebede attributes much of the success of Africa CDC’s programs to the authority and buy-in given to the agency by the member states, noting that they were able to convene heads of state 13 times in the last two years in a joint continental effort for pandemic response.

Integration across sectors also holds several advantages for surveillance efforts specifically. One is that, especially in the case of zoonotic disease, early signs of outbreaks can often be detected in animals long before people. Another is the potential cost savings when sectors share costly facilities and equipment, such as sequencing platforms or high-containment laboratories. And in many cases, a richer data set can be obtained by combining inputs from multiple sectors. Signs of antimicrobial resistance, for example, can be detected in animals, people, food products and the environment, such as in sewage, water or soil.

“It’s of mutual benefit,” Cognat said. “It’s not only the animal and environmental sector contributing to human health, it’s also the other way around.”

Within that, it is critical to remember that each organization at a table has its own agenda, priorities, mandates and regulations.

“We cannot really force them to see the same picture,” Prasarnphanich said. “But I think we can frame the big picture together—find a common goal.” COVID-19 is a perfect example: essentially, everyone would like the pandemic to be over. But how each agency and organization can contribute to—and benefit from—that goal will be different. Some organizations might be able to provide training and technical expertise, while others might provide access to new regions to expand surveillance reach. Still others might be best able to contribute financially. “We cannot expect all organizations to have the same level of funding, the same level of human resources or the same level of expertise to offer,” Prasarnphanich said.

“The world benefits by having better ways of sharing data in order to be able to detect [and] respond to emerging health threats”

Xenophon Santas

Likewise, different health sectors work in different contexts, and supporting them may require different approaches and investments. Thinking about surveillance in Thailand, for example, the public health sector already has established platforms to store health data but could benefit from help to utilize their data most effectively and efficiently. The animal health sector may have access to information but lacks a central data management system. And none of these mechanisms may be relevant for the wildlife health sector, where rangers need to be able to collect data in the field.



The Global Laboratory Leadership Programme includes a comprehensive framework of core competencies for building resilient, responsive laboratory systems.

True multi-sector partnerships must be built on mutual understanding and trust that each sector will contribute as it is best able to meet the collective need.

For a true One Health approach, all sectors need to have equally strong seats at the table, said Beth Skaggs, PhD, laboratory team lead of US CDC's Division of Global Health Protection Thailand office. That can be a challenge when the sectors do not have the same capabilities and resources, setting up inequities that must constantly be addressed. Continuity of funding is a constant challenge. In the absence of a threat like the pandemic, funding for surveillance gets cut, Skaggs said.

"We end up having to do patchwork approaches to sustain surveillance when things are calm—which is unfortunate, but that's the reality," she said. Continuous, reliable funding for sustained surveillance efforts would go a long way toward easing imbalances. So would an established data system compatible across sectors and data-sharing agreements that allow real-time analysis for decision making.

Data Modernization

Despite clear benefits to the global population when countries share surveillance data, such as through the [GISAID platform](#), data sharing is an ongoing challenge. Country-specific laws and concerns about data privacy and security can create barriers to transparency and openness, and there are often delays. For avian influenza surveillance work in Thailand, Prasarnphanich said, it took six months to a year to get the needed representatives from both public health and animal health sectors on board before establishing a platform for sharing information. That investment is key to building the foundational relationships, Prasarnphanich said. "Once that platform [and] the trust is established, I think it's easier to continue that kind of communication among different sectors."

It's well known, though, that infrastructure to support public health data collection—whether in support of surveillance, response or even research—is in dire need of updating and expansion.

Those needs have been exacerbated by the demands of COVID-19, said Xenophon Santas, associate director of Informatics at US CDC's [Center for Global Health](#). After years of advocacy work by the APHL and other partners, CDC's [Data Modernization Initiative](#) received a large infusion of funding through the CARES Act and the American Rescue Plan to update public health data infrastructure and processes throughout the US. In recognition that other countries face similar challenges, the American Rescue Plan included \$140 million over three years for a project that will expand similar efforts globally.

Now, Santas said, there is an opportunity to assist global partners to modernize and strengthen their data infrastructure and data governance to better enable data collection and sharing. "The world benefits by having better ways of sharing data in order to be able to detect [and] respond to emerging health threats."

The CDC-led and APHL-supported project adopts a two-pronged strategy: focus on solutions that can demonstrate immediate impact and serve as proof-of-concept for one or more countries, while simultaneously working on longer-term governance initiatives to ensure sustained and continued use of modernized systems and approaches. Multiple components will address data automation to streamline electronic capture of laboratory results internally within a testing laboratory and external reporting from a testing laboratory to relevant public health systems and stakeholders, electronic laboratory reporting, and data analytics for rapid and informed decision making.

APHL will help each host country government define a locally appropriate system architecture for consolidating data and systems. Importantly, the project will also draw on lessons learned from the US Data Modernization Initiative to address concerns around cloud hosting of laboratory data. These issues include the use of cloud infrastructure and shared services, required governance, and legal and policy development to protect data ownership, storage and sharing. Additional components will target workforce strengthening and coordination and communication, with efforts made to adapt progress from the US Data

Modernization Initiative. These efforts are underway in 10 countries: three in Asia, four in Africa and three in Latin America.

"Data infrastructure needs to be thought of as a consolidated activity, not a one-off, so that we're not reinventing the wheel every time we have a new public health threat," Santas said. An effective information management system should be in place before it's needed. "Prior to the current modernization efforts, it could take longer to design and stand up a system than it would for an outbreak to mature and peak and be finished."

Preparing for the Future

Ultimately, the global community is interconnected, and now is the time to build strong structures and partnerships to sustain that community through whatever is coming next. "You cannot spend much time building your capacity during wartime. During war, you go with whatever tools that you have," Kebede said. "We need to do a lot of investment during normal time."

That also means shaping preparedness efforts around the priorities that should be reflected during active outbreaks. Prasarnphanich's vision is to formalize One Health within the structure of organizations and integrate interagency coordination as part of the routine work of each: "To make One Health not just an approach, but more of the culture," she said.

Asked what a successful surveillance program would look like, Cognat emphasized real-time information sharing in support of decision making, with mutual trust across sectors. It's also essential, he said, to ensure that the people and systems who are providing the data receive equitable benefit from the vaccines, therapeutics and knowledge that emerge from that data.

"At the moment, we can't tell what will come next. Robust surveillance activities will be a key tool to identify potential future pandemics and elicit public health response in very good time," Otieno said. "There have been a lot of lessons learned from COVID-19, and we should not make a repeat of those same mistakes." ■

The “Greatest Threat Facing Humanity” and Public Health Labs

By **Nicholas Ancona**, MPH, specialist, Environmental Health and **Erin Morin**, MHS, associate specialist, Environmental Health

According to the World Health Organization, “**climate change is the single biggest health threat facing humanity.**” It increases the occurrence of heat waves and other extreme weather events such as storms, flooding and wildfires, which can all negatively affect the public’s health.

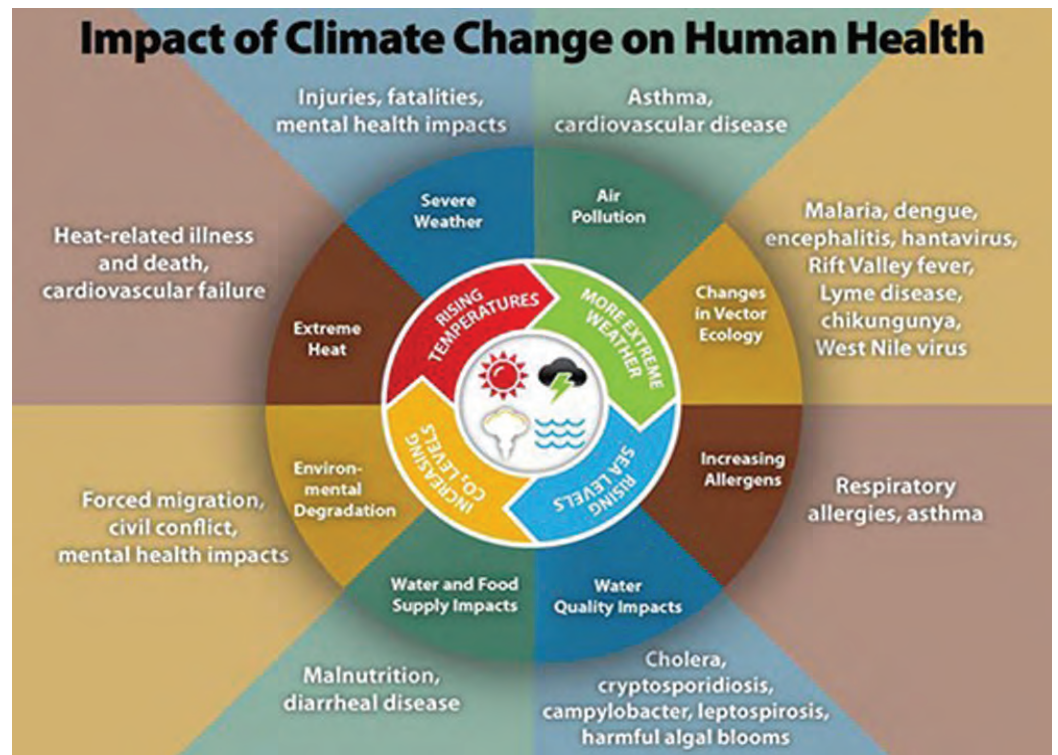
Many tests public health laboratories already perform will have even more relevance with the changing climate on regional, national and international scales. Given the established testing infrastructure and scientific expertise already in public health laboratories, they have the capability to **expand their testing to meet existing and future climate change data needs.**

But climate change testing and data needs require an enormous investment to start connecting federal, state and local efforts. While federal climate change funds were not expanded in 2022 as expected, APHL’s Environmental Health Committee (EHC) and Environmental Laboratory Science Committee (ELSC) have been incrementally exploring how public health laboratories can connect their everyday missions to address climate change.

Committees Discuss Climate Change

Public health laboratory testing was generally established to understand direct cause and effect. And while it appears obvious that public health laboratory data would help contribute to understanding how climate change affects health at local, regional and national levels, climate change is a giant entangled web of interconnected direct and indirect causes and effects. It takes work to begin understanding these connections and using them to justify expanding testing volume and capabilities.

At their annual EHC and ELSC meetings in September 2021, members learned about the **US Centers for Disease Control and Prevention’s (CDC’s) climate change initiatives:**



CDC National Center for Environmental Health, Impact of Climate Change on Human Health.
Source: <https://www.cdc.gov/climateandhealth/effects/default.htm>

- **Climate and Health Taskforce**, which is working to determine how climate change cross-cuts through all of CDC’s programs
- **National Environmental Public Health Tracking (NEPHT)** network, which has the potential to store state environmental health data all in one place by consolidating state dashboard information
- **Building Resilience Against Climate Effects (BRACE)** program, which helps communities prepare for health effects caused by climate change.

They also learned how the **US Department of Health and Human Services** is working to understand the environmental justice implications of climate change, and of the US Environmental Protection Agency’s **list of climate change indicators** and how health impacts are covered in its **Climate Change and Risk Analysis** project.

A major part of moving forward to connect public health laboratory data to these federal climate efforts is understanding which tests provide useful data for climate purposes. Based upon EHC and ELSC presentations and discussions, a list of climate change exposure pathways that have connections to public health laboratory testing was developed (see chart).

Access Information: Climate for Health Ambassadors Training Course

This training course can help public health laboratory scientists understand and communicate the connections between climate and health and potential actions to help address these challenges. Use the registration information included here to access the course free of charge.

Registration Link: <https://ecoamerica.litmos.com/self-signup/>

Registration Code: CfHAmbassadorAPHL

Public Health Laboratory Section	Examples of Increased or Expanded Tests Due to Climate Change Impacts
Environmental Health	Ambient air: ground-level ozone due to heat, particulate matter due to drought and wildfire, and toxins due to urban wildfires.
	Indoor air: mold due to floods and disasters and radon due to increased time inside because of extreme weather.
	Air, water and soil: chemical contaminants due to increasing pesticide use, chemical exposure during disasters, and increased concentrations in water and soil due to both drought and flood conditions.
	Drinking, ground and surface waters: fecal indicators and pathogens such as <i>Cryptosporidium</i> , <i>Giardia</i> , and <i>Naegleria fowleri</i> associated with warmer temperatures and potential biofilm growth.
	Surface and drinking water: cyano- and other toxins associated with increased harmful algal blooms from warmer and more nutrient-dense water from drought conditions or runoff. Marine biotoxins associated with shellfish toxicity will also be of concern.
	Sea surface temperature and salinity: used to assess vibriosis risk due to warmer ocean temperatures, rising sea levels and concomitant increased human exposure to ocean water.
	Temperature and precipitation data: can assess risks for microbe growth and accompanying disease, such as Valley fever.
	Human biomonitoring: test for markers indicative of chemical exposure, wildfires and biotoxins.
Infectious Diseases	Vector-borne diseases: malaria, dengue, encephalitis, hantavirus, Rift Valley fever, Lyme disease, chikungunya, West Nile virus, Zika virus due to expanded ranges of mosquitoes, ticks and other vectors.
	Zoonotic diseases: plague, rabies, bird flu, Ebola and anthrax due to warming temperatures and expanded populations of the disease-carrying animals.
	Fungal diseases: Valley fever, histoplasmosis, and <i>Candida auris</i> due to increasing geographic range and other effects such as dispersal and host susceptibility.
	Respiratory diseases: increased air pollution which compromises respiratory system function.
Food Safety	Shellfish, fish, meat, and other foods: waters with increased concentrations of microbiological (e.g., <i>Salmonella</i>) and chemical contaminants due to drought conditions or runoff from heavy rain events.
	Shellfish testing: cyano- and other toxins due to increased harmful algal blooms from warmer and nutrient dense water.
	Foodborne illness: warmer temperatures and other effects through clinical testing.
Public Health Preparedness & Response	Preparing for, and responding to natural disasters and chemical, biological, radiological and nuclear (CBRN) events.

Voices of Scientific Integrity

As public health laboratories understand the connections between their work and climate change, and the impact it may have, they may be able to capitalize on opportunities to speak with other public health stakeholders to support climate change indicator testing. EHC and ELSC met again in May 2022 to develop climate leadership and communication skills. APHL collaborated with EcoAmerica, a non-partisan, non-profit organization building public support and political resolve for climate solutions in the US, to provide [interactive, competency-based training](#) to EHC and ELSC members. The didactic training course focused on tailoring effective messages for multiple audiences, including professional colleagues, policymakers and the public.

Resources For Climate Change-Related Testing

The following resources developed by APHL and APHL committees for other purposes will help to support increased and expanded testing needs due to climate change:

- [Naegleria fowleri: Public health response to municipal water-associated cases in Texas and Louisiana](#), informs laboratories and other stakeholders about the amoeba that, while still very rare, has become more prolific in southern states. Cases have now occurred in states as far north as Minnesota, likely due to rising temperatures warming municipal and recreational waters.
- [Cyanotoxins: A Guidance Document for Public Health Laboratories](#) was recently updated due to occurrence of harmful algal blooms in all 50 US states.
- [APHL's Vector-Borne Diseases](#) as a general resource.
- [Testing Methods for Eastern Equine Encephalitis](#) fact sheet to highlight the importance of Eastern Equine Encephalitis virus (EEEV) and its impact on public health.
- [“So, You Want to Make Your Laboratory More Sustainable...”](#) webinar provides a variety of tips to laboratories on how to decrease their contributions to greenhouse gases due to high energy needs. ■

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Improving Foodborne Outbreak Response and Solving More Outbreaks

By Kirsten Larson, manager, Food Safety

Since 2006, [The Council to Improve Foodborne Outbreak Response \(CIFOR\)](#) has worked to improve methods to detect, investigate, control and prevent foodborne outbreaks (FBOs). As a founding member, APHL regularly proposes new projects based on opportunities for improvement as discerned by members and staff. APHL staff, member representatives and laboratory subject matter experts provide input on CIFOR product development to ensure that tools are accurate, up to date and applicable to the public health laboratory community.

Tools and Resources Designed with Public Health Laboratories in Mind

CIFOR recently developed and updated several tools and products to evaluate program performance, enhance collaboration among stakeholders, improve outbreak surveillance, and provide model practices in FBO investigations.

- [CIFOR Guidelines for Foodborne Disease Outbreak Response \(3rd Edition\)](#)

The CIFOR Guidelines are a comprehensive source of information on foodborne disease investigation and control for local, state, territorial, tribal, and federal health agencies. The guidelines describe model practices for detecting and responding to FBOs and outline the roles of key organizations. Several public health laboratory members contributed to the development of the Third Edition Guidelines, which incorporate significant changes, including:

- The implementation of whole genome sequencing (WGS) and culture-independent diagnostic tests (CIDTs).
- The integration of consumer-complaint systems with pathogen-specific surveillance.

- The importance of after-action reviews.
- Discipline-specific metrics pulled from existing programs such as [FoodCORE](#) and [OutbreakNet Enhanced](#).

APHL is developing web-based modules intended to provide a high-level overview of each chapter in the Third Edition Guidelines. The modules will be available on the [APHL Training Portal](#) in fall 2022.

• [Third Edition CIFOR Toolkit](#)

The 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. The toolkit will help local and state health departments to:

- Understand the CIFOR Guidelines
- Assess outbreak detection and investigation procedures
- Implement appropriate recommendations.

APHL recommends that public health laboratories meet with epidemiologists and environmental 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. Members who are interested in hosting local or regional CIFOR Toolkit meetings can contact Kirsten Larson at Kirsten.larson@aphl.org.

• [Outbreaks of Undetermined Etiology \(OUE\) Guidelines](#)

The OUE Guidelines are intended to provide adequate FBO specimens for second-tier testing and pathogen discovery should an etiology prove elusive. Recommendations include:

- Specimen collection and shipping protocols
- Suggested rule-out testing
- Storage criteria for negative specimens.

OUEs present an opportunity for public health investigators to expand our understanding of foodborne disease etiology, detect widespread CIDT failure due to mutations in detection targets, and identify practical and exciting pathways for public health laboratories to enter the world of metagenomics.

The [US Centers for Disease Control and Prevention](#) (CDC) is currently developing an advanced metagenomics pipeline for foodborne OUEs. Once developed and validated, the pipeline will be made available to state and local public health laboratories. APHL is excited to be working with CDC to help validate and roll out the pipeline in a phased approach, with Phase 1 beginning in the fall of 2022. Public health laboratories interested in participating in this project are encouraged to contact Kirsten Larson at Kirsten.larson@aphl.org.

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-discipline professionals working within their jurisdiction. ■

Leveraging Bioinformatics Tools to Dive Deep into *Clostridium perfringens*

by **Robyn Randolph**, senior specialist, Food Laboratory Accreditation and **Adom Yusuf**, associate specialist, Food Safety

Bill Wolfgang is usually knee-deep in whole genome sequencing (WGS) data while working in the Bacteriology Laboratory in the New York Department of Health's Wadsworth Center. This work covers many different pathogens, from *Salmonella* to *Mycobacterium* species to *Staphylococcus*. One day, when he was reviewing laboratory data on a *Clostridium perfringens* sample he began to wonder: why don't we sequence our isolates of this pathogen?

While *C. perfringens* doesn't get the publicity afforded to common foodborne pathogens such as *Salmonella* or *Listeria*, this bacterium is one of the most common causes of foodborne illness in the country. There is currently no national surveillance system for *C. perfringens*—these cases are investigated locally. Wadsworth laboratory scientists confirm that a sample is *C. perfringens* and the epidemiologists start the outbreak investigation—they typically find a linkage to a contaminated product, and that's it. "Why bother with sequencing?" was the traditional line of thinking.

Changing the Narrative

Wadsworth Center has long been a contributor to *PulseNet* and, more recently, to *GenomeTrakr*, two surveillance networks that use the power of whole genome sequencing to link human illnesses with contaminated product. Wolfgang and his team wondered if, by not sequencing *C. perfringens*, they could be missing critical information that could verify New York's illness case clustering. They decided to investigate this hypothesis that WGS-based subtyping can benefit epidemiological investigations of *C. perfringens*, as it has with other enteric organisms.

They used FDA funds to support this investigation, using *GalaxyTrakr* and *NCBI Pathogen Detection* for data analysis; these open-source bioinformatics tools do

not require a centralized national surveillance database for comparison and can be operated by personnel who have limited bioinformatics skills. While *GalaxyTrakr* and *NCBI Pathogen Detection* tools had historically been used only for *GenomeTrakr* organisms (e.g., *Salmonella*, *Escherichia coli*, *Listeria*), the Wadsworth team wanted to show their broader applicability in other pathogen investigations.

The laboratory retrospectively analyzed 52 patient and food isolates associated with 13 foodborne outbreaks, and 24 sporadic isolates collected over the past decade. They built phylogenetic trees using both *NCBI Pathogen Detection* and *GalaxyTrakr*'s *CFSAN* single nucleotide polymorphism (SNP) pipeline. This analysis helped determine if the phylogenetic clustering aligned with the epidemiologically defined outbreaks.

Surprising Results

They found that the genomic clustering lined up well with the epidemiologic clustering. The phylogenetic trees generated from both the SNP pipeline and *Pathogen Detection* agreed with one another. They discovered a restricted SNP distance between 0-20 SNPs within a cluster that is associated with an outbreak, which can help identify a sample that should be included in a cluster from a sporadic case. Out of the 13 outbreaks associated with these isolates, there were four outbreaks that had samples included epidemiologically that were not genetically related, as well as two "outbreaks" where none of the associated samples were genetically related. This WGS data was of great



Clostridium perfringens

value to their epidemiological colleagues, helping to better define outbreaks and hopefully find related cases and sources of contamination more quickly.

For Wolfgang and his team, this successful project relied on the great support received from *GenomeTrakr* and those supporting the *GalaxyTrakr* SNP pipeline. Their willingness to quickly and efficiently answer questions or issues that occurred allowed Bill's team to successfully move forward. Their findings were published in the January 2021 issue of *Journal of Clinical Microbiology*.¹ Based on this project, Wadsworth plans to use *GalaxyTrakr* tools during future *C. perfringens* investigations to assist their epidemiologists and protect public health. ■

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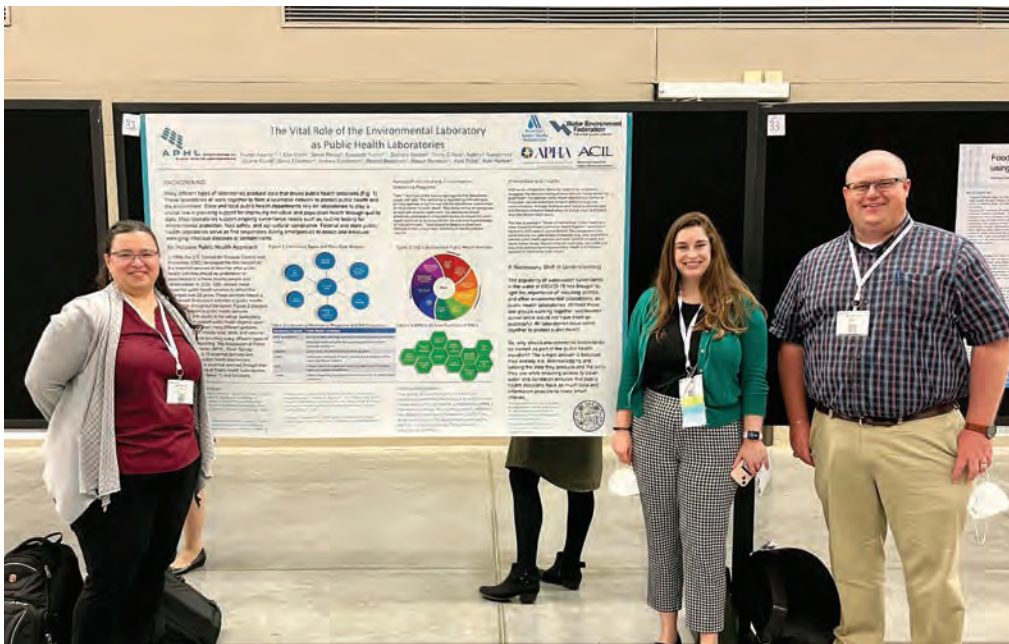
APHL Members Collaborate with Water Sector Partners

by **Hunter Adams**, environmental laboratory supervisor, City of Wichita Falls, TX; **Kathryn Wangsness**, quality assurance manager, Arizona State Public Health Laboratory; **Erin Morin**, associate specialist, Environmental Health; and **Sarah Wright**, manager, Environmental Laboratories

In 2015, the APHL Environmental Laboratory Science Committee began an informal collaborative partnership with the **Water Environment Federation (WEF)** Laboratory Practice Committee to identify collaborative opportunities within the environmental laboratory community to promote public health. They were joined by the **American Water Works Association (AWWA)** Water Quality Laboratory Committee in 2019. Through this partnership, overlapping topics of mutual interest were identified, resource dollars were maximized, and consistent messages were communicated across this important public health sector that has not historically been well-linked.

Webinars

- **“Coliphages: What Will You Need to Know and How Will Laboratories, the Regulatory Community, and the Public be Impacted?”** Since the majority of recreational water contact illnesses are caused by enteric viruses, data have shown that a viral structure such as coliphage may be more representative of the recreational water quality viral load. The webinar was held in preparation for the then-expected 2018 release of **US Environmental Protection Agency’s (US EPA)** recreational water quality coliphage criteria. While these criteria have still not been released, lots of preparation is needed to transition to test for, interpret and apply viral (coliphage) instead of bacterial indicators to recreational water quality management. Multiple speaker perspectives provided a comprehensive view into how this shift would affect the water laboratory sector.
- **“Calculating & Using Method Detection Limits” (MDLs).** MDLs are used to ensure laboratories measure analytes at a consistent sensitivity, because no two analytical instruments are



(from l to r:) Kathryn Wangsness, Erin Morin and Hunter Adams present a poster on the *WE&T* article “The Vital Role of the Environmental Laboratory as Public Health Laboratories” at APHL 2022

identical. Because US EPA updated the MDL procedure in 2016 (40 CFR Part 136), discussions between APHL, WEF and AWWA on how laboratories were incorporating and implementing the process determined that there was a need to better understand the updated process. The webinar’s goal was to define the importance of MDLs for use within the laboratory and as a basis of regulatory decisions, with an interactive component in which participants calculated MDLs utilizing a calculation spreadsheet and provided data.

- **“Ethics for the Environmental Laboratory.”** The **National Environmental Laboratory Accreditation Program (NELAP)** is the main US accreditation standard for laboratories performing water and wastewater analyses, with over 2,000 laboratories in 47 states and four countries participating. As part of this

accreditation, the 2016 TNI Standard Quality Systems General Requirements, Volume 1, Module 2, requires annual data integrity and ethics training for analysts. This webinar’s goal was to provide a recorded training that could be used to fulfill this requirement.

Publications

- APHL published **Cyanotoxins: A Guidance Document for Public Health Laboratories** in 2021 as an update to the 2017 edition, providing resources for environmental

AWWA and WEF are international US-based organizations that promote the research, training and dissemination of knowledge of water and wastewater practices. AWWA and WEF members include drinking water and wastewater utility laboratories, which do many of the same water tests as state environmental and public health laboratories.

and public health laboratories considering implementation of freshwater cyanobacteria and cyanotoxin testing. Federal, state and World Health Organization guidelines have been updated, as well as sections on sampling, methods, method limitations, partnerships, reporting and public messaging.

- Partnership members collaborated on “**Ethics Training is Essential for Environmental Laboratories**,” published in the November 2021 issue of *Journal AWWA*, to emphasize the importance of ethical practices for laboratories producing data used for public health purposes. Proper ethical practices help laboratories provide proof of data accountability and establish acceptable practices for laboratories to produce reliable and defensible data. It highlights steps to be taken to better ensure ethical laboratory practices, such as ethics training, data review, policies, codes of ethics and internal audits. This article, along with other ethics resources, can be found on [APHL's Environmental Laboratory Ethics Resources Page](#).
- In 2022, members collaborated with the [American Public Health Association](#) and [American Council of Independent Laboratories](#) on “**The Vital Role of the Environmental Laboratory as Public Health Laboratories**,” published in the March 2022 issue of *WE&T*. It outlines how state and local environmental, agricultural and food laboratories, and commercial laboratories are all integral parts of the public health system. While not commonly viewed in this light, the COVID-19 pandemic has brought wastewater surveillance to the forefront as a public health tool and has highlighted many other public health-related programs these laboratories participate in. It calls for a shift in this understanding by

acknowledging and valuing their role in producing quality data that provide the foundation for public health decision making.

Lab Chats

WEF started Lab Chats in 2021, connecting laboratory analysts across the United States to discuss a specific analysis. The first Lab Chat, covering total suspended solids, was attended by about 30 WEF members. Analysts were able to describe how they have the total suspended solids (TSS) analysis set up in their laboratories as well as ask questions about best practices. APHL members were invited to the second event in May 2022, which covered biochemical oxygen demand. This event was attended by over 50 WEF and APHL members and saw a high participation level as individuals asked questions and received input from laboratories all over the US. These hour-long events are planned to continue every quarter, and have provided an avenue for scientists to network, share ideas, questions and solutions on how to make processes in their own laboratories more effective.

Water and wastewater industry stakeholders may not always think of themselves as being a part of public health, but their data contributions are vital for protecting public health. The resources that have been produced from this partnership have been circulated to over 145,000 scientists, engineers, consultants, administrators, and water and wastewater system operators. If only a small fraction of these individuals use these resources and start to view their work through a public health lens, the industry will change for the better.

If you have questions, please contact Sarah Wright at sarah.wright@aphl.org. ■

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Building SARS-CoV-2 Molecular Testing Capacity in Kenya's Kakuma Refugee Camp

by **Nelson Akenga**, senior informatics specialist, Global Health; **Maria Landron**, senior specialist, Global Health; **Samantha Musumeci**, senior specialist, Global Health; **Rufus Nyaga**, deputy country director, Global Health; **Edwin Ochieng**, country director, Global Health and **Jully Okonji**, senior laboratory specialist, Global Health

The Kakuma Refugee Camp is the largest camp in East and Central Africa. Through an APHL initiative, in collaboration with the **US Centers for Disease Control and Prevention (CDC)** and the **International Rescue Committee (IRC)**, a molecular laboratory for **SARS-CoV-2** testing was established in May 2022 via molecular testing training and mentorship.

APHL procured the necessary equipment and supplies, ensured delivery and installation and trained the staff in proper protocols and procedures. To date, the laboratory has successfully processed more than 500 samples which have contributed to high throughput. Previously, a single run of four on

GeneXpert would take 45 minutes, compared to 93 samples in one hour using the QuantStudio5. The turnaround time for results was reduced from 72 hours to nearly 24 hours. And for the first time, the laboratory has the capacity to conduct real-time polymerase chain reaction (RT-PCR) molecular testing.

To ensure the quality of laboratory results, APHL initiated quality improvement initiatives that focused on quality manual development, standard operating procedure development, equipment maintenance and biosafety/biosecurity best practices adherence. APHL also trained the laboratory staff on how to report their data to the National COVID-19

Data Center, and carried out a laboratory information system assessment to establish core laboratory requirements and how they could be enhanced in the established electronic medical record system.

Using lessons learned in Kakuma, APHL is currently working towards establishing RT-PCR molecular testing capacity at the Dadaab Refugee Camp Laboratory. ■



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The new APHL Infectious Disease Laboratory Conference provides a forum to discuss the latest developments on detection and characterization of infectious diseases of public health concern. It's a new opportunity for public health laboratory professionals, clinical laboratorians, leading researchers and diagnostics developers to share their latest findings, technologies and developments and generate solutions to infectious disease problems.



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The New Era of Public Health Bioinformatics

by Joel R. Sevinsky, PhD, founder and CEO, Theiagen Genomics

Next-generation sequencing (NGS) and bioinformatics are transforming public health, and in no discipline is this more apparent than in infectious disease surveillance. It is now common practice for public health scientists to rely on variant distributions of SARS-CoV-2 along with case counts to help inform public health decision making during this pandemic. Additionally, if used properly, this kind of data can dramatically increase the efficiency of disease control work.

But two years ago, such uses of molecular epidemiology were limited to a more select group of pathogens. While strategic investments from the Office of Advanced Molecular Detection and the PulseNet program at the US Centers for Disease Control and Prevention (CDC) has dramatically improved access to and development of NGS technologies and bioinformatics for infectious disease surveillance, the current pandemic highlighted how much work still needs to be done. Many laboratories were ready to sequence this new pandemic pathogen, but were not ready to analyze the data to drive public health actions. Fortunately, cloud computing technology is rapidly accelerating, bringing new tools, paradigms and infrastructures that are dramatically transforming public health scientific computing.

A Revised Perspective

Key technologies contributing to this transformation are:

1. Encapsulation of standardized algorithms and their dependencies in containers (i.e., Docker, Singularity).
2. High level, reproducible data flows between containers using workflow description languages (i.e., WDL, Nextflow, CWL)
3. Workflow orchestration platforms to effectively manage the resources in a scalable fashion (i.e., Terra.bio, Nextflow Tower).



These technologies collectively provide an open source framework with community support. For example, bioinformatics workflow and container contributions from the State Public Health Bioinformatics group (StaPH-B) and Theiagen Genomics allow for the democratization of high-performance computing, so every laboratory can have access to a high-performance scientific computing environment for their public health needs.

The technical highlights of these systems include their scalability, portability and standardization. This allows for anyone, anywhere with an internet connection, to perform a reproducible analysis on their pathogen genomic sequence. There are non-technical benefits as well. In government systems where high-performance computing is traditionally associated with capital investments, information technology support contracts, and large upfront costs, these new infrastructure models transform scientific computing resources into an inexpensive consumable that can be budgeted based on specimen volume. Most analyses cost less than a dollar, a tiny fraction of the costs required to generate the raw data in the laboratory (i.e., sequencing instruments, service contracts, consumables and labor).

Change for the Better

We have clearly seen the effectiveness of these advances: in less than 18 months the US public health system has seen an adoption of Terra.bio into almost 50

laboratories at the state and local level for SARS-CoV-2 genomic surveillance. Much of this was achieved through collaborations between the Broad Institute and Theiagen Genomics, along with financial support from federal and state contracts. The simplicity of Terra.bio allowed many laboratories without any bioinformatics experience to be trained in less than 90 minutes on open source bioinformatics workflows in order to generate meaningful data from SARS-CoV-2 genomic sequences. Now that public health laboratories are realizing the potential of cloud resources for infectious disease surveillance, work is already underway to bring other pathogen surveillance systems into cloud-based infrastructures, including Enterics, Mycobacterium tuberculosis, HIV, healthcare associated infections (HAIs), Candida auris, Legionella and others.

The pandemic has taught us it is difficult to predict where things will go next. However, in the area of scientific computing for public health, the evidence is clear that containerization, workflow managers and workflow orchestration environments are here to stay. Public health leaders/scientists are excited that the traditional requirements for genomic surveillance (e.g., supercomputers and bioinformatics professionals on site) are no longer barriers to entry. Furthermore, as these technologies continue to mature and harmonization efforts between the two competing orchestration platforms—Terra.bio and Nextflow Tower—progress, it will usher in an era of standardized workflows that can be run anywhere, on any system, at any time, by anyone. ■

Theiagen Genomics is an APHL Platinum Level Sustaining Member.

SHARPening Efforts Against Antibiotic Resistance

by **Nikki Marchan**, senior specialist, Antimicrobial Resistance

In 2021, the **US Centers for Disease Control and Prevention (CDC)** received funding under the American Rescue Plan for the **Strengthening Healthcare Associated Infections/Antimicrobial Resistance (HAI/AR) Program Capacity (SHARP) supplement**. Originally funded at the beginning of the COVID-19 pandemic through the **Epidemiology and Laboratory Capacity for Infectious Diseases Cooperative Agreement (ELC)**, the SHARP supplement provides dedicated funding to leverage the existing structure of the **Antibiotic Resistance Laboratory Network (AR Lab Network)** to expand laboratory capacity for early detection of AR pathogens and public health infrastructure for rapid response to stop transmission.

“These funds support critical infrastructure vital to the success of expansion, including support for testing coordination, clinical laboratory engagement and data infrastructure,” said Stephanie Gumbis of CDC’s Antibiotic Resistance Coordination & Strategy Unit. “SHARP aims to reinforce public health capacity and is a vital component to ensure rapid detection and reporting to support prevention and response efforts to address AR threats.”

Increasing Capability and Capacity

SHARP will not only increase the local, state and federal responses to threats, but it also highlights the appreciation for the collaborative effort needed to effectively respond to growing threats. SHARP will allow the 56 jurisdictions of the AR Lab Network to increase their laboratory capacity to perform CLIA-compliant testing and characterization of pathogens deemed urgent and serious threats by the most recent **AR Threats Report**. Funding also provides the opportunity for non-regional laboratories to expand capacity even further through optional, specialized testing that previously resided within the seven regional laboratories. Non-regional laboratories who elected to



expand capacity further will now receive funds to:

- Support state-led epidemiologic investigations and HAI/AR prevention efforts focused on screening targeted AR threats.
- Perform whole genome sequencing to support containment priorities.
- Support surveillance activities and outbreak investigations.
- Monitor suspected treatment failures for *Neisseria gonorrhoeae* cases.

And with dozens of laboratories electing to bring on more advanced testing, the expansion provides for a stronger laboratory network.

Putting the Proper People in Place

In addition to funds provided for increased laboratory testing, SHARP also provides funds to all jurisdictions for dedicated staff to work on complementary AR activities to ensure a robust network. One funded position is the AR Coordinator, who works with laboratory staff, epidemiologists, healthcare facilities and regional laboratories to coordinate the many intricacies involved with AR activities, such as communication,

collaboration and outreach between the different groups.

“All of the testing that happens through the AR Lab Network requires so much more than the actual laboratory work,” said Emily Schneider, regional epidemiologist for the AR Lab Network West, who also coordinates epidemiological and testing activities within the West region. “With the high volume of testing, many submitters and the desire to get results out as quickly as possible, it is vital to have a dedicated point of contact, a clear and organized approach for coordination, and a strong laboratory-epidemiology relationship. The opportunity to bring on a dedicated AR Coordinator will be fundamental to building up laboratory capacity to effectively and efficiently serve local, state, regional and federal partners.”

In addition to supporting an AR Coordinator, AR Lab Network jurisdictions also received funding to support a dedicated AR informatics expert. This position will work to ensure information technology infrastructure for timely reporting to all relevant parties, work towards transitioning to HL7 messaging to CDC for all testing, and work directly with APHL to implement reporting via the **AIMS Platform** and a web-based electronic test order and result portal.

“As the AR Lab Network program grows, the need for informatics to work closely with [epidemiologists] and [laboratorians] has become much more apparent,” said Liore Klein, the Laboratory-Epidemiology Coordinator of the AR Lab Network Program at the **Maryland Department of Health Laboratories Administration**. “By carving out space within SHARP for an informatics expert (or experts), you can really have someone take ownership of AR informatics and make sure future development efforts not only make sense with the technologies we have but are best suited to the needs of laboratorians, epidemiologists and programmatic partners.” ■

APHL Supports Professional Growth Through Meeting Facilitator Workshop

by **Ruthanne Sheller**, MPH, manager, NewSTEPS; **Dara Plath**, associate specialist, Newborn Screening & Genetics; **Chenelle Norman**, MPH, ASQ CMQ/OE, manager, Newborn Screening Quality Improvement and **Fran Altmaier**, BSW, Arizona Department of Health Services

The ongoing COVID-19 pandemic is dramatically changing the landscape of in-person meetings, presenting new opportunities and challenges for organizers and attendees. In response to this shifting landscape, APHL hosted an in-person Professional Development Workshop in Silver Spring, MD May 5-6. The workshop equipped attendees, including APHL members and staff, with skills to effectively plan, deliver, manage and evaluate virtual and in-person meetings. Attendees were excited to reunite after a two-year hiatus and actively participated in group discussions, breakouts and exercises.

Workshop Overview

The professional development workshop was initiated at the request of APH Newborn Screening (NBS) Committee and workgroup chairs wishing to develop their facilitation skills and improve engagement. Workshop topics included types of meetings, tools for effective meetings, ideas for leveraging technology, strategies for starting meetings, managing dysfunction and meeting templates. After the workshop, APHL staff scheduled follow-up calls to ensure accountability and the continued success of attendees.

Professional Development

NBS relies on the workforce's commitment to continuous professional development and learning to ensure families everywhere receive quality care. Workshop attendees demonstrated this commitment to professional development—an APHL core value—by participating in brainstorming exercises and strategy discussions to confront challenges to meeting management. One exercise, in which attendees worked in a semi-circle, required them to build off words and phrases presented by their neighbor, with the goal of encouraging attendees to learn from each other.



“Something as simple as clearly stating a meeting purpose rather than just the topic really ensures meetings stay on course.”

Fran Altmaier, Co-chair, Quality Improvement Subcommittee

Service Excellence and Continuous Improvement

Delivering high-quality NBS services to patients and families while continually improving screening and follow-up processes are critical tasks. By focusing on topics including meeting management, leadership strategies and defining action items, attendees learned strategies for more effective meetings to ensure service excellence and continual improvement of NBS.

Innovation, Creativity and Leadership

Innovation, creativity and leadership were the foundation of the workshop's design, so a visually appealing space was chosen to encourage participants to move around and interact with each other. Additionally, workshop facilitators discussed characteristics of an outstanding meeting leader, which included finding a role for all attendees, being knowledgeable and enthusiastic

about the subject matter, and feeling empowered—and empowering others—to grow within their roles.

Accountability

As public health professionals, we are committed to the communities we serve. To demonstrate this commitment and accountability to diverse communities, including underserved groups, staff donated excess food from the workshop to local food pantry Shepherd's Table.

As public health laboratories continue to adjust to a new normal, now is an opportune time to seek opportunities for professional development that provides guidance for navigating engagement via virtual and in-person platforms. ■

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Supporting the Laboratory Response Network in Oregon

By **Rana Rahmat**, specialist, Laboratory Response Network

For some, the start of a career in and the passion for public health are purely accidental. One such individual is Robert Nickla, RBP(ABSA), QLS, M(ASCP), Biological and Chemical Threat **Laboratory Response Network** (LRN) coordinator, biosafety officer, and Select Agent Responsible Official for the **Oregon State Public Health Laboratory**. For Nickla, finding his way into public health was never intentional. His plans were to be a wildlife biologist, as that was the nature of his education and training, but one call back from the state public health laboratory in Arizona during a job search changed the entire course of his life. And he knew from day one that the field of public health was “it” for him, since it was immediately apparent as the “coolest job” and offered something new and exciting every day.

The novelty and excitement of the work have only diversified over the years. Not only does Nickla have several titles and responsibilities within the laboratory, but he also works with APHL as a member of the Public Health Preparedness and Response (PHPR) Committee and Academic Partnerships Workgroup. He also serves as chair of the Sentinel Laboratory Partnerships and Outreach Subcommittee (SLPOS), and co-chair of the Strategic Implementation Group for Recruitment and Retention. His other primary work includes engaging with **sentinel clinical laboratories**, emergency preparedness partners and local health departments, and acting as a laboratory subject matter expert in developing trainings and guidance. He additionally works with local student advisory committees and academic workgroups to lessen the struggles of those starting new careers in public health.

Making a Difference

Among the multitude of responsibilities, one of Nickla’s favorite aspects of his job are the opportunities to directly interact with clinical hospital teams.



Rob Nickla

“I’m 100 percent convinced that what we do as LRN coordinators directly helps the hospitals and has a positive impact on their laboratory preparedness and biosafety considerations.” And one of the facets of work he looks forward to getting back to beyond the pandemic is the return of in-person trainings and the future of virtual reality as it applies to training public health professionals. He is also eager to see what can be done further for his facility and public health department with respect to chemical threat response, outreach and training, since Oregon is a Level 3 LRN-C laboratory and does not have analytical chemistry testing capabilities. Chemical threat response has not received as much attention as its biological counterpart.

Another challenging characteristic of the job is that Oregon is a relatively small facility and, for Nickla, that means being a “one-person LRN department” with many responsibilities. Another issue which resonates with many other public health laboratories is the lack of sufficient time and resources in general to ensure everything that needs to be done is addressed.

When asked about how he thinks the future of public health will change as a result of the pandemic, Nickla stated that COVID-19 certainly helped shine a light on the value that public health laboratories

specifically bring in the world of public health. Yet the new visibility is not without its repercussions.

“The emerging challenge for us is still in the general public and how COVID-19 caused an unexpected public distrust in science.”

Nickla now has a new way to inspire the next generation of individuals joining public health: in September, he joined APHL as manager of the APHL-CDC Fellowship Program. The program seeks applicants interested in starting a new career in laboratory science and provides training to prepare the fellows for impactful careers in public health laboratories. With his background, years of experience and eagerness to assist future public health professionals, Nickla will be a valuable resource.

Taking Advantage of Opportunities

As for hobbies outside of work, Nickla has become more interested in gardening and horticulture in recent years, in addition to home projects that seem to constantly involve building and fixing things. One of his primary pastimes when not spending time outside hiking with his dogs is fixing his vintage motorcycle.

And the one piece of advice he would share?

“Get involved and volunteer with workgroups and committees. It helps you grow as an individual and it helps the overall work of public health.” Volunteering with committees and workgroups has opened a lot of doors to work in a wider variety of areas for Nickla. “Take as many opportunities as you can! ■

Fellows and Mentors Gather at APHL 2022



Fellows and mentors take a break from APHL 2022 and visit the House of Blues in Cleveland, OH

APHL fellows and mentors joined current and past participants in APHL's Emerging Leaders Program for a day of professional development before the start of APHL 2022. In addition to meeting in person for the first time since beginning their fellowships, they participated in an interactive training on giving high quality presentations. Following a discussion on networking methods and skills, they put what they learned into practice during a reception with APHL's Board of Directors and senior staff. Fellows who completed their program terms in 2022 were recognized at the APHL Awards Breakfast on May 19.

APHL completed a record-breaking recruitment cycle for new fellows who started in July 2022. A total of 120 fellows were matched to host laboratories in the following program areas:

- **Bioinformatics**
- **Biorisk Management**
- **Environmental Health**
- **Food Safety**
- **Infectious Diseases**
- **Informatics**
- **Newborn Screening**
- **Quality Management**

We look forward to working with these fellows and their host laboratories over the next year. ■

DIGITAL EXTRA:

For more information see www.aphl.org/fellowships.

APHLTM 2022

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BIOSAFETY

ISO 35001:2019 -- Is Biorisk Management Important to your Organization?

M Downing¹, M Marsico²; ¹ABSA International (American Biological Safety Association), ²Association of Public Health Laboratories

Post-COVID-19, the importance of a comprehensive biorisk management program should be obvious to all public health and clinical laboratories. The International Organization for Standardization (ISO), the world's largest developer of voluntary international standards, introduced ISO 35001:2019 (Biorisk Management for Laboratories and Other Related Organizations) prior to the pandemic. It is the first international standard that defines the requirements of a biorisk management system for laboratories that handle biological agents; it is also intended to complement other ISO standards for laboratories, including quality management systems and ISO 15190 (Medical laboratories—Requirements for safety). This poster will cover features of the standard, the benefits of ISO certification, continuous improvement and management engagement.

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

Building Biosafety Partnerships with Non-Traditional Testing Sites during COVID-19 and Beyond

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

Coronavirus disease (COVID-19) impacted almost every sector, including schools and nursing homes, with many locations opting to adopt waived tests to ensure the safety of its specific population.

Since a traditional laboratory setting is not required to conduct POC tests, non-traditional test providers typically lack the laboratory experience to successfully and safely perform tests. The staff performing these tests may not be able to conduct specific risk assessments to identify the appropriate biosafety mitigation measures to protect themselves from potential exposures along with the training to correctly package and ship infectious materials. In many instances, these non-traditional sites rely on public health laboratories for guidance and support, especially as it pertains to biosafety and testing.

This poster will discuss how APHL, CDC and public health laboratorians have successfully provided outreach, including biosafety training and other resources to non-traditional laboratory sites during the COVID-19 pandemic.

Presenter: Julianne Baron, Science and Safety Consulting, julianne@scienceandsafetyconsulting.com

Building Scientific Capability and Reducing Biological Threats: The Effect of Three Cooperative Bio-Research Programs in Kazakhstan

K Yeh¹, K Tabynov², F Parekh³, E Maltseva⁴, Y Skiba⁴, Z Shapiyeva⁵, A Sansyzbai⁶, S Frey⁷, S Essbauer⁸, R Hewson⁹, A Richards¹⁰, J Hay¹¹; ¹MRIGlobal, ²NSCED, ³EpiPointe, ⁴NCB, ⁵SPCSEEM, ⁶Kazakh National Agrarian University, ⁷Bundeswehr Research Institute for Protective Technologies and Chemical Biological Radiological Nuclear (CBRN) Protection, ⁸Bundeswehr Institute of Microbiology, ⁹London School of Hygiene and Tropical Medicine, ¹⁰Uniformed Services University of the Health Sciences, ¹¹Jacobs School of Medicine and Biomedical Science

Cooperative research programs aimed at reducing biological threats have increased scientific capabilities and capacities in Kazakhstan. The German Federal Foreign Office's German Biosecurity Programme, the United Kingdom's International Biological Security Programme and the United States Defense Threat Reduction Agency's Biological Threat Reduction Program provide funding for partner countries, like Kazakhstan. The mutual goals of the programs are to reduce biological threats and enhance global health security. Our investigation examined these cooperative research programs, summarizing major impacts they have made, as well as common successes and challenges. By mapping various projects across the three programs, research networks are highlighted which demonstrate best communication practices to share results and reinforce conclusions. Our team performed a survey to collect results from Kazakhstani partner scientists on their experiences that help gain insights into enhancing day-to-day approaches to conducting cooperative scientific research. This analysis will serve as a basis for a capability maturity model as used in industry and, in addition, builds synergy for future collaborations that will be essential for quality and sustainment.

Presenter: Falgunee Parekh, MRIGlobal, fparekh@epipointe.com

COVID-19

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 (MHDH). Of 2,098 specimens submitted to GISAID, 322 (15.3%) were from the two university campuses located within the MHDH 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, MHDH 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. MHDH 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

Application of Rapid Whole Genome Sequencing to Identify a SARS-CoV-2 Omicron Variant Outbreak Among Highly Vaccinated Wedding Attendees

DA Wadford¹, L Page², K Mosack², A Bauer³, M Khubbar³, N Balakrishnan³, J Elder¹, N Moss⁴, A Goeldner⁵, I Pray⁵, M Lambert^{5,6}, Y Cruz¹, S Gibbons-Burgener⁵, S Bhattacharyya³, K Johnson², R Westergaard^{5,6}; ¹California Department of Public Health, ²City of Milwaukee Health Department, ³City of Milwaukee Public Health Laboratory, ⁴Alameda County Public Health, ⁵Wisconsin Department of Health Services and ⁶University of Wisconsin Madison

Background: The SARS-CoV-2 Omicron variant was first described on Nov. 24, 2021, with the first detection in the US on Dec. 1, 2021. On Dec. 2, the California Department of Public Health (CDPH) detected SARS-CoV-2 with RT-qPCR spike gene target failures (a proxy for Omicron) from six vaccinated residents returning from a late-November wedding in Wisconsin. Rapid whole genome sequencing (WGS) subsequently confirmed these as Omicron on Dec. 3.

Methods: WGS analysis of available outbreak specimens was performed at the CDPH Laboratory and Milwaukee Health Department Laboratory (MHDH) using modified ARTIC-sequencing protocols, with Nanopore (ONT) technology on Clear Lab (Clear DxTM) or MiSeq (Illumina) platforms. WGS data were processed using the ARTIC bioinformatics pipeline with modifications to

generate consensus sequences, variant calls and perform phylogenetic analysis.

Results: Of 169 wedding attendees, 73 were tested for SARS-CoV-2 and 39 (53%) tested positive. Thirty-five (90%) cases had completed their primary immunization series and 10 had received a booster dose. WGS from 18 of the 35 PCR-confirmed cases found that 17 were Omicron (BA.1 sub-lineage) and one was Delta lineage. Phylogenetic analysis revealed that 13 cases shared identical BA.1 genomes, including the presumed index case who had recently returned from international travel.

Conclusions: The application of rapid WGS to one of the earliest Omicron outbreaks in the US allowed for rapid follow up across state jurisdictions for additional case findings, rapid confirmation and epidemiologic characterization. This multi-jurisdictional collaboration highlights the effectiveness of strong laboratory and epidemiology partnerships to investigate interstate outbreaks.

The findings and conclusions in this article are those of the author(s) and do not necessarily represent the views or opinions of the California Department of Public Health or the California Health and Human Services Agency, City of Milwaukee Health Department/Laboratory, or Wisconsin Department of Health Services and University of Wisconsin Madison

Presenter: Debra Wadford, California Department of Public Health, debra.wadford@cdph.ca.gov

Piloting Air Samplers to Assess SARS-CoV-2 Exposure and Improve Risk Mitigation in Congregate Settings for Public Health Actions

K Beversdorf¹, J Zimmer¹, M Khubbar¹, N Tomaro¹, L Schmidt¹, H Kraussel¹, E Durkes¹, M Ramuta², C Newman², S Brakefield², D O'Connor², S O'Connor², S Bhattacharyya¹; ¹City of Milwaukee Health Department, ²University of Wisconsin-Madison

Introduction: The COVID-19 pandemic has exacerbated concerns regarding the transmission of airborne illnesses, testing for respiratory pathogens and subsequent contact tracing, and employing enhanced genomic surveillance to support public health emergency preparedness and response. As such, there is great interest in developing new technologies and approaches to improve risk mitigation and public health actions. Clinical genomic sequencing and wastewater-based epidemiology have improved SARS-CoV-2 variant tracking and increased the understanding of dynamic community transmission. However, there is still a need for congregate surveillance (e.g., schools, transportation hubs and restaurants) and supporting data related to community spread and outbreak investigations. In collaboration with local and regional partners, the Milwaukee Health Department Laboratory (MHDH) placed aerosol samplers in select congregate spaces with the goal of monitoring SARS-CoV-2, thereby validating an air sampling method as an effective strategy for improving surveillance, assessing exposure and risk mitigation.

Method: Starting in December 2021, Thermo AerosolSense™ Samplers were deployed in several congregate settings in Milwaukee County to monitor for SARS-CoV-2 on a daily to weekly basis. Total genomic RNA from air sample substrates were extracted and tested using the Thermo TaqPath™ COVID-19 Combo Kit, which targets SARS-CoV-2 ORF1a/b, N gene and S gene. Samples with low cycle thresholds (CT < 30) were sequenced at MHDH using Nextera

kit in MiSeq platform (Illumina). Air samplers were decontaminated prior to cartridge replacement if SARS-CoV-2 RNA was detected to ensure there was no carryover contamination and deployed back in the field.

Results: Air sample substrates repeatedly tested positive for SARS-CoV-2, especially during the initial deployments, which coincided with the emergence of the Omicron variant. Samples with low CT values were sequenced and resulted in the confirmation of the Omicron BA.1 lineage. Positive results led to risk mitigation actions, such as increased rapid antigen testing and activating isolation protocols.

Conclusion: Air sampling surveillance of respiratory pathogens via air sampling is a promising technology to help develop risk mitigation strategies and strengthen the core concepts of emergency preparedness and response. This technology would be applicable in congregate spaces such as K-12 schools, childcares, shelters and high-traffic areas, such as airports and other public transportation hubs. While this pilot study focused on the detection of SARS-CoV-2, the method can be expanded to study other respiratory pathogens, such as the influenza and respiratory syncytial viruses. Lastly, air sampling is a promising low-cost approach to detecting airborne diseases in lieu of testing individuals in large groups.

Presenter: Lucas Beversdorf, City of Milwaukee Health Department, lbeversd@milwaukee.gov

Validation and Implementation of Clear Lab Dx Platform in Public Health Laboratory for SARS-CoV-2 Genomic Surveillance

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

Introduction: On September 23, 2020, FDA issued emergency use authorization for Clear Labs Dx in vitro diagnostic system for COVID-19 sequencing. The Clear Dx™ SARS-CoV-2 Test is intended for use by qualified clinical laboratory personnel trained in the real-time PCR and Whole Genome Sequencing (WGS) and in vitro diagnostic procedures. City of Milwaukee Health Department Laboratory (MHD) validated its use in SARS-CoV-2 genomic surveillance in October 2021 for real-time screening and detection of variants.

Methods: To evaluate the performance of the Clear Dx™ WGS SARS-CoV-2 Test, total nucleic acid was extracted on the Thermo Fisher Scientific KingFisher Flex Purification System and the bioMérieux NucliSENS® EMAG®. The purified nucleic acid is reverse transcribed, barcoded, pooled, purified and then sequenced using pre-packaged library prep and sequencing reagents and a MinION flow cell. Thirty SARS-CoV-2 positive specimens with published viral genomes in GISAID were chosen from the MHD specimen repository. Additionally, eight negative specimens and 14 non-SARS-CoV-2 respiratory pathogen positive specimens were analyzed for validation of this assay. Sequencing run data was analyzed via the Nextclade and Pangolin web applications to get genomic characteristics (lineage/clade etc.) for each specimen. Acceptance criteria was 90% genomic coverage and ≥100 depth.

Results: The overall accuracy was 93.3% (28/30). Sensitivity was 93% and specificity was 95%. With a panel of 14 specimens, 100%

repeatability and reproducibility were documented. Since October 2021, MHD has performed whole genome sequencing on 842 clinical specimens that tested positive for SARS-CoV-2 using the Clear Dx™ SARS-CoV-2 Test. Results are uploaded with de-identified patient information on to the national public database (Global Initiative on Sharing Avian Influenza Data (GISAID)) and reported to relevant public health authorities for the actionable outcome. The turnaround time has significantly decreased to an average of 3–6 days compared to other platforms where it takes a minimum of two weeks from the date of specimen collection.

Conclusion: Clear Labs Dx is a fully automated, next-generation sequencing platform and used at the MHD public health laboratories to characterize SARS-CoV-2 and its variants. The instrument plays a crucial role in near real-time genomic surveillance within our jurisdiction. The results are accurate, reliable and efficiently reported for epidemiological investigations to help prevent SARS-CoV-2 transmission.

Presenter: Manjeet Khubbar, City of Milwaukee Health Department, mkhubb@milwaukee.gov

Children Infected With SARS-CoV-2 While the Delta Variant was Dominant in Colorado Had Higher Viral Loads Than in Previous Time Periods

L Bankers¹, D Ir¹, D Tapay¹, E Ho, S O'Brien¹, S Dominguez², A Burakoff¹, S Matzinger¹; ¹Colorado Department of Public Health and Environment, ²Children's Hospital Colorado

After the SARS-CoV-2 Delta variant emerged in Colorado, it rapidly became dominant in June 2021. Adults infected by Delta exhibit higher viral loads and potentially increased disease severity than those infected by other variants. However, little is known about viral kinetics and severity in children infected with Delta compared to other variants. We evaluated relationships between the presence and severity of symptoms, viral load and SARS-CoV-2 lineage among hospitalized and non-hospitalized pediatric SARS-CoV-2 cases in Colorado to determine if children infected with Delta have different symptom severity or viral load compared to those infected by other variants. Viral lineage was determined by whole genome sequencing. To include samples for which we had Ct and symptom data but could not sequence, we performed analyses over two timeframes: pre-Delta (Jan. 1, 2021 – May 1, 2021; prior to prevalence of Delta reaching 80% in Colorado) and Delta (June 15, 2021 – Sept. 29, 2021; Delta prevalence ≥ 80%). We used Ct values as a correlate of viral load and symptomatology was determined by chart review (196 inpatients) or questionnaires (583 outpatients). Finally, we performed phylogenetic analyses of sequenced samples. Children hospitalized due to COVID-19 had lower Ct values (suggesting higher viral load) than those diagnosed with COVID-19 but hospitalized for other reasons (asymptomatic infections, Mann-Whitney U test, p=0.002). During days -3 to 30 of symptom onset, Ct values were significantly lower overall for the Delta compared to the pre-Delta timeframe (p = 0.02, Mann-Whitney U test). When separated into two age bins (0–4 years, 5–19 years), children aged 0–4 years had lower Ct values in the Delta than the pre-Delta timeframe (p = 0.005), while the difference was not significant for 5–19-year-olds (one-way ANOVA, Ad Hoc Tukey HSD). Of children hospitalized due to COVID-19, a larger proportion were in the PICU during the Delta (~35%) than the pre-Delta timeframe (~18%). A larger proportion of inpatients were

symptomatic due to COVID-19 during the Delta (~78%) than the pre-Delta timeframe (~67%); however, overall Ct values did not differ among symptomatic inpatients between timeframes. We found that children infected during the Delta timeframe exhibited higher viral loads (lower Ct) and a larger proportion were hospitalized in the PICU than children infected earlier in the pandemic or with other variants, suggesting Delta may result in more severe disease in children.

Presenter: Laura Bankers, Colorado Department of Public Health and Environment, laura.bankers@state.co.us

Introduction and Dispersal Dynamics of SARS-CoV-2 Lineages within a State Corrections Center and a County Detention Facility in Colorado

D Ir¹, M Hetherington-Rauth¹, L Bankers¹, L Warren², B Albanese², R Jervis¹, A Rossheim¹, T Mappe¹, S Matzinger¹, E Travanty¹; ¹Colorado Department of Public Health and Environment, ²TriCounty Health Department

SARS-CoV-2 outbreaks in correctional and detention facilities are difficult to prevent and contain because of the population-dense housing, limited space, resources for medical isolation and quarantine, and potential introductions by new people who are detained or incarcerated (PDI), staff members and visitors. However, little is known about the circulation patterns of SARS-CoV-2 within these facilities. PDI at state correctional facilities on average have longer incarceration stay periods whereas PDI at county detention facilities are typically temporarily detained and have shorter stay periods. These differences may affect both the prevalence and transmission dynamics of SARS-CoV-2 within these facilities. To better understand SARS-CoV-2 prevalence and transmission dynamics in correctional and detention settings, we analyzed 579 SARS-CoV-2 whole genome sequences from a state correctional facility and 1,079 SARS-CoV-2 sequences from a county detention facility in Colorado. Sampling was performed between the period of April 01, 2020 – November 30, 2021, coinciding with outbreak investigations. We generated SARS-CoV-2 whole genome consensus sequences and constructed maximum likelihood phylogenetic trees. We found that in the county detention facility, the outbreak was characterized by a larger range of SARS-CoV-2 lineages (27 different lineage calls) compared to the correctional facility (10) suggesting that, based on the genetic divergence of sequences, there were likely more overall introductions to the detention center during this time period. Our sequencing data show higher diversity in the detention facility than the correctional facility suggesting that the rate of PDI turnover was likely an important determinant in SARS-CoV-2 transmission in the two facility types. However, pairing sequencing data with epidemiological data, such as detailed contact tracing, is an important next step in confirming these results. Our results improve our understanding of SARS-CoV-2 transmission dynamics in population-dense housing scenarios and inform methods for surveillance tracking of pathogens of public health concern in correction and detention facilities.

Presenter: Diana Ir, Colorado Department of Public Health and Environment, diana.ir@state.co.us

Whole Genome Sequencing of SARS-CoV-2: Assessment of Multiplatform cDNA Preparation

A Rossheim, G Hareland, Jr., J Bello, S Baird, A Alford, V Nguyen, L Chaudhry, D Mallal, N Pysnack, L Bankers, M Hetherington-Rauth and S Matzinger, Colorado Department of Public Health and Environment

Streamlined, high throughput whole-genome sequencing (WGS) of the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) genome to track mutations and identify emerging variants is critical in supporting the public health response during the ongoing pandemic. Efficient and consistent WGS surveillance through volume surges and supply chain challenges is dependent on an adaptable and robust workflow that capitalizes on the strengths of the different available sequencing platforms. However, a limitation to this approach is that each platform has their own recommended cDNA preparation, which hampers the flexibility to redirect sequencing runs beyond early preparatory steps. Although the ARTIC cDNA preparation protocol can be used with both the Illumina and Oxford Nanopore Technologies (ONT) platforms, it is unclear if the Illumina specific COVIDSeq cDNA preparation protocol would yield comparable results when library prepped and sequenced on the ONT GridION. To assess the interchangeability of Illumina COVIDSeq cDNA preparation and the ARTIC cDNA preparation for use on the ONT GridION Platform, cDNA preparation was performed using either the Illumina COVIDSeq cDNA or the ARTIC cDNA protocol on four clinical samples with Ct values 20% and depth of coverage increased by an average of >1,500 for the ONT cDNA preparation relative to the COVIDSeq preparation. Therefore, a greater number of high-quality reads were successfully aligned to the reference genome and because of these increases, we were able to more reliably determine lineage information with the ONT cDNA preparation. These results suggest that the Illumina COVIDSeq cDNA preparation is best reserved for the Illumina workflow and not directly interchangeable with the ARTIC SARS-CoV-2 ONT GridION workflow. More broadly, our comparison provides valuable information for those developing, optimizing and streamlining whole genome sequencing protocols on multiple platforms.

Presenter: Alexandria Rossheim, Colorado Dept. of Public Health and Environment, alexandria.rossheim@state.co.us

Reducing the Curve: How Mobile Testing Can Mitigate the Spread of COVID-19 in a Congregate Setting

C Williams, K Tran and M McCarroll, DC Public Health Laboratory

Continuous testing for SARS-CoV-2 amongst the congregate setting was critical in identifying outbreaks. Polymerase chain reaction (PCR) tests are the gold standard for performing this testing; however, due to the surge of community testing, an increase in the length of time to get a report from a laboratory was observed. In the District of Columbia (DC), private laboratories were providing reports 3–7 days after the date of collection. Studies have shown that the COVID-19 latent period is between 4–5 days; therefore, residents of long-term care facilities could be exposed to positive cases before they were notified and isolated. This failing of the laboratory system led the DC Public Health Laboratory (PHL) to optimize testing efforts by providing rapid field screening. The DC PHL used a mobile laboratory truck, on loan from the CDC NHANES division, to provide on-site SARS-CoV-2 testing. Equipped with five Abbott ID NOW™ instruments, the mobile testing unit (MTU) had the capacity to

screen 10–20 specimens per hour, using nucleic acid amplification technology. Positive results were immediately reported, allowing the facility to isolate those individuals within one hour of specimen collection and contacts could be quarantined within 24 hours. According to the instructions for use (IFU), the Abbott ID NOW™ has a performance sensitivity of $\geq 95\%$ and specificity of 98.4%. While there was concern regarding this lower sensitivity and specificity when compared to PCR testing, the advantage of receiving a rapid result considerably outweighed that concern. The MTU was deployed to 16 different facilities performing a total of 1,483 tests, with a median turnaround time (TAT) of 21.5 minutes (range: 11–48 min). The CDC estimates the SARS-CoV-2 reproduction number is 2.5, when case contacts are identified in five days; however, using the MTU, case contacts are quarantined within 24 hours such that the R_0 could be reduced to less than 1 and stop the outbreak at the healthcare facility. This is clearly demonstrated at Facility A, which was dealing with an outbreak, where the dispatched MTU identified two additional cases. Immediately following rapid testing, Facility A experienced an 83.3% decrease in cases. During a second MTU deployment to Facility A, six cases were identified by MTU testing and a subsequent 87.5% decrease in cases immediately following testing. The timeliness that contacts are identified and quarantined is an important factor in reducing the spread of disease, such that testing can only be effective if contacts are identified before symptoms develop. The MTU rapid, on-site testing allowed facilities to identify contacts 48–72 hours sooner than private laboratory testing. In conclusion, the use of the MTU demonstrates how rapid field testing can aid in mitigating the spread of COVID-19 to residents of long-term care facilities by quarantining contacts within 24 hours after identifying a case.

Presenter: Channyn Williams, DC Department of Forensic Sciences, channyn.williams@dc.gov

Quantitating SARS-CoV-2 Nasopharyngeal Viral Load using Real-time PCR and Digital Droplet PCR in Acute COVID-19 Patients

K Gavina^{1,2,3}, D Bulir^{1,2}, S Chong², J Gilchrist², R Relich³, M Smieja¹; ¹McMaster University, ²St. Joseph's Healthcare, ³Indiana University School of Medicine

Background: Quantitation of SARS-CoV-2 viral load plays an integral role in diagnosis, infection prevention and control, and assessing COVID-19 infections. The aim of this study was to evaluate digital-droplet PCR (ddPCR) and reverse-transcriptase qPCR (RT-qPCR) for measuring SARS-CoV-2 viral loads in a clinical lab setting.

Methods: We evaluated quantitation using ddPCR compared to RT-qPCR in a point-prevalence cohort of 86 COVID-19 patients. Further, we followed and assessed SARS-CoV-2 viral loads after acute infection in a cohort of 21 COVID-19 patients.

Results: We observed high agreement between ddPCR and RT-qPCR for viral quantitation of SARS-CoV-2 targeting nucleocapsid genes (N1 and N2). In 21 patients with acute infection, SARS-CoV-2 viral loads were significantly lower at follow-up compared to enrolment

by both RT-qPCR (N1, $p < 0.0001$; N2, $p = 0.001$) and ddPCR (N1, $p < 0.0001$; N2, $p < 0.0001$).

Conclusions: ddPCR is effective at quantitating SARS-CoV-2 at low copy numbers, but given method agreement with RT-qPCR, increased cost and turn-around-time, RT-qPCR remains a more practical method for quantitating SARS-CoV-2 within clinical settings. While an important quantitative metric, clinicians should use viral load in conjunction with a symptoms-based approach for guiding patient care and assessing COVID-19 infections.

Presenter: Kenneth Gavina, Indiana University School of Medicine, gavinak@iu.edu

Evaluation of the INTEGRA Biosciences D-ONE Single Channel Pipetting Module on the Assist Plus Robot, Paired with the MINI 96 Portable Electronic Pipette for Semi-Automated Set-Up of the CDC Influenza/SARS CoV-2 RT-PCR Assay

C Loring¹, J Alexander², P Tirado Velez², D Deloge²; ¹Integra Biosciences, ²New Hampshire Public Health Laboratories

Background: INTEGRA Biosciences released the D-ONE single channel pipetting module for use on the ASSIST PLUS automated pipetting platform in February 2022. Used in conjunction with the INTEGRA Biosciences MINI 96, a 96-channel portable electronic pipette, the D-ONE on the ASSIST PLUS allows for automated master mix preparation, automated PCR plate setup followed by single step addition of 96 specimens/controls to a PCR reaction plate. This functionality was evaluated for use with the Centers for Disease Control and Prevention (CDC) Influenza/SARS CoV-2 rRT-PCR Assay.

Method: Serial dilutions of five previously tested SARS CoV-2 positive specimens were prepared, extracted and pooled. Ct values were evaluated and displayed linearity from 19–34. Programming of the ASSIST PLUS was accomplished using VIALAB Software (INTEGRA Biosciences). Master mix was prepared and plated using the D-ONE on the ASSIST PLUS. Samples were added using the MINI 96. Five replicates of each sample were tested over five days for a total of 125 tests per method. Test results were compared to results obtained from manual master mix preparation, plating and sample addition. Results were evaluated for accuracy, precision and analyst hands-on time.

Results: When compared to manual plate preparation, automated PCR plate set-up by the D-ONE/ASSIST PLUS and MINI 96 demonstrated 100% accuracy. Precision was evaluated by calculating the coefficient of variation (CV) of Ct values of all samples tested. Automated PCR plate preparation displayed a CV of 0.39%, while manual plate preparation displayed a CV of 0.52%. Ct values from the automated plate preparation averaged 0.431 higher compared to manual plate preparation. Initial data indicates that PCR plate preparation by the D-ONE/ASSIST PLUS and MINI 96 reduced hands-on technician time by 27%.

Discussion: Ongoing surges in SARS CoV-2 testing and high staff turnover has posed challenges to public health laboratories across the country. One strategy to address these challenges is to use more automation in the laboratory. The ASSIST PLUS is a low-cost, open platform pipetting robot in use in many public health laboratories. Until recently, a limitation of the ASSIST PLUS was

that it could only be used with a multi-channel pipette, resulting in limited functionality for tasks requiring single tube access (e.g., small volume reagent prep, hit-picking, normalizations). The release of the D-ONE single channel pipetting module provides this capability. This evaluation showed that the D-ONE on the ASSIST PLUS provides reliable, robust pipetting capability for automated preparation and plating of master mix. Used in conjunction with the MINI 96, a 96-channel portable electronic pipette to deliver extracted nucleic acid in one pipetting step, the system reduces the opportunity for manual pipetting errors, while freeing up technicians to perform other tasks.

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

Significance of High-Containment Biological Laboratories Performing Work During the COVID-19 Pandemic: Biosafety Level-3 and -4 Labs

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High containment biological laboratories (HCBL) are required for work on Risk Group 3 and 4 agents across the spectrum of basic, applied and translational research. These laboratories include biosafety level (BSL)-3, BSL-4, animal BSL (ABSL)-3, BSL-3-Ag (agriculture livestock) and ABSL-4 laboratories. While SARS-CoV-2 is classified as a Risk Group 3 biological agent, routine diagnostic can be handled at BSL-2. Scenarios involving virus culture, potential exposure to aerosols, divergent high transmissible variants and zoonosis from laboratory animals require higher BSL-3 measures. Establishing HCBLs, especially those at BSL-4, is costly and needs continual investments of resources and funding to sustain labor, equipment, infrastructure, certifications and operational needs. There are now over 50 BSL-4 laboratories and numerous BSL-3 laboratories worldwide. Besides technical and funding challenges, there are biosecurity and dual-use risks, and local community issues to contend with in order to sustain operations. Here, we describe case histories for distinct HCBLs: representative national centers for diagnostic and reference, nonprofit organizations. Case histories describe capabilities and assess activities during COVID-19 and include capacities, gaps, successes and summary of lessons learned for future practice.

Presenter: Kyle Parker, MRIGlobal, kparker@mriglobal.org

Fast Surveillance of Major SARS-CoV-2 Variants Without Sequencing: One Year's Experience From Advanced Diagnostics Labs in National Jewish Health

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Background: To promptly monitor the prevalence of SARS-CoV-2 variants and facilitate contact tracing, positive samples from COVID testing labs were subject to variant analysis by SARS-CoV-2 Variant Assay (Poster #89, APHL 2021) since early 2021.

Continued updates for the SARS-CoV-2 Variant assay enables us to continuously survey emergence, spread and disappearance of major variants in a one-year period. The current version is able to detect 19 mutations to identify eight major variants: Alpha, Gamma, Beta, Epsilon, Kappa, Delta, Lambda and Omicron with one multiplexed reaction.

Methods: The assay is based on reverse transcription PCR with end-point analysis on matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS). This combined platform eliminates the need for fluorescence-based detection and harnesses high sensitivities from both PCR and MALDI-TOF MS technologies (Poster #89, APHL 2021). New PCR and extension primers targeting mutations from new variants were quickly supplemented into the assay primer pools and optimized when Delta and Omicron first reported in June and November 2021. Specificities and sensitivities of new primers were optimized and verified through synthetic variants and Sanger sequencing.

Results: Of 2,028 positive samples analyzed since February 2021 through mid-January 2022, we identified 199 of Alpha (9.8%), 130 of Epsilon (6.4%); five of Gamma (0.2%); 1,008 of Delta (49.7%) and 261 of Omicron (12.9%). We were able to detect the very early emergence of new variants in Colorado, including Delta and Omicron, and observed phasing-out of variants like Alpha, Epsilon and Delta as well. In October 2021, we detected an unusual Delta variant with an additional Q677H mutation and 20 Delta variant samples carrying Q677H mutation were subsequently identified that came from the same facility. This provided valuable information for contact tracing of the virus origin within that facility.

Conclusion: From this one-year's experience of running the variant assay weekly, we demonstrated a major variant prevalence landscape since February 2021. The assay has a much faster turn-around time (eight hours in wet-lab) and much lower cost compared with other variant identification methods, such as SARS-CoV-2 NGS sequencing used by most public health labs for virus surveillance. Its flexibility with rapid expansion to include emerging variants makes this assay an ideal tool for ongoing detection of major SARS-CoV-2 variants of concern.

Presenter: Reeti Khare, National Jewish Health, kharer@njhealth.org

Routine Viral Load Assessment of SARS-CoV-2 in Northern Nevada Wastewater

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Monitoring the public infection rate of SARS-CoV-2 during the Coronavirus disease 2019 (COVID-19) pandemic has been crucial for public health decision making. Examining SARS-CoV-2 positivity rate through patient specimens is currently being utilized but has its limitations such as laboratory testing capacity and will of communal population to get tested. Studies with other viruses such as polio have demonstrated that monitoring wastewater is an effective and sensitive means to monitor pathogen presence within the area. Since SARS-CoV-2 infects gastrointestinal glandular epithelial cells and is excreted through feces into wastewater in both symptomatic and asymptomatic persons, wastewater surveillance for SARS-CoV-2 is a valuable tool to combat the limitations of patient diagnostic testing. Detection of SARS-CoV-2 RNA through wastewater provides a rapid, non-intrusive tool for monitoring new COVID infection rates

of entire communities sooner than patient diagnostic testing. This tool can also be used to mitigate SARS-CoV-2 disease outbreak and influence public health decision making. The Nevada State Public Health Laboratory has established a mainly automated same day workflow for routine viral load detection and quantification of SARS-CoV-2 in wastewater. This workflow consists of an automated 40-fold concentration of wastewater specimen using Ceres Magnetic Nanotrap Beads, automated viral RNA extraction using Thermofisher MagMax beads, and SARS-CoV-2 detection and quantification through Promega's RT-qPCR Kit for SARS-CoV-2 detection in wastewater. Weekly, wastewater RNA extracts are sequenced using Illumina technology. Sequence reads undergo bioinformatic analysis to calculate concentration of SARS-CoV-2 variants in the wastewater. Utilizing this workflow, the Nevada State Public Health Laboratory has organized a wastewater surveillance system for northern Nevada that is composed of four sites over the past year. In this poster, SARS-CoV-2 detection, quantification and variant data of these four sites would be presented. These sites are three wastewater treatment plants in three different northern Nevada counties and four separate sites on the University of Nevada Reno's campus (three dormitories and one student union). The next steps for this project include optimizing digital droplet PCR for direct quantification, analyzing wastewater metagenomics, and expanding this workflow to be able to identify other pathogens (mainly bacteria) than SC2 such as *Neisseria gonorrhoeae* and *Chlamydia trachomatis*.

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Establishment of Wastewater Sequencing Methods Allow for Surveillance of SARS-CoV-2 in the State of New Jersey

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Throughout the COVID-19 pandemic, a leading indicator of the presence and level of SARS-CoV-2 infection within a community has been the presence of viral RNA in wastewater. Fecal shedding of the virus by infected individuals can occur prior to the presentation of symptoms. This surveillance capability is an important tool for public health, indicating the presence of the virus in a community prior to individual testing, as well as the severity of infection and proportion of each variant strain that is present. Wastewater surveillance can also inform public policy decisions regarding the allocation of testing and treatment resources, in preparation for a potential outbreak within a community. As a pilot project, intended to develop methods for effective testing for the presence of SARS-CoV-2, two wastewater facilities in southern New Jersey were selected to provide samples to the New Jersey Public Health and Environmental Laboratories on a weekly basis. This study represents the first implementation of whole genome sequencing surveillance in wastewater by the New Jersey Department of Health. This project was undertaken as part of the FDA Center for Food Safety and Applied Nutrition's LFFM special wastewater project, with data being shared through the FDA's GenomeTrakr network. Virus concentration was performed using a polyethylene glycol precipitation, in combination with ultracentrifugation. Following extraction of viral RNA, RT-qPCR was used to detect the presence of SARS-CoV-2 in the wastewater samples. Prior to sequencing, the NEBNext ARTIC SARS-CoV-2 kit, including the VarSkip Short

primers to improve performance with variants, was used for library preparation. Samples testing positive for SARS-CoV-2 were then sequenced using the Illumina MiSeq sequencing platform. Quality assessments of raw reads were performed using the wastewater quality control workflow in GalaxyTrakr. Data passing quality control standards were submitted into NCBI public database and assigned accession numbers. Twice weekly testing allowed for regular surveillance of the presence of SARS-CoV-2 and population structure of SARS-CoV-2 strains circulating within the selected communities, along with a comparison of the level of RNA copies in wastewater with community nasal swab testing results. This project allowed for validation of methods that can be implemented on a larger scale throughout the state of New Jersey. The establishment of this testing infrastructure with wastewater treatment plants throughout the state of New Jersey will allow for surveillance of a broader array of pathogens of concern, under a One Health approach. This project has laid the groundwork for testing for organisms in wastewater that not only impact public health, but with the collaboration of other state agencies, those that impact plant and animal health, as well as the environment.

Presenter: Nicholas Palmateer, New Jersey Department of Health, nicholas.palmateer@doh.nj.gov

Improving Post-analytic Processes for COVID-19 Testing Through Automated Laboratory Information System (LIS) Solutions

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Introduction: Routine post-analytic lab processes can include several layers of manual result entry and checks both on paper and electronically to ensure accurate reporting of test results. Although these processes are functional, they are redundant and consume critical staffing resources. Increasing test volumes due to COVID-19 surges has highlighted the need to address these lab inefficiencies. LIS applications can be used to design custom algorithms to improve post-analytic processes. This study aims to develop, validate, implement and monitor LIS solutions that automate post-analytic processes for COVID-19 testing at NYC Department of Health operated labs.

Methods: LIS solutions were developed based on regulatory reporting requirements and the need for system flexibility to facilitate an automated electronic-based lab workflow. Automated LIS algorithms were developed with a result-based criterion to account for all possible test results. A thorough end-to-end validation process was used to ensure algorithms functioned as intended. Implementation was done in two phases: 1) automated release of only negative results and 2) automated release of negative and positive results. Automation Rate, which measures how many analyte results are directly reported by the system without user involvement, and turnaround time (TAT) from specimen receipt to release of results were monitored pre- and post-implementation.

Results: Automated LIS algorithms were developed and validation testing showed that all algorithms functioned as intended, but revealed end-user involvement would be required when a specimen needed retesting. Automation Rate and TAT were monitored to determine post-analytical process improvement. Pre-implementation data showed the Automation Rate was 0%, whereas post-

implementation data showed an increase to 98.4% and 99.0% for Phases 1 and 2, respectively. The average specimen-received to result-released TAT decreased from 99 mins (pre-implementation, n=144,796) to 71 mins (post-implementation, n=50,554).

Conclusions: LIS automated solutions were successfully implemented resulting in streamlined post-analytical processes by reducing manual processes for COVID testing. This also reduced TAT by 28 minutes and staff involvement with >98% of post-analytical processes being automated. Automation Rate and TAT metrics proved to be important quality indicators for monitoring the success of LIS automated algorithms.

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A Perfect Marriage: An Academic Lab and a Local Public Health Lab Work Together to Validate a New SARS-CoV-2 Test for Self-collected Saliva Specimens

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Introduction: The COVID-19 pandemic has strengthened partnerships between public health and academic labs. With surges in new COVID-19 cases, there is a significant need to provide simple, reliable and scalable SARS-CoV-2 tests to local communities. The Rockefeller University (RU) developed a real-time RT-PCR assay (DRUL test) that detects SARS-CoV-2 in self-collected saliva to address this issue. RU partnered with the NYC Public Health Lab (PHL) and 8 off-site PHL COVID labs to validate the DRUL test for submission for FDA EUA approval. This study describes the process developed to validate a new SARS-CoV-2 at an academic center and understand key takeaways and lessons learned for future collaborations.

Methods: NYC PHL and RU developed and submitted an application, protocols and personnel trainings for Institutional Review Board (IRB) review. Following IRB approvals, staff were provided in-person group trainings on patient enrollment, specimen collection and specimen/data workflows. Consenting patients had a nasopharyngeal (NP) swab collected by a collection specialist and were given a collection kit to self-collect a saliva specimen. NP and saliva were submitted to the NYC DOHMH Lab but only the NP was tested by the Xpert Xpress SARS-CoV-2 test and reported to the patient. Saliva specimens were shipped to RU for DRUL testing, with no results provided to the patient. Data collected included symptoms, test results and Ct values. Data requirements included collecting up to 50 SARS-CoV-2 positive NP specimens (25 symptomatic and 25 asymptomatic) with Ct values ≤40 and up to 100 SARS-CoV-2 negative NP specimens.

Results: Study goals were achieved quickly by focusing on only enrolling patients at 3/8 PHL COVID off-site labs which had the highest SARS-CoV-2 positivity rate (average < 2%) at the time. Due to staff turnover and inexperience, we faced slow enrollment and exclusion of specimens due to incomplete consent forms. To mitigate these challenges, refresher trainings and guided recruitment were provided to clinic and lab staff and the IRB was amended to expand patient enrollment at all 8 PHL COVID off-site labs. This increased enrollment and reduced the study timeline by 30 days. In total, 71 specimens positive for SARS-CoV-2 (32

symptomatic and 39 asymptomatic) and 203 specimens negative for SARS-CoV-2 had saliva specimens submitted to RU for DRUL testing.

Conclusions: This study demonstrates how important partnerships between public health and academic labs are in addressing the challenges of the COVID-19 pandemic. Key takeaways from this study included buy-in from all parties, clear and consistent communication among investigators, facilitators and clinic/lab staff, and having well-defined study goals. This collaboration resulted in exceeding study goals, facilitating the validation process of a new saliva assay and establishing a framework for future collaborations.

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Lessons Learned from “Pop-up” Testing Following the First SARS-CoV-2 Pandemic Wave in New York City

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A key component of pandemic response is the rapid mobilization of testing to identify infected individuals. This is particularly important in New York City, where population density is high and there is a large volume of international travelers. In the summer of 2020, the city's Department of Health and Mental Hygiene opened two community SARS-CoV-2 testing sites to provide increased access to testing. The temporary pop-up sites were quickly organized to provide rapid, point-of-care testing in neighborhoods with high positivity rates but low testing rates. We detail the decision making during a time when limited data was available. Although a rapid nucleic acid amplification test was deployed, a reflex testing workflow was also implemented. Our rationale for the initial workflow and why it was modified is discussed. The main advantage of the point-of-care testing is its turnaround time, which enabled immediate, on-site resource navigation for individuals who tested positive. However, this must be weighed against the potential disadvantages of rapid testing such as modified biosafety practices and higher cost compared to laboratory-based testing. We retrospectively review our approach to the challenges and share the lessons learned with the optimism that they may help to inform the early response to future pandemics. With thoughtful planning, pop-up testing can provide more timely resources where they are needed most.

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SARS-CoV-2 Pangolin Lineage Calling: Comparing pangoLEARN and USHER

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Pangolin lineage calling has been indispensable for tracking the evolution of SARS-CoV-2 and monitoring the rise and fall of variant caseloads. Due to the fast-moving nature of the pandemic, pangolin designations, which determine the defining traits of lineages, have been constantly updated and refined as more cases were sequenced, in turn requiring model updates to the pangoLEARN decision tree. As such, NYC DOHMH adopted the practice of rerunning historical data with any pangolin software

updates. Following the emergence of Delta (B.1.617.2) and its associated sublineages (AY), it was reported that these lineages may have increased transmissibility, infectivity, or virulence, and it became imperative to try to validate these claims using NYC data. However, it became apparent that pangoleARN lineage calls were not always monophyletic, making any associations of lineages to vaccine breakthrough or hospitalization potentially spurious. Thus, we sought to determine the extent of the miscalling in Delta and validate Ultrafast Sample placement on Existing tRees (USHER) as a potential alternative method to determine pangolin lineages. A phylogenetic tree containing 7229 Delta sequences from NYC, created by aligning consensus SARS-CoV-2 genomes using MAFFT and inputting it to IQ-TREE, was used to determine the phylogenetic relationship between sequences determined to be the same pangolin lineage. This analysis was conducted seven times between September 22, 2021 and November 4, 2021 and span the following versions of pangolin and its software dependencies: Pango designation v.1.2.76-91, pangolin v.3.1.11-16, pangoleARN 2021-09-17 to 2021-10-18. The multiple comparisons also allowed us to compare stability of pangolin lineage calls over time as new sublineages emerged and were defined (e.g., AY.4). Overall, both methods were able to correctly assign lineages to monophyletic clades in most cases. However, pangoleARN was more prone to outliers and most importantly had difficulty correctly calling newly defined lineages. This difficulty in first identifying emerging lineages negatively affected pangoleARN's stability through the study period as lineage calls were likely to be discordant with subsequent lineage calls. USHER results, on the other hand, were remarkably stable except in the case of reassignment of lineages as new sublineages were defined. As a result, NYC DOHMH has switched to USHER pangolin lineage calling.

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Detection of SARS-CoV-2 in Wastewater Samples in Ohio

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Wastewater-based epidemiology is potentially effective for an early detection of SARS-CoV-2 in wastewater before the outbreak is clinically detected. Four wastewater treatment plant sites in Ohio (Jackson Pike, Newark, Lancaster and Zanesville) were selected for SARS-CoV-2 surveillance based on the presence of SARS-CoV-2, population size, COVID-19 vaccination percent and proximity to Ohio Department of Health Laboratory. Wastewater samples (1 L) were collected twice per week for 7 weeks (mid-October to early December 2021) and stored at 4°C until processing. The samples were processed within 2-3 days of sample collection. Each sample was processed, extracted and analyzed in duplicate. The GT-Molecular method from GT-Digital Wastewater Surveillance Guide was employed for virus filtration, concentration, RNA extraction and SARS-CoV-2 quantification. Bovine Coronavirus (BCoV) was used as a matrix recovery control to normalize changes in viral recovery caused by the variable and complex nature of wastewater. The virus from wastewater samples were concentrated using the Innovaprep Concentrating Pipette Select. RNA was extracted from the viral concentrate using QIAGEN AllPrep PowerViral DNA/RNA Kit. The N2 region of the SARS-CoV-2 nucleocapsid gene was quantified to monitor SARS-CoV-2 in wastewater via digital PCR (dPCR) using GT-Digital SARS-CoV-2 Wastewater Surveillance Assay Kit and QIAcuity

One-Step Viral RT-PCR Kit. The initial BCoV percent recovery (BCR) amongst four sites was low ranging from 0.07 to 4.29% (average $1.37 \pm 0.099\%$) when 20 µl template volume was used. The initial BCR did not differ ($P = 0.96$) among the sites (0.95, 1.56, 1.67 and 1.22% for Jackson Pike, Lancaster, Newark and Zanesville, respectively). Low BCR was probably due to the presence of some inhibitory materials in wastewater extract and were present in all sites. However, this BCR improved significantly ($P < 0.001$), which ranged from 1.13 to 13.5 (average 6.28 ± 2.96) on reducing the template volume to 10 µl from 20 µl. The recovery rate was similar ($P = 0.77$) among the sites (6.70, 5.11, 6.96 and 6.34% for Jackson Pike, Lancaster, Newark, and Zanesville, respectively). In this study period, [N2] gene abundances (copies/L) increased from 1813 to 15355 in Jackson Pike, 8766 to 39538 in Lancaster, 7442 to 63050 in Newark, and 6772 to 23038 in Zanesville wastewater site, probably due to an increased incidence of COVID-19 in Ohio. Overall, gene abundance did not differ significantly ($P=0.87$) among wastewater sites during this time period. In conclusion, this study reveals that the use of 10 µl template volume increases BCoV recovery rate and the surveillance of the N2 gene abundance of SARS-CoV-2 in wastewater may be useful to early detect COVID-19 cases in the community.

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Performance of the VITROS® Immunodiagnostic Products Anti-SARS-CoV-2 IgG Quantitative Assay* Calibrated to the WHO 1st International Antibody Standard

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Background: This study was designed to assess the performance of the VITROS Immunodiagnostic Products Anti-SARS-CoV-2 IgG Quantitative assay (VITROS IgG Quant). The assay is for the fully quantitative detection of SARS-CoV-2 IgG antibodies with calibration traceable to the First WHO International Standard for Anti-SARS-CoV-2 antibody. Results are reported in both qualitative (reactive/non-reactive) and quantitative values (Binding Antibody Units/mL (BAU/mL)).

Methods: Reference calibrators (RC) were assigned values through a traceability study with the WHO 1st International Standard (NIBSC 20/136). A titration panel of the standard was used to assign values to an average calibration curve generated through 10 measurements of RC run on two VITROS systems. These RCs are then used to value assign sets of three Customer Calibrators on a reagent lot-to-lot basis for customer lab use. The assay reportable range was determined by calculation of the assay LoD/LoQ and demonstration of linearity. Clinical specificity was assessed using 533 samples collected prior to the start of the COVID-19 pandemic. Sensitivity (Percent Positive Agreement (PPA)) was evaluated using samples from 264 RT-PCR positive individuals collected ≥15 days since reported symptom onset. Reproducibility was evaluated consistent with CLSI EP05. Quantitative results of 45 vaccinated individuals were assessed prior to vaccine, after the first dose and after the second dose to demonstrate vaccine induced antibody response. An additional 74 fully vaccinated individuals were assessed months after vaccination to look for evidence of antibody waning post vaccination.

Results: The reportable range is 2-200 BAU/mL and with 1:50 dilution up to 10,000 BAU/mL. The clinical specificity of the VITROS IgG Quant assay for the 533 pre-pandemic healthy blood donors was 100.0% (533/533) [95% exact CI (99.3-100.0%)]. The PPA with RT-PCR for 264 PCR positive individual's samples collected 15 days or more from onset of symptoms was 92.4% (244/264) [exact 95% CI (88.5-95.3%)]. The observed total reproducibility for the 6 panel members ranged from 0.4 to 14.9 %CV. The assay demonstrated a quantitative increase in antibody titer throughout the course of vaccination and all vaccinated individuals demonstrated a result >600 BAU/mL after the second vaccine dose. Antibody response to the vaccine doses was greater in individuals with evidence of previous infection, based on nucleocapsid antibody reactivity prior to the first dose. Evidence of antibody waning post vaccination was also observed in fully vaccinated individuals tested months after vaccination.

Conclusion: The VITROS Anti-SARS-CoV-2 IgG Quant assay* demonstrates excellent clinical and analytical performance and can detect quantitative antibody response to COVID-19 infection or vaccination.

* For EUA Only. Note, dilution and vaccine response not authorized by the FDA

Presenter: Paul Contestable, Ortho Clinical Diagnostics, paul.contestable@orthoclinicaldiagnostics.com

Correlation of the VITROS® Anti-SARS-CoV-2 IgG Quantitative Assay* with SARS-CoV-2 Plaque Reduction Neutralization Test

B Hirsch¹, P Contestable¹, B Novick¹, L Hartson², I Ragan², R Goodrich², L Li¹; ¹Ortho Clinical Diagnostics, ²Colorado State University

Background: This study was designed to assess the correlation of the VITROS Immunodiagnostic Products Anti-SARS-CoV-2 IgG Quantitative assay (VITROS IgG Quant) to a plaque reduction neutralization test developed at Colorado State University (CSU PRNT). The VITROS IgG Quant assay is for the quantitative detection of SARS-CoV-2 IgG antibodies with calibration traceable to the First WHO International Standard for Anti-SARS-CoV-2 antibody. Results are reported in both qualitative (reactive/non-reactive) and quantitative values (Binding Antibody Units (BAU)/mL). PRNT is considered the gold standard method for determining neutralizing antibody (nAb) titers.

Methods: VITROS IgG Quant: The VITROS IgG Quant assay is a fully automated, high throughput method run on the VITROS family of immunoassay analyzers. First, antibodies to SARS-CoV-2 present in the sample bind with S1 subunit of the spike protein coated on wells. After washing, HRP-labeled murine monoclonal anti-human IgG antibodies are added. Following a final wash, bound HRP conjugates are detected using the VITROS signal reagent. The amount of conjugate directly correlates to the amount of SARS-CoV-2 IgG antibody present and is reported in BAU/mL.

CSU PRNT: Samples were heat inactivated for 30 min at 56 °C and serial two-fold dilutions were prepared in a 96-well plate. Viral stock (strain hCoV-19/USA/WA1/2020) containing ~200 pfu per 0.1 ml was added to each well containing serum dilutions. Following an incubation at 37 °C in 5% CO₂, 6-well plates containing recently confluent Vero cells were inoculated with the virus-serum mixtures.

After a second incubation at 37 °C, 2 ml of overlay was added to each well. After 24 h incubation at 37 °C, a second overlay containing neutral red was dispensed into each well and the number of plaques was counted 48–72 h after initial inoculation. The highest dilution of serum that inhibited plaque formation by 50% (PRNT50) was determined based upon the titer of the samples and the number of plaques present at each dilution. Samples with PRNT50 titers less than or equal to 1:20 are considered negative for nAbs. 25 samples were blind-tested with both the VITROS IgG Quant assay and the CSU PRNT, 20 known positive for SARS-CoV-2 antibody and 5 negatives. Correlation of the VITROS IgG Quant values to CSU PRNT50 was determined.

Results: The VITROS IgG Quant results ranged from < 2 to 1473 BAU/mL. The CSU PRNT50 results ranged from < 1:20 to 1:1280. Pearson correlation coefficient was calculated to be 0.912 demonstrating a good correlation between the VITROS IgG Quant results and the CSU PRNT50 titers.

Conclusion: The VITROS Anti-SARS-CoV-2 IgG Quantitative assay demonstrates a strong correlation to PRNT50 for measurement of SARS-CoV-2 nAb titers.

* For EUA Only. Note, correlation to PRNT not authorized by the FDA

Presenter: Paul Contestable, Ortho Clinical Diagnostics, paul.contestable@orthoclinicaldiagnostics.com

SARS-CoV-2 Detection by the VITROS® Immunodiagnostic Products SARS-CoV-2 Antigen Assay* is Unaffected by Mutated Variant Nucleocapsid Proteins

L Li, C Noeson, P Hosimer, B Hirsch, P Contestable and P Lundberg, Ortho Clinical Diagnostics

Background: Ortho Clinical Diagnostics continuously monitors emerging SARS-CoV-2 variants for their potential impact on the VITROS® SARS-CoV-2 tests, including the VITROS Immunodiagnostic Products SARS-CoV-2 Antigen assay (VITROS CV2Ag assay) used to detect acute infection through the detection of SARS-CoV-2 nucleocapsid antigens. Monitoring comprises: 1) in silico analysis of variant proteins to evaluate for potential interference, 2) monitoring of customer false negative (FN) reports across the globe and 3) wet lab testing of recombinant mutant proteins and patient samples. Here, we focus on mutation monitoring in nucleocapsid and testing of recombinant nucleocapsid protein using the VITROS CV2Ag assay, with a particular emphasis on B.1.1.28.1 (Gamma), B.1.617.2 (Delta) and B.1.1.529 (Omicron).

Methods: To date, seven specific mutations near the epitopes for the monoclonal antibodies used in the VITROS CV2Ag assay have been tested. Testing was performed at 15 pg/mL, which is 3X the level of detection (LoD; 5 pg/mL) for the assay. Matching supplier wild-type (Wuhan-Hu-1 nucleocapsid) was used to normalize signals. Data are presented for select mutations and variants. At the time of the abstract submission, clinical nasopharyngeal samples from patients infected with SARS-CoV-2 Omicron were being procured and results will be shown and contrasted with data from non-Omicron samples.

Results: The VITROS CV2Ag assay uses recombinant wild-type Wuhan-Hu-1 (WT) nucleocapsid calibrators. Potentially interfering mutations from in silico analysis were ordered as recombinant proteins and tested using the WT nucleocapsid as control. In all instances, recombinant mutants were detected to the same extent,

or better than, WT control nucleocapsid protein. Specifically, the recombinant point mutations identified gave relative signal/cutoff (S/C) values of 110-380% compared to WT. Additionally, the fully mutated Omicron nucleocapsid protein was tested, carrying its three mutations and a single three-residue deletion. As was the case with the individual recombinant point mutations tested, the Omicron nucleocapsid protein was detected with higher S/C than the WT control.

Conclusion: The performance of the VITROS Immunodiagnostic Products SARS-CoV-2 Antigen assay is unaffected by mutations found among emerging variants to date, up to and including the Gamma, Delta and Omicron variants.

**For EUA only RD-00024*

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HiFiViral SARS-CoV-2: A Mutation Tolerant, Fully-kitted Solution for COVID-19 Surveillance

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The COVID-19 pandemic is an ongoing global challenge, with the repeated emergence of new variants that are more contagious, more virulent, drug resistant or evade vaccine-induced immunity. In response, the HiFiViral SARS-CoV-2 kit was developed as a scalable solution with increased resilience against virus mutations, designed for use on the Sequel IIe system. Unlike PCR-based amplicon methods, the HiFiViral SARS-CoV-2 kit relies on ~1,000, densely tiled Molecular Inversion Probes (MIPs) such that every genomic position is covered by ~22 probes, resulting in robust genome coverage of all circulating variants, including the mutation-dense Omicron lineage, across a broad range of Ct values. The HiFiViral kit offers many benefits for high-throughput surveillance. Sequencing 675 bp fragments with highly accurate HiFi reads enables comprehensive variant detection, including single nucleotide variants, indels, structural variants and identification of multi-strain samples. The kit is scalable, containing all reagents needed to enrich and barcode 384 samples, in batches ranging from 24 – 384, for sequencing in one SMRT bell library. For high throughput labs, up to 8 SMRT Cells may be loaded on an instrument with no subsequent touch points, for up to 3,072 samples per run. The enrichment assay is also simpler to execute than PCR-based assays, consisting of just 4 add-only, color-change indicated steps. Barcoding primers come pre-mixed in a 384-well, resealable plate. The simple assay design uses fewer plastics, limiting the impact of supply chain issues. In addition, methods for running the HiFiViral kit were established on a mosquito® HV Genomics pipetting robot and assessed for their performance. Finally, SMRT Link data analysis is one touch, and reports include variant calling, genome coverage, multi-strain detection and a plate performance summary. File outputs include consensus sequences ready for database submission and reads and consensus sequences aligned to the Wuhan reference. In this study, we demonstrate consistent recovery of >95% complete SARS-CoV-2 genomes using the commercially available HiFiViral kit at 4 different sites performing routine genomic surveillance. The evaluation runs included a broad range of sample

Ct inputs across control and nasopharyngeal samples in batches up to 384. The runs also demonstrated consistent performance against Alpha, Delta, Omicron and other variant lineages, without the need for probe updates. In summary, the HiFiViral for SARS-CoV-2 kit is a cost-effective, convenient, accurate method for viral sequencing and well-suited for scalable surveillance of a rapidly evolving virus to inform public health decision making.

Presenter: Meredith Ashby, Pacific Biosciences, mashby@pacificbiosciences.com

Extraction-free Nucleic Acid Detection by DirectDetect™ Technology on Gargle Samples

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Background: Nasal or throat swab followed by nucleic acid extraction, RT-qPCR is the most common procedure for diagnosis of COVID-19. This report provides a feasible and non-invasive sample collection, extraction-free method for SARS-CoV-2 detection.

Methods: Saline gargle samples were self-collected and stored at 4°C. Samples were processed the same day using PerkinElmer's DirectDetect™ technology without any nucleic acid extraction or additional heat lysis steps. Inactivated SARS-CoV-2 virus was spiked in pooled negative gargle samples for performance evaluation.

Results: The technology can reach detection sensitivity (limit of detection) as 5,400 cp/mL. **Conclusion:** The method takes ~2hrs from sample input to results. It provides a cost-efficient screening solution for SARS-CoV-2 with a non-invasive sample collection procedure.

This product is under development.

Presenter: Michael Miller, PerkinElmer, michael.miller@perkinelmer.com

A Convenient Nucleic Acid Extraction Procedure for Wastewater-based Epidemiology

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The COVID-19 pandemic has spurred investigation on the use of wastewater-based epidemiology (WBE) as a tool for monitoring the appearance and spread of COVID-19 in communities. The SARS-CoV-2 genetic signal is present at low concentrations in wastewater, making sample concentration a prerequisite. Most of the existing viral concentration and processing methods were originally developed to concentrate non-enveloped viruses. We hypothesized that since the SARS-CoV-2 genetic material in the wastewater is primarily present in nucleoprotein complexes and not in intact virions, a direct capture method that renders total nucleic acid conducive to binding to affinity resin may be able to overcome cumbersome viral concentration steps. This led to development of a simple, rapid and modular alternative to existing purification methods from wastewater. In this approach, chaotropic agents are added to raw sewage allowing nucleic acid binding to a silica matrix. The captured nucleic acid is then washed by successive alcohol washes to remove inhibitors that may have co-purified, followed by subsequent elution with water. The eluted

nucleic acid is then processed using either a spin column or using an automated nucleic acid purification system. The process recovered $63.13 \pm 4.16\%$, $40.09 \pm 10.89\%$, $39.67 \pm 10.66\%$, of HCoV OC43, 229E and bacteriophage MS2. Using this workflow, we monitored wastewater samples from three WWTP in Dane County, Wisconsin, serving a combined population of over 300,000 people, for one year. As wastewater often contains RT-qPCR inhibitors, we formulated enzyme mixes that demonstrated resistance to PCR inhibitors commonly found in wastewater such as humic acid and urea. We developed a SARS-CoV-2 RT-qPCR assay using US CDC N1, N2 (nucleocapsid) and the E (envelope) gene fragments. Pepper Mild Mottle Virus (PMMoV), a fecal indicator RNA virus present in wastewater and used as a normalization tool. SARS-CoV-2 variants of concerns (alpha, beta, gamma, delta) and enteric viruses (Norovirus GI, GII and Hepatitis A) were also analyzed using the method. The workflow has a limit of detection of 1.6 GC/ ml with 40 ml of raw wastewater sample for SARS-CoV-2. Our analysis was compared to the COVID-19 cases declared by the municipalities, showed strong correlation between the SARS-CoV-2 viral load present in wastewater and clinical cases. The alpha and delta variant started appearing in wastewater samples in March and May, 2021 respectively. Norovirus GII was detected in all samples and GI in 25% of the samples tested. HepA was not detected in any sample.

Presenter: Subhanjan Mondal, Promega Corporation, subhanjan.mondal@promega.com

Intelligent Routing and Challenges of COVID-19 Electronic Lab Reporting and Applicability to Future Pandemics

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As COVID-19 breaking news began to flood our media, we were thrown into the midst of a pandemic and teams across the country pulled together to strategize and take action. The US Department of Health and Human Services (HHS) released guidance to ensure appropriate COVID-19 Electronic Lab Reporting (ELR) data reached Public Health Agencies (PHAs), requiring all testing facilities to comply with the requirements found in the CARES Act. One issue in particular that was becoming increasingly transparent was the lack of accessible infrastructure, data transport and data quality validation for private laboratories that found themselves in the COVID-19 testing space. In response to the challenges surrounding COVID-19 test results reporting, the Association of Public Health Laboratories (APHL) launched the COVID-19 Centralized ELR project utilizing the AIMS Platform (AIMS). AIMS is a secure, FISMA-compliant, cloud-based environment that is widely-adopted by state, local and federal public health stakeholders. These factors accelerated getting higher-quality COVID-19 ELR data exactly where it needed to be received. For mandated ELR to PHAs such as COVID-19, AIMS acts as a hub, allowing the sender to transmit ELR messages to a single connection where AIMS securely receives, routes and delivers this data to PHAs through already-established connections. This single connection significantly decreased the time-to-production from months to weeks and alleviates burden by reducing the reporting burden on testing laboratories, leveraging the existing AIMS infrastructure and connections, facilitating collaboration and communication with PHAs, facilitating quality control and COVID-19 standards preservation, and reducing point-to-point connections with PHAs. APHL's Centralized ELR project allows

private laboratories to deliver secure, critical SARS-CoV-2 results to 55 PHAs. The project is in a continuous state of onboarding new COVID-19 Centralized ELR reporters, facilitating communication and improvements among reporters and PHAs, and addressing challenges that have come-up along the way like multiplex, variant and school-based COVID-19 test results reporting. We will share information on the Centralized ELR intelligent data flow, challenges encountered, solutions to address those challenges, discuss impact and benefits to public health, and metrics quantifying the scope and scale of that impact.

Presenter: Caitlyn Simpson, Ruvos/AIMS+/APHL, Email: csimpson@ruvos.com

Demonstration of the Usefulness of a Quantitative SARS CoV-2 Human IgG Multiplex Antibody Assay

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Understanding the kinetics of antibody response to SARS-CoV-2 (CoV2) after infection and vaccination is critical to assess the utility of serological testing at individual and population levels during the COVID 19 pandemic, which continues to cause a significant burden on global public health. Several serological tests available today provide a qualitative result for the presence or absence of antibody reactivity to a single antigen in a sample. Although CoV2 is a novel coronavirus causing severe respiratory illness, four common cold human coronaviruses (HCoV 229E, OC43, NL-63 and HKU1) have circulated in the human population for a prolonged duration and have become endemic. We developed multiplex assays based on Luminex® xMAP® technology to estimate circulating IgG antibodies to multiple recombinant proteins from wild-type and variant CoV-2, SARS-CoV, MERS-CoV and four other HCoVs. We evaluated over 200 samples following infection, 50 samples after vaccination and 168 samples from PCR negative or normal healthy individuals. A qualitative 7-plex assay used three wild-type CoV-2 specific antigens—Receptor Binding Domain (RBD), Nucleocapsid Protein (NP) and a hybrid RBD-NP protein. Quantitative human IgG antibody response to three CoV2 recombinant antigens, RBD, NP and trimeric spike protein, was tested in these samples using WHO quantification standard. Higher levels of antibodies were found in vaccinated samples compared to the infection. We performed analytical validation of the multiplex quantitative IgG antibody assay to determine its performance characteristics. Results showed that multiplex quantitative antibody assay could be used very effectively for measuring immune responses to SARS-CoV-2 antigens after infection or vaccination. The multiplex assay has great potential to differentiate immune response induced by natural infection from vaccination. Multiplex quantitative antibody assay can serve as a surrogate test to indicate correlates of protection following SARS-CoV-2 infection and vaccination.

Presenter: Neeraja Venkateswaran, Tetracore, Inc., nvenkateswaran@tetracore.com

Utilizing the Terra.Bio Platform to Rapidly Develop and Distribute Resources for SARS-CoV-2 Sample Characterization and Genomic Epidemiology to Public Health Laboratories

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SARS-CoV-2 (SC2) sample characterization and genomic epidemiological analysis has become an increasingly critical function for public health laboratories. Integration of these practices, however, has been a major challenge due to the technical workforce and compute infrastructure development required to access the many SC2 bioinformatics solutions. Best-practice approaches for SC2 sample characterization and genomic epidemiology were captured into a series of workflow description language (WDL) workflows—deemed the Titan workflow series. The Titan workflow series was made accessible through the Terra.Bio platform, a bioinformatics web application that connects non-technical users to bioinformatics WDL workflows and dynamic cloud compute resources through a clean and intuitive graphical user interface. Public health scientists were introduced and trained on the Titan workflows to enable routine SC2 sample characterization and genomic epidemiological analysis in laboratories with otherwise limited bioinformatics capabilities. The Titan workflow series has been adopted in over 40 public health laboratories in the United States and over 15 countries internationally. To date, the US laboratories have analyzed an estimated 400,000 SC2 samples using the Titan workflows on the Terra platform.

Use of WDL workflows and the Terra.Bio platform has allowed various public health laboratories to overcome the major barriers to accessing advanced bioinformatics solutions for SC2 genomic analysis. Continued use of the Terra.Bio platform may also provide value in developing and distributing bioinformatics solutions for other pathogens of concern.

Presenter: Kevin Libuit, Theiagen Genomics, kevin.libuit@theiagen.com

Rapid Next Generation Sequencing of the Complete SARS-CoV-2 Genome using the Ion AmpliSeq SARS-CoV-2 Insight Research Assay

A McGeachy, N Li, W Zhang, X Duan, L Pickle, J Ni, X Meng, L Guo, Y Zhong, S Ambriz, Y Lin, B Hsaw, S Roman, A Shah and F Hyland, Thermo Fisher Scientific

Whole genome sequencing of SARS-CoV-2 is essential to monitoring the emergence and spread of variants such as Delta and Omicron. The Ion AmpliSeq SARS-CoV-2 Insight Research Assay is a targeted NGS solution that enables specific amplification, analysis and monitoring of the complete SARS-CoV-2 genome. We report the intelligent design for robust performance and utility in a wide variety of SARS-CoV-2 research applications. The design covers 99.6% of the total bases of SARS-CoV-2 genome. Approximately 62% of the viral genome is covered with two independent amplicons, giving the panel exceptionally robust performance in the face of naturally occurring variation. Amplicons were designed to cover all serotypes of SARS-CoV-2 with high analytical specificity and demonstrate no crosstalk to other Coronaviruses. Panel performance was assayed

using synthetic controls or heat-killed virus. Library preparation was performed manually or on the Ion Chef™, sequenced on GeneStudio™ S5 or Genexus™ sequencers, and evaluated for sequencing uniformity, variant calling and consensus sequence assembly with built-in plugins. Automated workflows reduce hands-on time to as little as 45 minutes using the Ion Chef™ system or 5 minutes with the turnkey Ion Torrent™ Genexus™ sequencing system, reducing the risk of operator induced errors. With the 540 chip on Ion GeneStudio™ S5, up to 80 samples can be multiplexed for high throughput. The panel exhibited strong targeted sequencing performance, with on-target reads above 95% and coverage uniformity above 90%. The panel also demonstrated high analytical sensitivity, with a limit of detection for variant calling and lineage assignment as low as 20 viral copies. The high sensitivity of the assay allows for sample inputs as low as 1 ng total RNA, increasing the eligibility of low viral load samples. Using the Ion AmpliSeq™ SARS-CoV-2 end-to-end analysis solution, we successfully detected known variants in synthetic controls and assembled the genomes from heat-killed virus. The Ion AmpliSeq™ SARS-CoV-2 Research panel requires minimal sample input and as little as 24 hours turnaround time with the Genexus system for comprehensive genomic analysis, genome assembly and variant annotation. Together, this uniquely positions the Ion AmpliSeq™ SARS-CoV-2 assay to provide rapid, high-quality results for tracking viral spread and evolution with robust performance across viral variants. To date, over 124k sequences have been deposited into GISAID using these workflows, including over 81k Delta genomes and 3.8k Omicron genomes with on average ≥99% sequence identity. The panel has proven to be robust to natural variation, making it usable in a wide array of applications including contact-tracing and viral spread with a diversity of sample types such as nasopharyngeal swabs, wastewater and post-mortem FFPE tissue.

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Presenter: Anna McGeachy, Thermo Fisher Scientific, anna.mcgeachy@thermofisher.com

Rapid, High-frequency SARS-CoV-2 Wastewater Sequencing with the Genexus™ Integrated Sequencer

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Environmental wastewater surveillance is becoming a powerful and minimally invasive genomic sequencing tool to monitor the emergence and spread of SARS-CoV-2 variants such as Delta and Omicron. A key advantage of wastewater sequencing is its ability to potentially provide early, comprehensive surveillance data within a community, especially with frequent sampling and sequencing. Rapid sequence data availability offers the potential for earlier public health messaging, advice, or interventions to help decrease the severity of outbreaks. However, current approaches are hindered by pooled sampling and long sequencing turnaround times, which can delay results. Wastewater sequencing can also be impacted by poor sample quality due to degradation, low viral titers and mixed samples for analysis. To address these challenges, we report a protocol for SARS-CoV-2 wastewater surveillance using the Ion AmpliSeq SARS-CoV-2 Insight Research Assay on the Genexus Integrated Sequencer. Data were obtained by sequencing archival wastewater samples from University of

California, San Diego (UCSD). We achieved >95% 20x coverage for samples with Ct values up to 35 with a one-day turnaround time. Using a sample de-mixing tool, Freyja, (<https://www.medrxiv.org/content/10.1101/2021.12.21.21268143v1>), we identified mixed strains matching expected percentages, with sensitivity as low as 1%. The automated workflow of the Genexus Integrated Sequencer enables quick turnaround times of one day with minimal hands-on time, which is ideal for high-frequency SARS-CoV-2 surveillance with wastewater samples.

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Presenter: Jingwei Ni, Thermo Fisher Scientific, jingwei.ni@thermofisher.com

Identification and Quantification of Bioactive Compounds Suppressing SARS-CoV-2 in Wastewater using Metabolomic Approach

M Bayati¹, C Li¹, S-Y Hsu¹, H-Y Hsieh¹, L Wilfing¹, J Klutts², S Zemmer², M Reynolds³, E Semkiw³, H-Y Johnson³, T Foley⁴, C Wieberg², J Wenzel³, M Johnson¹, C-H Lin¹; ¹University of Missouri, ²Missouri Department of Natural Resources, ³Missouri Department of Health and Senior Services, ⁴Missouri Department of Corrections

Understanding the full scope of the COVID-19 pandemic is an ongoing challenge for public-health officials. Diagnostic testing capacity may be insufficient; hospitalization lag infections by weeks and do not report on people with mild or asymptomatic disease. With all the limitations, wastewater-based epidemiology (WBE) has been used as a potential tool for assessing and managing the pandemic. In our study, SARS-CoV-2 viral material has been detected in raw wastewater throughout the COVID-19 pandemic and a positive relationship has been found between the amount of viral material in sewage and the number of reported COVID-19 cases in the area that collects and treats wastewater for a community, called a "sewershed." Although there is a clear correlation between the number of COVID patients in a sewershed and the level of viral material in the wastewater, there is also a clear variability among treatment facilities. Specifically, some facilities have low recovery in viral material per diagnosed case (suggesting suppression of the genetic material from the sewershed). Therefore, the wastewater samples were tested with UPLC-HRMS, the raw data were processed with XCMS online platform, and the features were annotated using METLIN library, which resulted in the putative identification of 30 compounds. The LC-MS/MS analyses with authentic standards was used for the absolute quantification of the compounds identified in the first approach. Fourteen compounds out of thirty have been detected and quantified. Finally, four compounds were used for suppression experiments and the results confirmed their responsibility in suppressing the genetic material in the wastewater.

Presenter: Mohamed Bayati, University of Missouri, mbb229@mail.missouri.edu

Biomarkers Selection for Population Normalization in SARS-CoV-2 Wastewater-based Epidemiology

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Wastewater-based epidemiology (WBE) has been one of the most cost-effective approaches to track the SARS-CoV-2 levels in the communities since the COVID-19 outbreak in 2020. Normalizing SARS-CoV-2 concentrations by the human fecal biomarkers in wastewater can be critical for interpreting SARS-CoV-2 load, comparing viral load between sewage samples over time and identifying the vulnerable communities. In this study, five population biomarkers, caffeine (CAF), its major metabolite paraxanthine (PARA), creatinine (CRE), 5-hydroxyindoleacetic acid (5-HIAA) and pepper mild mottle virus (pMMoV) in the wastewater were investigated for their utility for normalizing the SARS-CoV-2 loads. Their utility for estimating the real-time population contributing to the wastewater were also assessed for future WBE applications. The best candidate identified from this study was further assessed for its capacity for improving the correlation between normalized SARS-CoV-2 loads per capita and the clinic cases reported in the City of Columbia, Missouri, a university town and a community with constantly fluctuated population. Our results showed that PARA is the most reliable population biomarker for determining the SARS-CoV-2 load per capita due to its higher accuracy, lower variability, higher stability, excellent recovery rates and higher temporal consistency to reflect the change in population dynamics and dilution in wastewater. Both direct and indirect normalization approaches derived from the regression functions allow accounting for the changes in wastewater dilution and differences in relative human waste input over time without the requirement of flowrate and population at given WWTP. The PARA also demonstrates its excellent utility for real-time assessment of population contributing to the wastewater. Finally, the correlation between SARS-CoV-2 load per capita and prevalence data per capita normalized by PARA-estimated population was significantly improved ($\rho=0.5878$) suggesting that the population dynamics determined by a reliable biomarker should be always taken into the consideration for the SARS-CoV-2 WEB. The chemical marker PARA offers an excellent alternative to the currently recommended pMMoV genetic marker to help us understand the size, distribution and dynamics of local populations for forecasting the prevalence of SARS-CoV-2 within each sewershed.

Presenter: Shu-Yu Hsu, University of Missouri, shgm5@umsystem.edu

The Impact of COVID-19 on Laboratory Operations and Mitigation Strategies at the Utah Public Health Laboratory

K Christensen, A Atkinson, J Harbour, A Rohrwasser and A Rossi, Utah Public Health Laboratory

Introductions of SARS-CoV-2 infections in workplaces performing essential functions, such as public health laboratories, represent a formidable challenge to their continuity of operations. Here we describe the impact of COVID-19 on the Utah Public Health Lab (UPHL) workforce between September 2020 and January 2022, as well as the mitigation measures that were implemented over time. UPHL is an institution currently employing approximately 140 individuals. COVID-19 illnesses were first detected at UPHL in September 2020 and continued sporadically until January 2021. During this period, coinciding with the first big wave of cases in Utah, 10 lab members were infected. Vaccination with the Moderna mRNA vaccine was provided to the whole staff in January-February 2021 and additional cases were not observed until July 2021. During the period between July and November 2021, coinciding with the surge of the Delta variant, 8 cases were detected. The highly infectious Omicron variant was first detected in Utah on December 3rd 2021 and began an explosive ascent around December 20th, quickly becoming the prevalent circulating strain. Between December 29, 2021 and January 19, 2022, 16 employees were infected, most likely with Omicron. No severe COVID-19 outcomes have been seen among UPHL workforce, probably reflecting the high percentage of employee vaccination (now 86% for 2 mRNA vaccine doses). Secondary transmission within UPHL has been rare, with only four instances of likely or probable "employee to employee" transmission. Only five employees have experienced re-infections with SARS-CoV-2 over the course of the pandemic. UPHL's leadership has strived to ensure a safe workplace environment and protect the integrity of the lab's operations. Mandatory masking was the first measure introduced in June 2020. Mandatory vaccination was introduced early in 2021. Voluntary employee saliva testing via RT-PCR was made available in January 2021. The arrival of Omicron in the US has led to the implementation of more stringent mitigation measures and contingency measures to maintain critical functions. 3rd Booster doses, introduced to contrast waning immunity against Delta, were made available to UPHL employees in November 2021 and are now part of the SARS-CoV-2 vaccination requirement. Higher quality masks such as KN95 have been provided and cloth masks have been entirely banned. Daily NAT testing of critical staff has been recommended. The CDC's isolation/quarantine updated guidance for healthcare workers issued in December 2021 was applied to UPHL critical teams with some modifications, such as a shortened isolation contingent on a negative antigen test. While the increase of Omicron cases at the lab has been striking and expected, emphasis on mitigation and workforce engagement have so far allowed UPHL to continue operating at normal capacity.

Presenter: Kim Christensen, Utah Public Health Laboratory, kchrise@utah.gov

Data Lake in the Cloud: Centralizing Public Health Data in Response to the SARS-CoV-2 Pandemic

C Jossart and K Florek, Wisconsin State Laboratory of Hygiene

The SARS-CoV-2 pandemic has demonstrated the value of genomic epidemiology and the need to build capacity to manage and process the growth of data. In order to meet the demand for variant surveillance, laboratories have expanded sequencing efforts and focused their efforts on reporting in near real-time. At the Wisconsin State Laboratory of Hygiene (WSLH) and the Wisconsin Department of Health Services, the existing data infrastructure was inadequate to meet the needs of integrating genomic and epidemiological data to inform public health partners. To support the integration of numerous data sources, the WSLH has developed a cloud-based centralized data repository for raw and disparate data, known as a data lake. A data lake consists of multiple layers for data storage and processing, which include an ingestion layer for raw data, a curated layer for processed and transformed data, and an application or production layer, which serves cleansed data to end-users. This unbiased approach to data collection allows joining genomic, clinical and epidemiological data from a multitude of sources providing a framework for a highly adaptable data repository. Leveraging a data lake infrastructure to contextualize genomic data will support valuable SARS-CoV-2 analytics including identifying associations between vaccine breakthroughs and changes in health outcomes. Additionally, a cloud-based approach offers a scalable and cost-efficient platform that will allow the WSLH to immediately expand capacity to meet data expansion needs while only paying for the resources and services used. The scalability and affordability of this platform increase access to pre-built cloud-based tools and computationally intensive analytical methods, such as machine learning, allowing the WSLH to operationalize advanced analytical methods to inform public health partners. Cloud-based platforms also ensure data security through durable data infrastructure and data redundancy to prevent data loss and provide the WSLH with total control of the data lake governance to manage data usability and accessibility for users and services. This cloud-based data lake will prepare the state of Wisconsin for future outbreaks of disease, increase the data and analytical capacity, and more effectively apply genomic data to public health disease surveillance.

Presenter: Christopher Jossart, Wisconsin State Laboratory of Hygiene, cjjossart@gmail.com

ENVIRONMENTAL HEALTH

Assessing the Feasibility and Acceptability of using Residual Newborn Dried Blood Spots (DBS) for Prenatal Opioid Exposure Surveillance

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Rationale: This project sought to inform the Council of State and Territorial Epidemiologists (CSTE) and Centers for Disease Control and Prevention National Center for Birth Defects and Developmental Disabilities (CDC NCBDDD) about efforts to enhance laboratory surveillance of prenatal opioid exposures. Using newborn DBS to determine the prevalence of late-pregnancy opioid exposures can provide data on to estimate prevalence, guide resource allocation and service delivery to families affected by opioid use, and support public health interventions.

Objective: To explore the feasibility and acceptability of using anonymized, residual newborn DBS as a specimen type for assessing late in utero exposure to opioids and/or their metabolites.

Methods: The Association of Public Health Laboratories (APHL), in collaboration with CSTE and CDC NCBDDD, conducted an assessment and key informant interviews with state public health laboratory newborn screening programs. An assessment with 10 questions was sent to 53 programs. Key informant interviews were conducted with four programs to learn more about possibly using residual newborn DBS for surveillance of late prenatal opioid exposure, terms for specimen and data sharing, and interest in participating in a pilot project.

Results: Overall, 22 (42%) programs completed the assessment. Four programs that indicated possible interest in participating in a pilot project were interviewed and reported a range of available hole punch (0–58,000) and DBS card (0–62,000) specimens. Three key informants stated that their specimens were likely collected from hospitals providing care for an elevated number of newborns prenatally exposed to substances. All four key informants already had existing systems or working relationships for opioid biosurveillance. Barriers to participating in a pilot project included extended timelines to revise formal processes (e.g., Institutional Review Board, consent forms, collecting new specimens versus using residual specimens) and to gain support from leadership (e.g., health department, newborn screening programs, genetics advisory committee). Two had a strong interest in piloting residual DBS. Two programs expressed concerns about the potential to reduce public trust in newborn screening programs and to discourage participation in newborn screening programs in general. Most key informants noted that performing testing on site would be preferred to shipment of specimens, as this would be more efficient and likely more acceptable to individuals consenting and policy. All key informants expressed concerns about rising numbers in prenatal opioid use and most were enthusiastic about the benefits of using a novel method to perform prenatal opioid exposure surveillance.

Conclusions: Collection of a separate newborn blood spot may be feasible and acceptable for the purposes of prenatal opioid surveillance.

Presenter: Nicholas Ancona, Association of Public Health Laboratories, nicholas.ancona@aphl.org

Overcoming Per- and Polyfluorinated Alkyl Substances Sample Preparation Challenges in Drinking Water

M Ebitson, Biotage

Per- and Polyfluorinated Alkyl Substances (PFAS) have been an interest for the last few years and will continue to be as new analytes are being explored. They are highly resistant to degradation, which allows them to persist in the environment and accumulate in many matrices, including our drinking water sources. Exposure to these compounds has been found to negatively impact human health, which motivated the US EPA to add these analytes to newly developed methods to be monitored in drinking water. When working with selected per- and polyfluorinated alkyl substances in drinking water, one faces several challenges, such as how to minimize background contamination, eliminating unknown sources of interferences and failing results of compounds based on the methodology quality control. These analytes are becoming one of the fastest growing classes being monitored today, and the methodology has been evolving to larger lists to be monitored as we learn more about these analytes. The methodology we will discuss will be based on EPA 537.1 guidelines and will highlight a turnkey solution for extracting and quantifying analytes from drinking water for 18 per- and polyfluorinated alkyl substances. This work demonstrates a method compliant streamlined workflow for PFAS compounds from extraction, concentration to analysis. Drinking water volumes were demonstrated with 250 mL and processed using a 6 mL cartridge-based extraction platform. The cartridge of choice with this methodology was a 6 mL 500 mg unmodified polystyrene-divinylbenzene copolymer. This media choice provides excellent retention for the extraction of a range of polar analytes from aqueous samples using a non-polar retention mechanism. This cartridge was an excellent choice which demonstrated low system background well below the EPA 537.1 methodology recommendation limits of 1/3 the method reporting limit (MRL). The extraction platform provided consistent flows and, when coupled with an ISOLUTE® 101 PS-DVB 6 mL 500 mg PS-DVB cartridge, demonstrated an excellent initial demonstration of accuracy and precision turnkey solution. The results of the analytes were within 15% of their normal values and had a relative standard deviation (RSD) under 11% for each analyte. The recovery data will be presented, along with tips for troubleshooting interferences that are commonly experienced in the lab and for complying with blank contamination issues, as well as areas to focus on to improve and yield consistent recoveries and concentration optimization, and what to avoid.

Presenter: Paul Cannon, Biotage, paul.cannon@biotage.com

Detection of Synthetic Opioids using High Resolution Mass Spectrometry

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Introduction: In 2020, opioid usage in the United States was involved in nearly 69,000 overdose deaths, 80% of which involved synthetic opioids. Novel synthetic opioids are reported frequently,

with 12 new compounds identified by the DEA in the past two years. To increase surveillance regarding these issues and better detect emerging synthetic opioids in clinical matrices, a high-resolution mass spectrometry method, validated using ANSI/ASB Standard 036, has been developed that can identify over 250 novel and synthetic opioids. This method is easily expandable to include new novel drugs for future and retrospective analysis, with the mass spectral library publicly available for use by any lab.

Method: The development of a mass spectral library and effective opioid detection are accomplished using a high-performance liquid chromatography-high resolution mass spectrometry (HPLC-HRMS) method. Analytes in clinical matrices (urine, plasma and whole blood) are extracted and screened against the existing in-house library for tentative identification. For increased confidence, identifications are confirmed through comparison to a reference standard. The lower limit of detection, carryover, interference, extraction efficiency and matrix effects of select analytes are validated. The curated in-house library created using the Traceable Opioid Material Kit® (TOM Kit®) product line can be easily transferred between instruments and laboratories, and the library files are available for download free-of-charge from the TOM Kits® website.

Results and Conclusions: This method has the capability to detect over 250 novel and synthetic compounds within a 16-minute time of analysis. Its validation processes have reflected high confidence in detection capability, as it has been used in four external proficiency tests with a 100% success rate. It expedites the detection and identification of synthetic opioids in clinical matrices over existing targeted analytical methodology, with the ability to increase its analytical capabilities through addition of new analytes. To date, over 100 new compounds have been added since initial method creation in early 2020. This method enables comprehensive synthetic opioid detection and supports public health activities related to surveillance, health studies and emergency response.

(Traceable Opioid Material and TOM Kits are registered marks of the US Department of Health and Human Services)

Presenter: Anisha Saddy, Centers for Disease Control and Prevention, shv8@cdc.gov

Detection of Opioids in Public Health Studies by High Performance Liquid Chromatography Coupled with Tandem Mass Spectrometry

A Tuachi, R Shaner-Forster, E Hamelin and M Carter, Centers for Disease Control and Prevention

Substance abuse is an ongoing threat to public health domestically and internationally. There is accumulating evidence about the harmful effects of opioids abuse; however, studying long-term health effects of abuse is complicated. This is due, in part, to the lack of available longitudinal data, discrepancies in the ability to study abuse consistently, and a lack of information about the best way to characterize and measure long-term opioids abuse. We developed a high-sensitivity laboratory detection method to support the opioids public health emergency. This method identifies 26 fentanyl analogs and related synthetic opioids in human whole blood using high performance liquid chromatography coupled with tandem mass spectrometry (HPLC-MS/MS). The integration of dried blood spots (DBS) allows for a simple sample collection protocol with high

extraction efficiencies. The Opioid Certified Reference Material Kit (Opioid CRM Kit) from the Traceable Opioid Material® Kits (TOM Kits®) product line supplies this method with 22 emerging synthetic opioids and their paired isotopically-labeled internal standards (carbon-13; nitrogen-15) free-of-charge. This method was validated following SWGTOX guidelines and boasts sensitive detection limits ranging between 0.25 and 0.50 ng/mL for all analytes. With measurable long-term stability, low matrix effects and high reproducibility, this method is invaluable in our laboratory's ability to support the opioids public health emergency and provide laboratory testing in stakeholder health and surveillance studies.

Presenter: Abraham Tuachi, Centers for Disease Control and Prevention, qtr4@cdc.gov

Strengthening Regional Environmental Health Systems Partnering to Improve Water Pathogen Surveillance and Public Health Communication

K Schieble, L Beversdorf, B Pfothner, J Plevak and S Bhattacharyya, City of Milwaukee Health Department

Introduction: The water-related exposure of microbial pathogens is a significant determinant of human health. The disease burden from water-associated communicable diseases is influenced by water use, chemical pollution, pathogens and other disease vectors. The Milwaukee Health Department Laboratory (MHDHL) hosted a meeting with 50 system partners in Southeastern Wisconsin to share information and evaluate knowledge gaps regarding drinking, waste and recreational water pathogen surveillance. The goal of this multi-sector partnering approach was to educate environmental health system (EHS) stakeholders on current surveillance efforts to continuously improve public health essential services.

Method: A planning committee comprised of representatives from drinking and wastewater utilities, public health laboratories, academia, local businesses and community organizations was tasked with refining the objectives, identifying subject matter experts as presenters and establishing evaluation criteria for the meeting. The water pathogen surveillance presentations included laboratory technology use and innovations, regulations and processes regarding pathogen surveillance and how these data are shared with local stakeholders and amongst regional partners. The breakout groups, which consisted of drinking water, recreational water and wastewater, evaluated system performance using the Association of Public Health Laboratories (APHL) Laboratory System Improvement Program (L-SIP) essential public health services: Monitor Health Status, Mobilize Community Partnerships and Assure a Competent Workforce.

Results: In general, observations were consistent between the drinking water, recreational water and wastewater groups: 1) a major strength is community partnering and opportunity for collaborations in the areas of water pathogen surveillance, cross-sector partnerships and fellowship opportunities; 2) improving public education and outreach, including robust and sustainable public data sharing, would help establish trust in the community; and 3) statewide community of EH practice system improvement would benefit from having a system "champion" to guide and coordinate regional efforts.

Conclusion: A participant evaluation was performed, recommendations were made and a final report was issued back to

all regional partners for input on prioritizing the next steps for EHS improvement. Participants discussed what has been working well and identified knowledge gaps and opportunities for improvements. By continuing to collaborate with this multi-disciplinary, system-wide partnering, MHDL and regional stakeholders will identify the next steps for advancing efforts toward water pathogen surveillance, research and workforce enhancement.

Presenter: Kristin Schieble, City of Milwaukee Health Department, Email: kschieb@milwaukee.gov

The Vital Role of the Environmental Laboratory as Public Health Laboratories

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Public water and wastewater systems in the United States are responsible for analyzing samples in their laboratories or ensuring samples be analyzed to meet regulatory standards. The laboratory's role in collecting, analyzing and reporting sample results is especially important as data generated by utility samples drives decision-making for public health. Water utility laboratories protect the public health of those that drink produced water and of those downstream of the wastewater effluent discharge. Utilities and laboratories both protect the public health as a core function, while also protecting animals, plants and the environment. While water and wastewater utilities and laboratories are essential stakeholders in the public health system, they are not widely promoted within the industry. Equally integral and often overlooked, other laboratory types include: food, agriculture, commercial and state environmental labs. This interconnected mission is an excellent example of the CDC One Health Concept (OHC), which recognizes interconnection of human, animal and environmental health. The OHC has become a globally distinguished way to promote public health collaboration, involving multiple levels of expertise from scientists to the general public. The 10 Essential Public Health Services (EPHS) also link utilities and laboratories to public health. The role of utilities and laboratories fall under several EPHS goals:

- Assess and monitor population health status, factors that influence health, and community needs and assets – wastewater surveillance
- Communicate effectively to inform and educate people about health, factors that influence it, and how to improve it – regulated contaminants
- Build and support a diverse and skilled public health workforce – utility and laboratory personnel
- Improve and innovate public health functions through ongoing evaluation, research and continuous quality improvement – innovation instead of stagnation
- Build and maintain a strong organizational infrastructure for public health – partnerships with public health departments

Understanding the utility's role as a public health entity is key to promoting and fostering a culture of public health awareness. If they are not viewed this way, funding, equipment quality and personnel will be affected. Utilities participate in many public health-related activities and provide an invaluable prospective for solving problems. The public health system should work with utilities and laboratories to develop an understanding of their role in the protection of public health and build partnerships and interdisciplinary collaboration. The National Wastewater Surveillance System (NWSS), developed in response to the COVID-19 pandemic, shows how these partnerships can be utilized in existing and future events. Utilities and laboratories are an integral part of public health.

Presenter: Hunter Adams, City of Wichita Falls, hunter.adams@wichitafallstx.gov

Automating Sample Extraction for High-throughput Environmental Chemistry Testing

Z Cao, A Frost, X Hu and S Urban, Maryland Department of Health Laboratories Administration

Achieving high and reliable throughput in complex extraction regimens remains a bottleneck in environmental chemistry testing programs. This inherent challenge has been exacerbated by the coronavirus pandemic causing staffing shortages. To mitigate these challenges, we adapted a new, 8-channel automated solid-phase extractor (SPE-03) across three sophisticated EPA Methods for water testing (525.2, 537.1 and 1633). Optimized conditions reduced extraction time, nearly halved costs, maintained or improved recovery and consistency, and dramatically increased throughput. Specifically, evaluating extractor line composition, disc type, disc holder composition and extraction parameters ultimately led to increased recoveries and/or decreased carryover for EPA Method 525.2 analytes. Remarkably, deploying SPE-03 to EPA Method 537.1 for quantifying PFAS at 1 ppt reporting levels resulted in an increase of capacity to ~1,600 drinking, ground or surface water extractions per year with only a 20% FTE involvement. Our studies suggest the automated SPE-03 extractor to be a versatile, reliable and accessible means to increase environmental testing quality and capacity in a state public health laboratory setting.

Disclaimer: Mention of specific commercial products in this work does not in any way constitute endorsement.

The first three authors contributed approximately equally to this work.

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REDCap as a Data Exchange and Storage Platform for Biomonitoring and Surveillance

O Latona and A Perry, Michigan Department of Health and Human Services

The Michigan Department of Health and Human Services (MDHHS) has established a novel surveillance program to determine firefighters' average exposure to per- and polyfluoroalkyl substances (PFAS)–the PFAS in Firefighters of Michigan Surveillance (PFOMS)

system. PFAS are a group of chemicals that have been used in many industrial applications and consumer products. Firefighters are often exposed to PFAS through the use of firefighting foam containing PFAS. Participants in PFOMS provide blood samples for serum PFAS lab testing and complete an exposure survey. Research Electronic Data Capture (REDCap®) is a secure web application for building and managing online surveys and databases. In establishing the data systems in REDCap® for this surveillance program, the PFOMS data team have addressed challenges related to data standardization and transfer capability, restricted data issues, multiple users from an interdisciplinary team and data security. The PFOMS data system was designed as six linked REDCap® projects, each a relational database. The database allows the project team to recruit participants, track communication, link participant information across projects using unique identifiers, store laboratory data and automate communications between project team members and participants. REDCap® projects utilize simple workflows that funnel data from three different sources into self-contained databases. These include internal data entry forms used by 20 different staff members and external data collection surveys completed by an estimated 350 participants at multiple distinct time points. After extraction from STARLIMSTM, R scripts are used to automatically clean and standardize data for 39 PFAS analytes through several quality assurance and control processes before upload into a REDCap® project. An application programming interface (API) within R allows for uploading and extracting data to each REDCap® project, automating much of the ETL (extract, transform, load) process from one system to the next. The exposure survey data is ultimately linked to these lab testing data which is collected using REDCap® project in the database. REDCap®, alongside the R coding software as implemented in the PFOMS project, allows for easy data collection, storage, retrieval and processing of laboratory results. This allows for improved communication and coordination within the project team.

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NJHANES–Implementation, Progress, Learned Lessons

CH Yu, E Bind, E Cook, E Lax, M Chaves, D Riker and Z Fan, New Jersey Department of Health

The New Jersey Department of Health (NJDOH) is conducting a probability-based population surveillance study, NJHANES, that collects basic health and exposure information, nutritional status, and specimens (blood, urine and serum). The survey will measure physical health (height, weight, blood pressure and heart rate) at participants' homes and ~130 environmental contaminants in collected specimens at NJDOH labs. NJHANES's design has been significantly modified since its inception to address COVID-19 brought challenges and limitations. For example, all activities with NJHANES participants are primarily established through non-contact online approaches, including mailing out recruitment package, screening eligible household members and conducting questionnaires through REDCap (HIPPA-complaint survey platform),

consenting individual participants through virtual meetings over MS Teams, and scheduling the home visit and results reporting through an NJDOH secure email account. Only physical measurements and specimen collection are conducted at participant's home by the contracted mobile phlebotomist service. We have adapted specimen collection to be efficient, accommodating and COVID-19 safe. Each staff member is required to wear PPE and use sanitized equipment. COVID-19 has complicated the scheduling of home visits and we prioritize participant's comfort. The specimens are dropped off at NJDOH for testing through the contracted courier service. As of March 31, 2022, 49 households from the 2,000 randomly selected households contacted have responded with interest and 34 participants are consented to participate in the study. 29 home visits and specimen collection were made by simultaneously administering NJHANES surveys. Participants' specimen analyses were complete for metals (n=26) and individual metal results with established health limits (lead, mercury and cadmium in blood and urine; arsenic in urine) were reported to study participants (n=23). We noted COVID-19 brings unanticipated recruitment challenges. We also note a low response rate due to the COVID-19 pandemic and that the participating population leans toward certain sociodemographic subdomains. By comparing NJHANES data with NJ reference data from US Census, we found statistical differences in NJHANES participants vs. NJ reference population, which were derived by higher participation rates from educated and older people. We will address these sampling biases by exploring recruitment strategies that invite nonresponse households including revising recruitment materials, door-to-door recruitment and increased incentives.

Presenter: Zhihua (Tina) Fan, New Jersey Public Health & Environmental Laboratories, tina.fan@doh.nj.gov

Ultra-Sensitive SLE-LC-MS/MS Method Optimization and Validation for Nicotine Metabolites Measurement in Human Serum

C Xu, CH Yu, S O'Leary and Z Fan, New Jersey Public Health & Environmental Laboratories

Tobacco smoking remains a leading cause of preventable disease and premature death in the United States and other countries. Its active ingredient, nicotine, has long been recognized to be an addictive substance. Cotinine (COT) and trans-3'-hydroxycotinine (HC) are two primary metabolites of nicotine after exposure to tobacco smoke. Because their concentrations in body fluids are greater and their half-lives are longer than nicotine, these two metabolites are generally preferred over nicotine as biomarkers of tobacco smoke exposure and are often used for biomonitoring in general population study. A bioanalytical method for the measurement of COT and HC in human serum has been developed by CDC (Method 2017). The CDC method has been used for large-scale biomonitoring study such as the National Health and Nutrition Examination Survey (NHANES) that is ongoing. Due to a wide range of tobacco exposure in general population (e.g., smokers and non-smokers simultaneously), a higher sensitivity/selectivity and a wider range of linearity are required for a population study. The Environmental and Chemical Laboratory Services (ECLS) within the New Jersey Department of Health (NJDOH) conducted experiments to optimize the method based on the CDC method #2017, including

Liquid Chromatography (LC) conditions and Mass Spectrometry (MS) parameters as well as Supported Liquid Extraction (SLE) procedure to enhance overall method sensitivity/selectivity. By using new mobile phase compositions and switching to different ionization source, we achieved much higher signal to noise ratio (S/N), which resulted in LOQ (Limit of Quantitation) improvement from 0.02ng/mL to 0.005 ng/mL. In addition, we further refined the sample preparation steps that resulted in recoveries greater than 95% for both analytes in complex human serum matrix. This optimized method was validated to achieve excellent accuracy (90-105%) and precision (< 5%). This validated method will be used to support the NJ Health and Nutrition Examination Survey (NJHANES), ongoing NJ population surveillance study for the NJ State Biomonitoring Program. The method optimization process and validation results will be discussed in this poster.

Presenter: Carrie Xu, New Jersey Public Health & Environmental Laboratories, carrie.xu@doh.nj.gov

The Analysis of Hydroxy Polycyclic Aromatic Hydrocarbons by Isotope Dilution Online Solid Phase Extraction Followed by High Performance Liquid Chromatography Tandem Mass Spectrometry

E Cook, CH Yu, AM Marcel, S O'Leary, Z Fan and L Zhong, New Jersey Public Health & Environmental Laboratories

The New Jersey public health laboratory is a recipient of the national biomonitoring grant for the second year. The goal of the grant is to perform biomonitoring in New Jersey (NJ). Surveying concentrations of toxic elements and chemical compounds in NJ is important to understand human exposure. Polycyclic aromatic hydrocarbons (PAHs) are produced from incomplete combustion processes. These PAHs are widespread and many are suspected carcinogens with no established threshold levels for carcinogenicity. Biomonitoring of PAHs is relevant for environmental public health because of the widespread exposure of PAHs to humans. Some PAHs are metabolized in the human body and excreted in urine. Eight hydroxy polycyclic aromatic hydrocarbons in human urine can be quantitated by isotope dilution, online solid phase extraction (SPE), followed by high performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS). The concentration of eight PAHs (1-hydroxynaphthalene, 2-hydroxynaphthalene, 2-hydroxyfluorene, 3-hydroxyfluorene, 1-hydroxyphenanthrene, 2- & 3-hydroxyphenanthrene, 1-hydroxypyrene) in human urine can be determined for 500 subjects as part of the New Jersey biomonitoring program. The reference CDC method⁴ is adapted for our protocol. 100 µl of human urine is combined with 50 µl internal standard, 50 µl enzyme buffer, 20 µl ascorbic acid solution and 200 µl MeOH. This mixture is shaken for 10 minutes at a frequency of 23/s. After incubating the mixture for 18 hours at 37 °C, 200 µl of MeOH is added and centrifuged for 15 min at 7500 rpm to remove the enzyme. 200 µl of the supernatant is added to a 500 µl autosampler vial containing 350 µl water. This solution is shaken for 10 minutes at a frequency of 23/s, before injection. Sample SPE, separation and analysis is performed on a ABSciex Qtrap 6500 and spark Holland SPE/HPLC system. SPE is performed on 450 µl of specimen. The analytes are separated using by an Agilent Zorbax Eclipse PAH column analyzed in the mass spectrometer. The

chromatography of the samples showed good separation of all eight analyte by the gradient program employed. The calibration range for 1&2-hydroxynaphthalene is between 200–0.0488 ng/ml and all other analytes are between 25.0 – 0.00610 ng/ml. The r² values for all analytes are 0.99 and greater. The method blanks are less than the lowest calibration for each analyte. The limit of detection, accuracy and precision is currently being determined.

Presenter: Linbin Zhong, New Jersey Public Health & Environmental Laboratories, Linbin.Zhong@doh.nj.gov

South Carolina Opioid Bio-Surveillance Outreach Program

M Tredway, South Carolina Public Health Laboratory

South Carolina (SC) was awarded grant funding from the Centers for Disease Control and Prevention (CDC) to gather data on suspected opioid overdoses. With funding from the Overdose Data to Action Cooperative Agreement (OD2A), the SC Public Health Laboratory (PHL) is working with hospitals to test de-identified urine specimens from suspected opioid overdose patients. The SC PHL tests the specimens for twenty-one fentanyl analogs and reports the data to participating hospitals and stakeholders. The data is helping our state in detecting and addressing opioid misuse in collaboration with our Injury and Substance Abuse Prevention Division and partners statewide. An aggressive recruitment campaign was developed to partner with local hospitals to assist SC PHL by providing residual urine specimens for analysis.

Objectives: To enter into a partnership with hospitals and free-standing Emergency Departments under a formal Memorandum of Agreement (MOA). Train hospitals on specimen submission, provide logistical support and report data. These partnerships allowed for data collection that will provide information on opioid usage and trends in SC.

Methods: Initially, SC PHL staff attempted to get hospital participation by working with hospital laboratory directors, with limited success. After redesigning the approach and targeting hospital executive staff and key stakeholders, we were able to achieve engagement and MOA partnerships at a rate above 90%.

Results/Conclusions: The SC PHL has successfully recruited 37 out of 63 hospitals and two free standing Emergency Departments. The recruitment has resulted in the SC PHL receiving over 5,300 specimens for analysis, thus providing the state with valuable data in addressing the opioid epidemic. Our recruitment campaign has provided data that is used by hospitals, communities, law enforcement, public health officials and other state agencies to fully understand the true impact of opioid use in SC. Our ongoing engagement efforts will continue to build relationships that will help retain partnered hospitals and expand the testing capabilities of the SC PHL.

Presenter: James LaPalme, South Carolina Public Health Laboratory, Email: lapalmjh@dhec.sc.gov

Detection of Mitragynine and 7-Hydroxymitragynine in Keratinized Specimens

A Thompson and J Jones, United States Drug Testing Laboratories, Inc.

Background/Introduction: *Mitragyna speciosa*, known as kratom, is a native tropical tree to southeast Asia, with leaves that contain compounds that can have psychotropic effects. The species has been used in herbal medicine and is used in religious traditions. The leaves of the kratom plant can be chewed, brewed in tea, smoked, or eaten in food. However, most people take kratom as a pill, capsule or extract. Two compounds in kratom leaves, mitragynine and 7-hydroxymitragynine, interact with mu-opioid receptors in the brain, producing sedation, pleasure and decreased pain, especially when users consume large amounts of the plant. When kratom is taken in low to moderate doses it produces stimulant effects. The legality of the plant versus the legality of the specific analytes is becoming increasingly complicated as states take different legal actions as well as the federal government.

Objective: The objective of this study is to develop and validate a method for the detection of mitragynine and 7-hydroxymitragynine in keratinized specimens using a Laser Diode Thermal Desorption Tandem Mass Spectrometry (LDTD-MS/MS) screening analysis and a Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS) confirmation analysis.

Results: The Standard Practices for Method Validation, was followed. The LDTD-MS/MS analysis had a cutoff of 100 pg/mg, with a limit of detection of 30 pg/mg. Both the extracts and dried LazWell plates were stable for 48 hours and no carryover or obvious interferences were observed during the validation. Significant ion suppression was observed for 7-hydroxymitragynine, however, significant ion enhancement was observed for mitragynine. Authentic specimens were analyzed and presumptive positive specimens were forwarded to LC-MS/MS for confirmation. The confirmation method was validated with a cutoff of 100 pg/mg and a limit of detection of 5 pg/mg for 7-hydroxymitragynine and 3 pg/mg for mitragynine. Precision and accuracy were challenged at 40 pg/mg (Low), 125 pg/mg (Mid) and 1000 pg/mg (High) following our standard in-house procedure for setting up assays and results were acceptable. Extracts were stable for up to 7 days, there was significant ion suppression, >25% for both analytes and no obvious interferences or carryover were observed. Linear range was determined to be between 40 pg/mg and 2000 pg/mg. All authentic specimen analyzed showed 100% correlation with the screening analysis.

Discussion/conclusion: Kratom has found an increased use among people who are looking at alternatives to traditional opioids. The validated method described here allows accurate identification of usage of kratom, allowing for objective proof that can be used by rehab and diversion programs to intervene and provide more successful outcomes. On a federal level, the FDA has no approved use for kratom, while the DEA has listed kratom as Drug and Chemical of Concern.

Presenter: Andre Sukta, USDTL, andre.sukta@usdtl.com

FOOD SAFETY

Utilizing APHL's Learning Management Portal to Promote the PulseNet USA BioNumerics v7.6 Training

A Yusuf and K Kubota, Association of Public Health Laboratories

Background: PulseNet, the national molecular subtyping network for foodborne disease surveillance, was established in 1996 by the Centers for Disease Control and Prevention (CDC), United States Department of Agriculture (USDA), United States Food and Drug Administration (FDA), four state public health laboratories and Association of Public Health Laboratories (APHL). The PulseNet program works with federal, state, local and international partners to create a globally integrated surveillance network in response to foodborne disease outbreaks. APHL works with federal partners to provide training in the wet-bench and analytical methods (BioNumerics) for public health laboratories participating in the PulseNet network both domestically and globally. Due to the SARS-CoV-2 pandemic, in-person trainings were paused, which led APHL and the Enteric Disease Laboratory Branch at the CDC to collaborate and develop online training modules.

Methods: APHL Food Safety department worked closely with the Training Workforce and Development at APHL to develop P.A.C.E. (Professional Acknowledgment for Continuing Education) accredited training webinars. The P.A.C.E. material application checklist was followed to ensure the training was aligned with the P.A.C.E. guidelines. Our CDC partners created the assessments (quiz questions) for each part of this series. Participants are required to score 80% or above to receive P.A.C.E. credit. A series of Zoom calls were made with the CDC to record each presentation for the training. APHL also used the transcription service REV to help transcribe the recordings. All training materials, such as flyers, presentations, transcripts and assessments, are live on the APHL Learning Management Portal.

Results: Data gathered from July 2021 to March 2022 shows that 143 participants registered for the BioNumerics training. Of these, 54 participants fully completed the training, 51 participants have not started the training and an additional amount of 38 participants are still completing the training. Data found that most public health laboratorians completed the training between September and October of 2021. A total of 23 state public health laboratories, eight local public health laboratories and two federal laboratories completed the training. The PulseNet BioNumerics Advanced training series went live on the APHL Learning Management Portal in March 2022. The intended audience will be those who are familiar with BioNumerics or have completed the PulseNet BioNumerics v7.6 self-study.

Conclusion: Due to the SARS-Cov-2 Pandemic, in-person training was paused. However, APHL and CDC were able to efficiently adapt and utilize the Learning Management Portal for the delivery of training materials in 2021. This portal allowed our participants to complete the training virtually and at their own pace. In just a few months, we were able to enroll over 100 participants in the BioNumerics v.7.6 training series. Despite the obligations of working the front lines of the public health response to SARS-Cov-2, PulseNet participating laboratory scientists found time to complete these training modules, demonstrating the importance of this

training series. In the future, we look forward to providing more PulseNet training using our Learning Management Portal.

Presenter: Adom Yusuf, Association of Public Health Laboratories, adom.yusuf@aphl.org

Evaluation of a Novel One-tube Nested Real-time Quantitative Polymerase Chain Reaction Assay to Detect *Cyclospora cayetanensis*

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Cyclospora cayetanensis is a coccidian parasite that causes outbreaks of cyclosporiasis, a food-borne gastrointestinal (GI) disease, in North America and other regions. In 2021, domestic diagnosis of *C. cayetanensis* was made 38% of the time by microscopy, 50% by syndromic tests and 3% by PCR assays. Microscopy is cheap and time efficient but requires a highly skilled technician. Syndromic tests are powerful diagnostic tools but often require specialized equipment and are expensive. It is imperative to develop sensitive, reliable and inexpensive assays for the diagnosis of *C. cayetanensis* to protect the health of individuals and keep fresh produce safe for consumers.

In this study, we compare a one-tube nested qPCR targeting 89bp of the mitochondrial cytochrome B (CytB) gene to the 18S rRNA (18S) diagnostic qPCR currently used by the US Centers for Disease Control and Prevention. The one-tube nested qPCR approach was evaluated due to the increased sensitivity and specificity usually afforded by nested assays, without the risk of amplicon contamination and lengthy hands-on time usually associated with nested PCR formats. A panel of 151 specimens was used to assess the performance characteristics of this assay, including 101 clinical specimens positive by the BioFire[®] gastrointestinal panel, which has a reported 100% sensitivity and 100% specificity for *Cyclospora* and 50 specimens negative by microscopy and PCR for *Cyclospora*. These negative samples were positive for other related human pathogens, such as *Cryptosporidium* and *Giardia*. Difference in sensitivity was assessed with a chi squared (χ^2) test to determine significance. The novel CytB qPCR test detected 81/101 (80%) *C. cayetanensis* positive specimens while the 18S qPCR detected 58/101 (57%) of the positive specimens. Neither test reacted to the negative specimens. The increase in positive specimens detected by the CytB qPCR compared to the 18S qPCR was statistically significant ($p=0.0007$). While BioFire[®] is more sensitive, the novel CytB assay could be useful in settings where proprietary machines and supplies are not available or accessible due to cost and is a marked improvement over the 18S assay. It is also useful as the one-tube nested qPCR method requires no additional training other than what is already required to run traditional qPCR assays. The CytB qPCR test should make detection of *C. cayetanensis* more accessible. Further evaluation of this assay will be performed using sorted oocysts for a limit of detection study.

Presenter: Travis Richins, Centers for Disease Control and Prevention, richinstravis@gmail.com

Foodborne Outbreak Detection and Control using an Automated Whole Genome Sequencing Platform

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Foodborne illnesses impact over 600 million people worldwide each year, leading to more than 420,000 deaths and the loss of 33 million healthy life years. Detection and serovar identification of foodborne pathogens such as *Salmonella* and *Listeria* can help limit the impacts of foodborne illness, but these methods lack the resolution needed for public health surveillance. Whole genome sequencing of bacterial isolates (iWGS) provides the highest level of strain resolution, determines relatedness to other strains, and can fully characterize the virulence and antimicrobial resistance profiles of a pathogen. CDC's PulseNet and NCBI/FDA's collaboration, GenomeTrakr, use iWGS data for pathogen identification, which facilitates tracing outbreaks to their origins and sharing that information in real time with the FDA, USDA, CDC and public health departments to protect consumers. Over 700,000 food pathogen isolates have been sequenced and added to public databases through these programs. Revealing pathogen relatedness at the DNA level enables greater resolution of strain and lineage, thereby increasing more appropriate responses and interventions to contain food safety outbreaks establishing iWGS as the gold standard for foodborne disease surveillance and outbreak investigation. However, universal adoption of iWGS is challenging due to the specialized expertise and hands-on time that is required with preparing libraries for sequencing and bioinformatics data analysis. Clear Labs fully automated iWGS platform can reduce manual hands-on time from multiple hours to less than 30 minutes and narrow the number of human touch points from multiple steps down to just one. This is achieved through automation of DNA extraction, normalization, library preparation, sequencing and bioinformatics analysis; helping to democratize genomics so that it can be more widely adopted across any laboratory to support foodborne disease surveillance. To evaluate the performance of the Clear Labs automated iWGS platform, *Salmonella enterica* Typhimurium isolates from the same FDA SAFE outbreak cluster collection and similar isolates not implicated in the outbreak were analyzed. Genome assemblies were generated with these strains using the Clear Labs end-to-end automated workflow alongside a manual sequencing workflow. The sequences generated were then used to build phylogenetic trees that equally and correctly clustered the *Salmonella* outbreak strains. Thus, automated iWGS platforms, through generation of near real time genomic data will play a key role in the rapid generation of actionable data for food safety surveillance and investigations.

Presenter: Michael Balamotis, Clear Labs, michael.balamotis@clearlabs.com

Rapid Sequencing: A Tale of iSeq and MinION

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Whole genome sequencing of foodborne pathogens allows public health agencies to characterize bacterial isolates and identify similarities between strains. The bulk of bacterial genome sequencing done by the US Food and Drug Administration and GenomeTrakr network laboratories is performed using the Illumina MiSeq platform. In this study, we evaluate the utility of two more recent sequencing instruments, the iSeq from Illumina and the MinION from Oxford Nanopore. Both instruments are well-suited to the sporadic sequencing of real-time isolates as they rapidly produce sequence data on a smaller number of samples than the MiSeq, and less consumables are used per sequencing run. Reference strains of *Escherichia coli*, *Salmonella enterica*, *Listeria monocytogenes*, *Campylobacter jejuni* and *Vibrio parahaemolyticus* were sequenced on the iSeq using 2 x 150bp library preparation and the MinION using 1D library preparation. We found that the iSeq produces sequencing data in ~18 hours with quality metrics similar to MiSeq (>80% Q-Score 30). In contrast, the runtime of the MinION can be adjusted as desired, but we found that the bulk of data was produced within 12 hours. The MinION reads were much longer with an average length greater than 10kb, but the quality was greatly reduced (average Q-score 10). Sequence data from both instruments was sufficient for bacterial species identification. Genome assemblies of the iSeq and MinION data were generated individually as well as combined into a hybrid genome assembly using Unicycler. The iSeq assemblies were very accurate yet fragmented into many contigs while the MinION assemblies were contiguous but contained regions of low-quality sequence. The hybrid genome assembly method resulted in complete and accurate genomes that provide greater detail than either method individually. Overall, the results of our studies show that both iSeq and MinION can be used to rapidly confirm and characterize the genetic identity of bacterial isolates.

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Where in the World is *Salmonella* Sandiego?

E Thomson, P O'Brien, K Kuwazaki, J Takiguchi, D Ornellas and M Ching-Lee, Hawaii State Department of Health

Introduction: Since 2017, multiple cases of *Salmonella* Sandiego have been reported to the Hawaii State Department of Health (HDOH), primarily among Maui residents. No source has been identified. Hawaii has not previously had *Salmonella* Sandiego cases reported. An epidemiological investigation was launched in 2021 to investigate this cluster.

Methods: Case demographics and risk factors were assessed using a standardized *Salmonella* interview form. A case is defined as a person who experienced diarrhea and/or fever and had S. Sandiego confirmed by culture or culture-independent diagnostic test and serotyping from 2017–present. Four cases with urine specimens were included. Specimens positive for *Salmonella* were sent to the State Laboratory for further analysis. Serotyping was conducted via conventional agglutination methods. From 2019–2021, cluster isolates were characterized using whole genome sequencing, while isolates from 2017–2018 were characterized using pulsed-field gel

electrophoresis. Environmental samples collected include four 2-L water samples from locations along the Kihei coastline.

Results and Discussion: Of 38 *Salmonella* Sandiego cases reported to HDOH from 2017–present, ages ranged from 0–82 years with a median age of five years old. Fourteen (37%) were ≤2 years old at time of illness. Twenty-two cases (58%) were female and 16 (42%) were male. Five cases (13%) were hospitalized and no deaths reported. Sixty-eight percent of cases resided in a single coastal community; 24% who resided elsewhere reported spending time in that area. Three cases (8%) were non-residents. The most recent case was in December 2021. Of six (43%) cases ≤2 years old in 2021, 33% were formula-fed and 67% were breast-fed. Additional exposures assessed for cases included local produce (3/14, 21%), recreational water (4/14, 29%) and animals at home (4/14, 29%). Two out of three national isolates (UT,WA) reported exposures on west Maui. All *Salmonella* Sandiego isolates were within 0–14 alleles of each other. Cluster isolates received at NARMS indicate intermediate interpretation to colistin. After review of water quality reports on Maui, two consecutive samples had increased levels of indicator organisms at an ocean swimming area in September 2021. Water samples taken in December 2021 from the Kihei coastline were filtered, enriched and isolated a Group B *Salmonella*, H-Factor I.

Conclusion: HDOH continues to investigate a *Salmonella* Sandiego cluster now comprising of 38 cases over a four-year period, with common exposure to a coastal Maui community. Standard investigative techniques have been unsuccessful in identifying the source. Next steps we are considering include open-ended interview techniques, applying the CDC hypothesis generating questionnaire, inquiring on food sampling from the cases' household and consultation from our foodborne illness center of excellence.

Presenter: Ellen Thomson, Hawaii State Department of Health, ellen.thomson@doh.hawaii.gov

Development and Validation of a Multiplex Real-time Quantitative PCR Method for the Detection and Speciation of *Vibrio* Bacteria

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Vibrios are marine bacteria that are highly abundant in coastal waters worldwide. *Vibrio* bacteria are found both free living in aquatic environments and associated with marine organisms including fish, crustaceans and algae. While most of the over 100 identified *Vibrio* species are not human pathogens, approximately 12 *Vibrio* species are the causative agents of human illness. In the United States, the CDC estimates that approximately 80,000 cases of *Vibrio* illness occur each year, of which approximately 52,000 cases are caused by eating foods contaminated with *Vibrio* bacteria, often raw or undercooked contaminated seafood. Most *Vibrio* infections result in mild or moderate gastroenteritis, while severe cases can lead to septicemia. One species, *Vibrio vulnificus*, can cause both foodborne illness and severe wound infections. Because Vibrios favor warm waters, most *Vibrio* infections occur during summer months, and several studies have correlated rising ocean temperatures with increased *Vibrio* concentrations and, likewise, infections. Here, we develop a multiplex real-time TaqMan PCR method for the rapid detection and speciation of *Vibrio* bacteria.

Two multiplex PCR panels with five primer and probe sets each have been designed to be tested sequentially. The first PCR panel differentiates whether a tested clinical specimen is a member of the *Vibrio* genus or of four additional genera that commonly cause foodborne illness: *Listeria*, *Salmonella*, *Shigella* and *Yersinia*. If the first PCR panel indicates that a tested clinical isolate is a *Vibrio*, a second multiplex PCR reaction is used to determine if the bacterium is one of five *Vibrio* species that commonly cause disease: *Vibrio alginolyticus*, *Vibrio cholerae*, *Vibrio fluvialis*, *Vibrio parahaemolyticus* and *V. vulnificus*. For the PCR panels, the oligonucleotide primer sets and TaqMan probes for *Vibrio* detection were designed to amplify sequences that are highly conserved among the *Vibrio* genus or among specific *Vibrio* species but are absent in other genera or species, respectively. Validation of the PCR primer and probe sets were performed individually and in multiplex on five clinical isolates of each *Vibrio* species. Future directions include adapting the PCR method for the detection of *Vibrios* on contaminated seafood and within clinical specimens, quantifying the detection limit of the method and testing the ability of the method to detect mixtures of *Vibrio* species.

Presenter: Michaela Eickhoff, New Jersey Department of Health, Public Health and Environmental Laboratories, mjeickhoff2@gmail.com

Analysis of Pesticides in Fruit Juices by Modified QuEChERS Extraction and Analyzed by High Performance Liquid Chromatography Quadrupole Time of Flight

D Wene, S O'Leary, A Lin, CH Yu and Z Fan, New Jersey Public Health and Environmental Laboratories

The New Jersey State Public Health and Environmental Laboratories (PHL) was the recipient of an FDA grant to enhance public health by developing a method to test pesticides in juices. The method will enhance public health by providing a new method for the New Jersey public health laboratories. Recent studies report concerns in finding increasing quantities of pesticides in juices. Pesticides can be extremely harmful to humans. Carbamates and organophosphates negatively affect the nervous system. Research data supports findings that other groups cause cancer as well as detrimentally affect the endocrine system. This method was adapted from a liquid chromatography (LC) triple quad mass spectrometer (QqQ MS) method to detect pesticides in marijuana based on literature. The method was setup to detect harmful pesticides regulated in the US as well as pesticides banned in the US because fruit juices can be imported from other countries. Juices from Spain and the UK have relatively high level of pesticides compared to the US. Presently there are limited regulations set by the US government to monitor fruit juices for pesticides. The aim of the research is to develop a method to test for pesticides in apple and grape juices utilizing a quadrupole time-of-flight (Q-ToF) instrument. The method was optimized for ToF instrumentational analysis and utilizes ultra high-pressure liquid chromatography (UHPLC) to separate compounds. The extraction process was modified from the standard QuEChERS method. Experiments were performed on the Q-ToF instrument to determine optimal gas flows and temperatures, voltages on the capillary and nebulizer pressure. UHPLC conditions were optimized as well as modifications with the mobile phase. A fully comprehensive method was developed and validated. The method

will be able to detect all pesticides at levels below ten parts per billion, though most are more sensitive. All limits of detection are between 0.023 and 3.23 ppb. The laboratory was able to develop the method for 64 pesticides in multiple brands of apple and grape juices. Baseline separation was achieved for all compounds. The limits of quantitation, between 0.07 and 9.7 ppb, were below tolerances set by the US government. Linearity was achieved with all pesticides using six calibration points and a coefficient of determination greater than 0.995. Spike recoveries were performed utilizing seven spikes. Spikes recoveries were evaluated and results support a recovery range of 60-130%. Reproducibility was within 22%. Interference determination was analyzed for three different apple and grape consumer brands with five extractions for each brand. All interferences were below the lowest standard. The validated method will be utilized by to run samples of fruit juices for any agency that requests testing. The results of development and validation will be discussed in the poster.

Presenter: Daniel Wene, New Jersey Public Health and Environmental Laboratories, daniel.wene@doh.nj.gov

The Limit of Detection of the BioFire® FilmArray® Gastrointestinal Panel for the Foodborne Parasite *Cyclospora cayetanensis*

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Cyclosporiasis is a foodborne diarrheal illness caused by the parasite *Cyclospora cayetanensis*. Since 2018, >1000 laboratory confirmed cases of cyclosporiasis have been reported annually in the United States. Diagnosis of cyclosporiasis has primarily relied on parasite specific methods, though new detection tools are now available including syndromic panels such as the BioFire® FilmArray® Gastrointestinal (GI) panel, a molecular multiplex panel that tests for 22 unique gastrointestinal pathogens in a single stool sample, including detection of *C. cayetanensis*.

In 2021, the Centers for Disease Control and Prevention (CDC) received 251 specimens from patients diagnosed with cyclosporiasis using the BioFire® FilmArray® GI panel for genotyping through our novel workflow. However only 71% of these successfully genotyped, raising questions about potential differences in the limit of detection (LOD) of the BioFire® FilmArray® GI panel in comparison to the CDC genotyping workflow. The published BioFire® FilmArray® GI panel LOD is 180 genome equivalents (GEq)/mL, though it is unclear how this relates to the number of *C. cayetanensis* oocysts present in a clinical sample. Thus, we sought to determine the LOD of the BioFire® FilmArray® GI panel in terms of numbers of *C. cayetanensis* oocysts. We used a BD FACSARIA™ II Cell Sorter to sort specific numbers of *C. cayetanensis* oocysts previously purified from three different human stool sources. We sorted 50, 40, 30, 20, 10, 5 and 1 oocyst(s) from each of the sources in triplicate, for a total of nine samples at each of the seven values. We added 200µL of negative stool matrix in Cary-Blair to the sorted oocysts and ran samples on a BioFire® FilmArray® Torch instrument according to the manufacturer's instructions. We found that the BioFire® FilmArray® GI panel consistently detected ≥20 *C. cayetanensis* oocysts (9/9

samples positive at each level), but false negative results were obtained among samples with 1, 5 or 10 *C. cayetanensis* oocysts (11-67%). These results provide a parasitologically relevant LOD for the BioFire® FilmArray® GI panel that should enable comparison to non-molecular *C. cayetanensis* detection techniques (such as acid-fast staining) that are still widely used for cyclosporiasis diagnostics. This information may also help to inform and improve outbreak investigations by providing insight into specimens that fail to genotype in the CDC genotyping workflow.

Presenter: Anna Peterson, Oak Ridge Institute for Science and Education, yyi6@cdc.gov

Partnerships and the Epidemiological Advancements of Cyclosporiasis detection facilitated by Next Generation Sequencing

SL Butler, D Dunbar, Y Sun, C Wang and R Lee, Texas Department of State Health Services Laboratory

Pathogenic coccidia, *Cyclospora cayetanensis*, are intestinal protozoans directly responsible for cyclosporiasis. Cyclosporiasis is a disease known to cause intermittent prolific diarrhea, low grade fevers, myalgia, anorexia, fatigue, weight loss and vomiting. Onset of illness typically begins a few days after ingestion of fecal contaminated food such as fruits and vegetables and last as much as three weeks. The occurrence of other species is recognized; However, *C. cayetanensis* is thought to be the only species to cause human illness and has no known animal hosts. Morphologically, species of this genus are indistinguishable. Still the gold standard for medical parasitology, microscopy, is used as a diagnostic tool for suspected cyclosporiasis cases. With a worldwide geographical distribution, outbreaks of cyclosporiasis have occurred every year since the early 1990s with peaks between May and August. However, this nationally reportable disease is difficult to investigate. There are currently no immunodiagnostic tools available, subtyping technologies are very limited and outbreaks can contain a mixture of species. No successful in-vitro culture of *Cyclospora* exists and there are no known animal hosts, thus leaving stool samples collected from infected humans to be the model source. In 2014, the CDC partnered with the Texas Department of State Health Services (DSHS) Laboratory and several other state public health laboratories in an effort to develop a real-time epidemiological tool for the detection of *Cyclospora*. During peak seasons, anonymized human stool samples were routed to the CDC as part of surveillance. These samples were speciated to help develop a more efficient subtyping tool. Using a CDC-developed next generation sequencing (NGS) method, our laboratory collaborated with the CDC in 2017 to initiate targeted NGS testing of eight genetic markers for *Cyclospora* and submit sequence data generated to CDC for analysis. A total of 720 samples were tested from 2018-2021. This poster will depict our role in the CDC *Cyclospora* Surveillance Project, illustrate how different areas of the DSHS Laboratory and epidemiologists work collaboratively, and the surveillance results from Texas. The accumulation of genomic data is expected to facilitate development of a repository, provide case and cluster linkage and, consequently, aid in the investigation of future *Cyclospora* outbreaks.

Presenter: Susan Butler, Texas Department of State Health Services Laboratory, Susan.butler@dshs.texas.gov

Bactopia v2: Highly Scalable, Portable and Customizable Bacterial Genome Analyses

R Petit, Theiagen Genomics & Wyoming Public Health Laboratory

Generation of sequence data for bacterial genomes has become standardized to the point that bioinformatic analysis is the major bottleneck. There is now, more than ever, a need for bioinformatic workflows to be highly scalable and adaptable to the user's needs. In order to address this we created Bactopia, a Nextflow workflow to provide efficient and flexible comparative genomic analyses for any bacterial species or genera. Bactopia can process Illumina and/or Nanopore sequences with more than 100 bioinformatic tools and many publicly available datasets. Furthermore, Bactopia includes more than 20 additional workflows to select high quality samples for downstream comparative analyses, such as pan-genome, phylogenetic and functional analyses. We have rewritten the original workflow to take advantage of Nextflow DSL2, allowing users to customize Bactopia by including different tools for each step and simplifying integrations with other bacterial analysis workflows from the popular nf-core community. Bactopia v2 is a significant step forward, as it allows users to easily adapt Bactopia to fit their organism-specific needs. To demonstrate this, we implemented Staphopia as a custom Bactopia workflow for the analysis of *Staphylococcus aureus* genomes. This sub-workflow supplements the standard Bactopia workflow with additional *S. aureus* specific analyses such as methicillin-resistance (MRSA), spa typing and agr typing. In addition, we created the Merlin sub-workflow, which automatically executes species-specific analyses for many public health pathogens. Bactopia (v1 and v2) is an open source system that has been shown to scale from projects as small as one bacterial genome to tens of thousands of bacterial genomes. In the latest release of Bactopia, the scalability remains and we have introduced even greater flexibility to meet the needs of our users. The documentation and code for Bactopia can be accessed at <https://bactopia.github.io/>.

Presenter: Robert Petit, Theiagen Genomics, robert.petit@theiagen.com

Vibrio cholerae Infections Following Consumption of River Water in Nebraska

T Wahlig, University of Nebraska Medical Center

Introduction: Although cholera caused by toxigenic *Vibrio cholerae* was historically noted to pose a significant threat to Indigenous peoples and European settlers in the US in the 1850s, recent cases of cholera in the US are sporadic and almost always associated with international travel. With the introduction of culture independent diagnostic tests (CIDT) to detect non-toxigenic cases of *V. cholerae*, diagnosing gastrointestinal (GI) vibriosis has become more common, especially following consumption of undercooked shellfish. This report describes two cases of GI vibriosis in individuals who consumed untreated river water in Nebraska.

Case Reports: Two cases of *V. cholerae* were identified in two partnered individuals in July 2021. The patients were a male (33 years old) and female (35 years old), who were experiencing homelessness and had spent a month camping near the Platte River in Scottsbluff, NE. The patients reported drinking untreated river water while camping. The patients experienced diarrhea, vomiting, cramps, fever and muscle pain, starting on 20 July 2021,

which prompted them to seek medical care on 25 July. At this time, stool specimens were collected and sent to the local laboratory for testing.

Bacteriology: Stool samples from both patients were reported out as detected for both the *Vibrio* and the *V. cholerae* targets using the FilmArray Gastrointestinal Panel (BioFire Diagnostics). These stools were subsequently forwarded to the Nebraska Public Health Laboratory where presumptive *V. cholerae* was isolated from each stool using TCBS agar and other phenotypic testing. Whole genome sequencing was subsequently performed on the isolates using an Illumina MiSeq platform. Bioinformatics analysis for assessing sequencing quality, genome assembly, organism identification and toxin detection were performed with software available on <https://github.com>, using default parameters. Bioinformatics analysis showed the isolates to be unrelated by SNP analysis but confirmed the identifications of *V. cholerae* and the absence of the cholerae toxin genes. Further analysis of the isolates at the Centers for Disease Control and Prevention showed that the isolates were not serogroup O1 or O139.

Conclusion: These cases demonstrate the use of CIDT, along with confirmatory culture testing, in the assessment of risk factors to improve public health detection and case management. Additional studies are needed to better define what role various serogroups of *V. cholerae* play in disease progression in the U.S.

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Differences in the Spatiotemporal Factors Associated With *Salmonella* and *Listeria monocytogenes* Likelihood in Environmental Samples From Virginia Produce Farms

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Introduction: Foodborne pathogen contamination risk is not uniform within produce growing environments; thus, managing contamination risks of preharvest produce is challenging. This study aimed to identify spatiotemporal factors associated with *Salmonella* and *Listeria* isolation from soil, drag swab and water samples from Virginia produce farms.

Methods: Soil (N=3400), drag swab (N=3400) and water (N=3120) samples were longitudinally collected from ten Virginia produce farms. All samples were tested for *Salmonella* and *Listeria* and PCR confirmed. Presumptive *Listeria* isolates were identified to species-level by sigB typing. Conditional forest analysis and Bayesian mixed models were used to identify associations between spatial (e.g., growing region) and temporal (e.g., weather) factors, and the likelihood of *Salmonella*, *L. monocytogenes* and *Listeria* spp. (excluding *monocytogenes*) isolation. Surrogate trees were used to visualize conditional forest results.

Results: *Salmonella* was isolated from 5.3% (49/920) of samples, with the majority coming from Virginia's Eastern Shore (46/49). Of the 49 *Salmonella* positive samples, 14 unique serovars were isolated with Newport yielding the predominant serovar (32.7%; 16/49) followed by Braenderup (10.2%; 5/49). *L. monocytogenes* and *Listeria* spp. were isolated from 2.3% (21/920) and 1.8% (17/920) of samples, respectively. Based on the mixed models, the likelihood of *Salmonella* detection was significantly higher in water

compared to terrestrial samples (Odds Ratio=6.5, 89% Credibility Interval=3.3, 14.9). Additionally, the likelihood of isolating *L. monocytogenes* and *Listeria* spp. was significantly higher in samples collected in winter, compared to fall, spring and summer (P=9.4 °C (P< 0.001). *L. monocytogenes* was most likely to be isolated from water samples collected in winter from sites where forest and wetlands accounted for < 36% of land cover (P< 0.001). *Listeria* spp. were most likely to be isolated from drag swabs samples collected in winter when no rainfall occurred 1-2 days before sample collection (P< 0.001).

Significance: These findings identify factors that increased the likelihood of isolating *Salmonella* and *Listeria* in preharvest environments and are helpful in development of control strategies within small scale growing regions for specific scenarios to minimize produce contamination.

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GLOBAL HEALTH

The Global Laboratory Leadership Programme Provides Lessons for One Health Collaboration and Laboratory System Building

J Isadore, S Emery, S Musumeci, B Wilcke and L Maryogo-Robinson, Association of Public Health Laboratories

One Health was recently defined by a joint tripartite [Food and Agriculture Organization of the United Nations (FAO), the World Organisation for Animal Health (OIE) and the World Health Organization (WHO)] and United Nations Environment Programme statement as “an integrated, unifying approach that aims to sustainably balance and optimize the health of people, animals and ecosystems.” (1) The approach seeks to engage and mobilize multiple sectors and disciplines to address threats to health and ecosystems. Throughout the world, the sectors that make up national laboratory systems are often siloed with minimal communication or collaboration among sectors. Through a unique collaboration, six international partners came together to support a One Health approach to laboratory professional development and laboratory system building. The Association of Public Health Laboratories (APHL), the Centers for Disease Control and Prevention, the European Centre for Disease Prevention and Control, FAO, OIE and WHO collaborated on the development of the Laboratory Leadership Competency Framework and a One Health professional development program, the Global Laboratory Leadership Programme (GLLP), designed to support the leadership competencies. Through this on-going collaboration, the partners developed an inclusive leadership program designed to connect laboratory sectors and encourage their engagement. The partners are currently supporting program development and implementation in 23 countries across the globe and continue to support the One Health approach by engaging multisectoral participation in the programs and collaborating among partners to allow for engagement of all sectors. Eight countries have initiated program activities and engaged their first cohorts, including 126 laboratorians from across the sectors. Sixty-seven percent of participants are from the human health sector, 29% from the animal health sector, 9% from food/water/environmental health sector and 3% from research laboratories. As part of the GLLP, participants

collaborate on projects and develop a community of practice to encourage relationship building, on-going communication and collaboration amongst sectors. APHL's Global Health Program and domestic Training and Workforce Development Program will also collaborate to transform GLLP modules into eLearning sessions to support APHL's domestic workforce development goals. As laboratories navigate the ever-changing challenges ahead, multisectoral communication and collaboration will be key to success and the GLLP will provide laboratory leaders with the knowledge and skills necessary to support a One Health approach to laboratory system building.

(1) <https://www.who.int/news/item/01-12-2021-tripartite-and-unep-support-ohhlep-s-definition-of-one-health>

Presenter: Shannon Emery, Association of Public Health Laboratories, shannon.emery@aphl.org

INFECTIOUS DISEASES

Multi-Plex STI Testing & Co-infection Prevalence at a Reference Laboratory

K Lundgren¹, K Calvert¹, W Crown²; ¹Alverno Laboratories, ²Abbott Laboratories

The Abbott Alinity m STI Investigation Use Only (IUO) assay is a qualitative PCR test that simultaneously detects *C. trachomatis* (CT), *N. gonorrhoeae* (NG), *T. vaginalis* (TV) and *M. genitalium* (MG) from a single sample, allowing labs to consolidate molecular-based STI testing on a single, fully automated, random-access platform. In this study, we compared the performance of the Alinity m STI IUO assay to the predicate methodology across multiple sample types in a large reference laboratory. Tested in this study were derived samples and clinical samples previously characterized via Abbott RealTime CT/NG assay on the m2000 platform (394 & 391, respectively) and 220 TV samples using Cepheid Xpert TV assay or wet mount in the following samples: urine, cervix/vagina, rectum, urethra, throat and ThinPrep. No reference MG testing was performed. Of 394 CT samples, 154 were positive on both m2000 and Alinity m, with percent positive agreement (PPA) 93%, negative percent agreement (NPA) 99% and overall percent agreement (OPA) 96%. Of 391 NG samples 89 were positive on m2000 and Alinity m, PPA (99%), NPA (100%), OPA (100%). Of 220 TV samples, 73 were concordant on Cepheid and Alinity m, PPA (95%), NPA (99%), OPA (97%). Although there is no method comparison for MG, of 399 samples, 87 were positive across sample types. Out of 1,404 total results across all targets and sample types, 21 were discordant. Although CT PPA was

< 95%, this is likely attributable to freeze/thaw cycles and the stability of Alinity m RNA targets when stored in m2000 collection device media, which preferentially stabilizes the RealTime STI assay DNA targets. Also evaluated was the incidence of coinfection on the Alinity m. Percent coinfection was calculated from clinical samples only based on total detected results for each target with one or more additional pathogens detected. Thirty-nine percent of 109 CT+ samples, 53% of 40 NG+ samples and 44% of 36 TV+ samples were coinfecting. MG coinfection was the highest observed,

with 70% of 37 MG+ samples being coinfecting. Seventeen of 26 (65%) were coinfecting with CT. Out of 348 total clinical samples that produced a result for all 4 targets, 14% were coinfecting with at least 2 STIs. This study explored the utility of consolidating a multi-plex STI assay on a single platform and collection device in a high-throughput reference lab. The Alinity m STI IUO assay showed good concordance with comparator platforms across sample types and targets. Moreover, Alinity m STI IUO assay multi-plex design enabled the simultaneous identification of coinfections across all four targets, which is not possible using our current testing platforms. Finally, the Alinity m STI IUO assay's ability to selectively detect any of the four targets also supports current CDC guidelines recommending testing specific populations for MG as well as opt out screening of females < 25 y/o. for CT/NG.

Presenter: Katherine Lundgren, Alverno Labs, katherine.lundgren@alvernolabs.com

Hepatitis A Whole Genome Sequencing Using IonS5TM AmpliSeqTM Technology for Enhanced Outbreak Analysis

N Cleary, P Bryant, D Lamson and K St. George, Wadsworth Center, New York State Department of Health

Hepatitis A incidence has been increasing in the United States in general and New York State specifically from 2015–2019, with endemic transmission and outbreaks since mid-2019. These outbreaks have been largely comprised of individuals with risk factors such as intravenous-drug use, persons experiencing homelessness and men who have sex with men. A vaccine-preventable liver disease, hepatitis A is usually a self-limited disease-causing mild symptoms but can be severe or fatal, especially in susceptible populations. Of the six genotypes that infect humans, three are circulating in the United States (IA, IB and IIIA). Currently at the Wadsworth Center, Sanger sequencing of a 315nt section of the VP1/2A region is used for hepatitis A genotyping. To generate hepatitis A whole genome sequences (WGS), a custom Ion AmpliSeqTM panel was designed in collaboration with the White Glove Team at Thermo Fisher and validated at the Wadsworth Center. Using the AmpliSeqTM panel, a total of 132 near whole genome sequences (~7360nt) were generated on hepatitis A samples at Wadsworth. Two gaps at positions 3,925 and 4,931bp, approximately 21bp and 9bp respectively, were filled using Sanger sequencing. The samples had previously tested positive on a real-time RT-PCR detection assay and were collected between February 2019 and November 2021 from residents of NYS (125), Florida (1), Pennsylvania (1) and Virginia (5). They included specimens with genotypes IB (102), IA (28) and IIIA (2). Phylogenetic trees generated with data from the two methods showed that the AmpliSeqTM panel provided greater resolution compared to that achieved with the current Sanger genotyping method.

WGS analysis offers greater resolution for enhanced outbreak analysis and infection control.

Presenter: Nora Cleary, Wadsworth Center, New York State Department of Health, noragcleary@gmail.com

Whole Genome Sequencing (WGS) of Carbapenem-Resistant Organisms that are PCR negative for Major

Carbapenemase-encoding Genes

J Socea, V Stone, C Moore, L Thomas, JA Burks, X Qian, P Gibbs and R Steece, Tennessee Department of Health Laboratory Services

The Tennessee Department of Health (TN DoH) serves as the Southeast Regional Laboratory for the Antibiotic Resistance Laboratory Network (AR Lab Network). A central goal of the AR Lab Network is to enhance the detection of antimicrobial resistance (AR) genes in various pathogens to aid in the generation of public health solutions that can work toward addressing the building problem of antimicrobial resistant infections. One target of this arm of detection are genes that encode carbapenemases, including KPC, NDM, VIM, IMP and OXA, coined “the big 5.” These genes are often found in genera within the Enterobacterales and Pseudomonadales order. To screen for carbapenem-resistant organisms, patient samples are tested using the Modified Carbapenem Inactivation Method (mCIM). This phenotypic assay is a quick and cost-effective method for visualization of isolates that produce carbapenemases. Those that are considered positive (inactive antimicrobial disks) are then subject to PCR (polymerase chain reaction) to confirm the presence of one of the target genes. Interestingly, there are instances where organisms present an mCIM positive result, but none of the target genes are detected by PCR. The focus of this project was to identify other AR genes within the genome of these organisms that may be responsible for the phenotypic result observed by using whole genome sequencing (WGS). Upon analysis of the WGS data, we may be able to identify additional AR genes that play a role in generating an mCIM positive result but were not detected through PCR because of their dissimilarity to the target carbapenemase genes. Additionally, there is scope to gain understanding of the sources from which the AR genes were acquired by these organisms through phylogenetic analysis. Twenty-one isolates submitted to the TN DoH that were mCIM positive but PCR negative were sequenced using Illumina MiSeq, and paired-end sequencing data were analyzed with an in-house pipeline. Antimicrobial resistance genes were identified using the NCBI AMRFinderPlus. Gene annotation was performed by Prokka v1.14. The resulting data was used to identify additional potential carbapenemase resistance mechanisms. The overall goal of these studies is to create a clearer picture on AR within these pathogens and the importance of accurate detection to monitor this growing public health problem.

Presenter: Jillian Socea, Tennessee Department of Health Laboratory Services, jillian.socea@tn.gov

WBE of Enteric Viruses using Bio-Rad Droplet Digital PCR Expert Design Assays

D Spina, Bio-Rad Laboratories

ddPCR assays have been developed using Bio-Rad’s Droplet Digital Expert Design tool for the following enteric pathogens: Adenovirus, Norovirus, Enterovirus, Respiratory Syncytial Virus A/B, HF183, & Legionella. Bio-Rad’s ddPCR offers many benefits over traditional qPCR for wastewater testing, including its high sensitivity and accuracy in quantifying DNA and RNA viruses found in very low concentrations, as well as the ability to handle inhibitory substances. Droplet Digital PCR also offers a method of absolute quantification without relying on standard curves, as is the case with

qPCR. The data presented shows absolute quantification and assay dilution series for a selection of two known DNA virus (Adenovirus and Enterovirus) and an RNA virus (Norovirus) commonly found in wastewater in varying concentrations. The data illustrates that the Norovirus, Adenovirus and Enterovirus assays are able to detect concentrations as low as a 1 cp/μL of viral pathogen in wastewater. The results demonstrate how Droplet Digital PCR accurately quantifies these DNA and RNA viruses in wastewater and can serve as an accurate warning system to monitor disease outbreaks in a particular community long before the first cases are reported in health facilities.

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HantaNet: A MicrobeTrace Application for Variant Classification and Outbreak Investigations of Hantaviruses

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Introduction: Hantaviruses cause human infections worldwide and are transmitted by infected rodents that shed virus in urine and feces. Genetic evidence suggests that viral sub-strains are geographically confined, but the lack of an organized clearinghouse for hantavirus data integration and sharing hinders virus surveillance and outbreak response. To overcome this challenge, we developed HantaNet, a new MicrobeTrace application for rapid variant classification of hantaviruses. CDC and public health labs use MicrobeTrace for HIV and COVID-19 cluster detection and response by combining genomic and epidemiologic data for visualization and analysis of transmission networks.

Methods: We built three reference modules in MicrobeTrace for the nucleocapsid (S), glycoprotein (M) and RNA-dependent RNA polymerase (L) for the hantavirus segmented genome. First, we searched the NCBI nucleotide database for hantaviruses collected since 1982, curated the metadata in Tableau and performed sequence alignments using MAFFT. Hantavirus strain-specific references were selected based on good sequence quality and completeness and were imported into MicrobeTrace to generate clusters with the Tamura-Nei 93 (TN93) nucleotide substitution model. To validate the HantaNet reference modules, the genetic threshold was changed to modify cluster size and specificity compared to published S, M and L gene phylogenies. Transmission networks in HantaNet were validated using contact tracing data and hantavirus sequences collected during a recent Andes virus (ANDV) outbreak in Argentina.

Results: We determined a TN93 distance threshold of 15–20% (0.15–0.20 nucleotide substitutions/site) was optimal for viral strain-specific clustering in HantaNet. Lower genetic thresholds resulted in specific clustering of ANDV viruses isolated during the 2018–19 outbreak in Argentina. With HantaNet, users can classify, build and visualize outbreak clusters and transmission networks, and save and share datasets.

Conclusions: HantaNet is a new adaption of MicrobeTrace for the classification of hantaviruses and analysis of transmission networks making it a versatile tool for rapid deployment during surveillance and outbreak response of viruses containing segmented genomes, such as hantavirus and influenza.

Presenter: Roxana Cintron-Moret, Centers for Disease Control and Prevention, vrw7@cdc.gov

Evaluation of Etest, Liofilchem and Neo-Sensitabs for Phenotypic Detection of Metallo- β -lactamases

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Background: Carbapenemase-producing organisms are an urgent public health threat. They limit antibiotic treatment options and can spread rapidly in healthcare settings. The rise of metallo- β -lactamase (MBL) type carbapenemases are of particular concern since these enzymes confer resistance to almost all β -lactam antibiotics. Early detection of MBLs is critical for effective patient management, including infection prevention (IP) and treatment. Molecular detection requires either PCR, using a validated assay that can detect the targeted genes, or whole genome sequencing (WGS), which costs additional time and money. Our goal was to identify a phenotypic MBL screening test to enhance detection of MBL enzyme production through routine testing, thereby alerting for rapid IP responses.

Methods: We compared performance of three commercial phenotypic screening tests for MBL detection: the Rosco Neo-Sensitabs combined disk test (imipenem/imipenem+EDTA) and two agar gradient diffusion tests (bioMérieux MBL Etest and Liofilchem MBL MIC Test System (MTS)). Gradient tests included both the high-range imipenem/imipenem+EDTA (IP/IPI) and low-range meropenem/meropenem+EDTA (MP/MPI) strips. Sixty well-characterized carbapenemase-producing *Acinetobacter* (n=9), *Enterobacteriales* (n=34) and *Pseudomonas* (n=17) isolates were selected for this study; thirty produced an NDM-, VIM-, IMP-, SIM-, or SPM-type MBL. Testing was performed in accordance with the manufacturer's instructions and repeated when results were discordant with WGS.

Results: The accuracy of Neo-Sensitabs, IP/IPI Etest and IP/IPI Liofilchem was 90%, 82% and 67%, respectively. Adding low-range MP/MPI strips increased overall accuracy to 90% for Etest and 73% for Liofilchem. The lower accuracy for Liofilchem was due to 27% off-scale indeterminate results. Sensitivity and specificity for the Neo-Sensitabs were 83% and 97%, respectively, with five false-negatives (3 IMP, 1 NDM and 1 VIM) and one false-positive (OXA-66/72) observed. Both sensitivity and specificity were 90% for Etest and 83% and 63%, respectively, for Liofilchem. One IMP isolate gave a false-negative for both Etest and Liofilchem and one false-positive OXA-66/72 was observed for Etest.

Conclusions: The combination of IP/IPI and MP/MPI MBL Etest had the best sensitivity and specificity for detecting MBL carbapenemase production in this study. Although Neo-Sensitabs are less expensive than Etest, their lower sensitivity makes them less ideal for MBL detection. Phenotypic screening can enhance MBL detection, particularly for novel carbapenemase genes not targeted by routine PCR testing, without having to wait for WGS information. Early detection of MBLs through screening can inform infection control to help prevent their spread to other patients.

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Integrating Epidemiological and Laboratory Data into Informative Visualizations with MicrobeTrace

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Challenge addressed: Laboratory data aids outbreak investigations, but few tools integrate laboratory and epidemiological data and fewer can be used on novel organisms (e.g., SARS-CoV-2) or can explore relationships with other data types. Many tools require specialized bioinformatics or coding skills, which hinders use. We describe MicrobeTrace, a pathogen-agnostic data integration and visualization tool, for outbreak investigations and response for emerging pathogens and a special-purpose version of MicrobeTrace for hantavirus classification and outbreak studies.

Methods: Although MicrobeTrace was originally developed for analysis of HIV data, the MicrobeTrace team rapidly responded to changing priorities and adapted the tool for other pathogens, including SARS-CoV-2. We developed specific training documents and provided ad-hoc training for epidemiologists to use during active investigations. The team also developed bioinformatic methods for exporting phylogenetic trees and viral genetic distance matrices from the widely used viral tracking tool NextStrain and using the output for rich visualization in MicrobeTrace. We also developed a special-purpose MicrobeTrace version, HantaNet, to classify hantaviruses based on a set of reference sequences and to assist with outbreak investigations.

Results: Public health partners have adapted MicrobeTrace by itself or in combination with Nextstrain for analysis of SARS-CoV-2 and COVID-19 data. MicrobeTrace has provided public health support and guidance to state and local health labs working with various pathogens, including SARS-CoV-2, HIV, TB and hantaviruses, helping them generate data visualization to inform targeted response and policy.

Conclusions and impact on public health: MicrobeTrace provides an intuitive, flexible and powerful tool with broad applicability. This tool can be used to integrate and visualize laboratory, epidemiological and/or contact-tracing data to understand disease transmission networks and dynamics.

Presenter: Anupama Shankar, Centers for Disease Control and Prevention, ikb6@cdc.gov

Laboratory Surveillance for Disseminated Gonococcal Infection - A Critical Role of SURRG-Center of Excellence Laboratories to Support Statewide *Neisseria gonorrhoeae* Antimicrobial Susceptibility Testing

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Introduction: As one of eight SURRG project sites (Strengthening the US Response to Resistant Gonorrhea), the Milwaukee Health Department Laboratory (MHDL) has been performing GC-AST using the Etest method since 2017. MHDL also participates in CDC's Gonococcal Isolate Surveillance Project (GISP) for urethral GC-AST surveillance. In the second five-year funding cycle, expansion of Antibiotic-Resistant Gonorrhea (ARGC) surveillance in higher

morbidity areas of the state of Wisconsin will include support as a reference lab and SURRG–Center of Excellence (COE) to perform *N. gonorrhoeae*-Antimicrobial Susceptibility Testing (GC-AST) for patients with suspect treatment failure, and for culture confirmed cases of gonococcal disseminated infection (DGI).

Methods: *N. gonorrhoeae* is identified from InTrays with oxidase positive colonies, gram-negative diplococci and confirmed by api NH. AST is performed using Etest with Ciprofloxacin (CI), Ceftriaxone (TX), Cefixime (IX) and Gentamicin (GM) gradient strips. Surveillance for ARGC from genital, rectal and pharyngeal sources conducted at six designated SURRG clinics–STD clinics and five community-based clinics in Milwaukee. Isolates are sent to Tennessee Antibiotic Resistance Lab Network (ARLN) for confirmatory AST using agar dilution. Isolates from patients reported with DGI are sent to CDC for genomic characterization.

Results: A total of 390 patients (445 isolates/2779 positive cultures), were identified with ARGC. Majority of ARGC isolates (440; 98.9%) isolates had reduced susceptibility (RS) to Azithromycin. During five years of SURRG Cycle 1, very few were identified with RS to cephalosporins (2 Ceftriaxone; 3 Cefixime). No treatment failures have been observed. During CY2019–CY2021, 29 cases of culture confirmed DGI were reported in Wisconsin. Of the 17 isolates shared with MHDL for GC-AST, with culture confirmed DGI, two isolates had RS to Azithromycin. None of the DGI isolates were resistant to cephalosporins.

Conclusions: The Southeastern Region of Wisconsin has the highest reported rate of GC in the state (183 per 100,000 in CY 2020). MHDL serves a critical role in this high morbidity region as it continues to provide GC-AST surveillance activities employing rapid methods to detect ARGC in patients in diverse set of select SURRG clinics with high rates of reported GC morbidity. As MHDL expands its capacity as a SURRG-COE reference laboratory during SURRG Cycle 2, these PHL services will be an important statewide resource to support GC-AST for patients with suspect treatment failure and patients with culture confirmed DGI. While genomic analyses of DGI isolates at CDC help understanding and characterization of disseminated gonococcal infections, building local culture-independent and genomic capacity will allow timely addressing increasing trends of reported DGI observed in Wisconsin and across the country.

Presenter: Suzanna Budiono, City of Milwaukee Health Department, sbudio@milwaukee.gov

Comparison of Environmental and Clinical isolates of *Legionella pneumophila* Associated with a Long-Term Care Facility using Next Generation Sequencing

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Legionella pneumophila is an opportunistic pathogen that infects amoebas in freshwater environments. The ability to persist in these protozoan hosts lends it the ability to survive as intracellular parasites in humans. Clinical diseases associated with *Legionella* species are often found in immunocompromised patients such as neonates and the elderly. Genomic surveillance of *L. pneumophila* is limited in comparison to well established pathogen surveillance programs such as foodborne pathogens with PulseNet. Here, we detail the use of next generation sequencing methods to identify

genetic links between an environmental isolate of *L. pneumophila* with a clinical isolate collected from a long-term care facility in the District of Columbia and a resident with confirmed legionellosis in 2021. The long-term care facility has had a history of *Legionella* infections and additional isolates collected in 2018 were also compared. Sequence-based typing (SBT) of these isolates identified the *Legionella* as ST224, a member of *L. pneumophila* serogroup 1. Additionally, the use of a Single Nucleotide Polymorphisms Pipeline (SNP) analysis showed the environmental isolate and the clinical isolate had no SNP differences, indicating an environmental source for legionellosis cases at this facility. Our study demonstrates the use of next generation sequencing analysis to determine relatedness among environmental and clinical *Legionella* isolates.

Presenter: Scott Nguyen, DC Department of Forensic Sciences, scott.nguyen@dc.gov

Improved Genomic Characterization of Clinical Bacterial Isolates of Unknown Identification in Florida using Hybrid Assemblies from Short- and Long-read Sequencing Platforms

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The Microbiology and Molecular Departments at the Florida Bureau of Public Health Laboratories (BPHL) regularly receive clinical bacterial specimens from unknown etiologies as culture for identification (CFI). Whole-genome sequencing is readily performed for genetic identification and characterization of the bacterial specimens that traditional culture and biochemical methods cannot identify. BPHL has sequenced and assembled over 20 draft genomes from CFI specimens using short-read sequencing data, generated on Illumina platforms, that continue to remain difficult to identify. This is likely due to minimal representation at the genus or species level in public genomic repositories. To improve the genomic characterization of CFIs and to increase the representation in public genomic databases, long-read sequencing was used to improve the draft assemblies. Hybrid assemblies were generated for each CFI using short-read and long-read sequencing data, generated from the Oxford Nanopore Technologies minION, to improve characterization of these minimally represented organisms. With both the accessibility to more complete regions of the genome and the continuing progress in accuracy, long-read sequencing has the potential to enable improved CFI profiling to aid in the identification of hard-to-identify clinical bacterial specimens. Improved genetic characterization and identification of these specimens facilitates more effective public health responses to the related clinical cases.

Presenter: Tassy Bazile, Florida Bureau of Public Health Laboratories, Tassy.Bazile@flhealth.gov

Clinical Evaluation of a Commercially Available Chromogenic Agar for Detection of *Candida auris* at a Large Academic Health Center Clinical Microbiology Laboratory

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Background: *Candida auris*, an often multi-drug resistant yeast of global health significance, can cause nosocomial outbreaks and easily be misidentified using standard laboratory methods. Global case counts may be underestimated and there is a need for more accurate, reliable, affordable and accessible identification test methods. HardyCHROM™ *Candida auris* is a selective and differential medium used to identify *C. auris*, *Candida albicans*, *Candida glabrata*, *Candida krusei* and *Candida tropicalis*. We evaluated HardyCHROM™ *Candida auris* media and assessed how it could be used to improve laboratory efficiency and reduce potential workplace hazards by relying on manual visualization of colonies without exposing personnel.

Methods: Clinical isolates (n=30) of *C. auris* (appear white with a teal bullseye center and a fluorogenic reaction under UV light), *C. albicans*, *C. glabrata*, *C. krusei* and *C. tropicalis* were tested, as well as six clinical isolates and reference strain (ATCC 22019) of *C. parapsilosis*. Current lab protocol for positive identification of *C. auris* (SAB + HardyCHROM™ *Candida* plates) was used as the reference identification method. Colonies with indeterminate results by manual visualization were identified by mass spectrometry.

Results: Clinical isolates of *C. auris*, *C. albicans*, *C. glabrata*, *C. krusei* and *C. tropicalis* were identified as expected using the manufacture's procedure under ambient lighting. *C. auris*, *C. albicans* and *C. parapsilosis* clinical isolates fluoresced under UV light. *C. parapsilosis* (the clinical isolates and ATCC strain), grew as a mixture of white or teal with colonies that exhibited a teal bullseye center, fluoresced under UV light, and were subsequently misidentified as *C. auris*.

Conclusions: We found that HardyCHROM™ *Candida auris* plates can be used to easily distinguish among *C. albicans*, *C. glabrata*, *C. krusei* and *C. tropicalis* and from *C. auris*. However, *C. parapsilosis* was not easily distinguished from *C. auris*. Using fresh and frozen clinical isolates and our ATCC strain of *C. parapsilosis*, we could not rule out *C. auris* growth on the plates, phenotypically. Considering that *C. parapsilosis* is often encountered, HardyCHROM™ *Candida auris* plates should be utilized with caution and alongside additional methods of identification to distinguish it from *C. auris*.

Presenter: Brynne Whitacre, Indiana University Health, bewhitac@iu.edu

Identification of *Blastomyces dermatitidis*, *Blastomyces gilchristii* and *Histoplasma capsulatum* using Multiplex Real-time PCR

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Blastomyces dermatitidis, *Blastomyces gilchristii* and *Histoplasma capsulatum* are dimorphic fungi that belong to the Ajellomycetaceae family. Dimorphic fungi switch between filamentous growth in the environment to yeast-like growth in the animal host. *Blastomyces*

and *Histoplasma* are responsible for significant respiratory mycoses in North America, share similar geographic distributions and patients can present with similar symptoms. Culture-based methods of identification require conversion to the yeast phase which requires lengthy incubation and extensive expertise in mycology. Molecular diagnostics using the AccuProbe® system rely on nucleic acid hybridization using probes complementary to ribosomal RNA. These assays are being phased out by the manufacturer. Therefore, the development of alternative methods for identification of *Blastomyces* and *H. capsulatum* are needed. No commercially available methods exist to differentiate between *B. dermatitidis* and *B. gilchristii*. Data regarding potential differences in pathogenicity, epidemiology and ecology between *Blastomyces* species are also lacking. Based upon previously published methods, we validated a multiplex real-time PCR (RT-PCR) assay to distinguish between *Blastomyces* species and *H. capsulatum* by amplification of the BAD1 and GADPH loci. Sequence variations within BAD1 were used to differentiate between *B. dermatitidis* and *B. gilchristii*, while GADPH was used to identify *H. capsulatum*. Isolates tested for validation of this assay were speciated by AccuProbe® and culture confirmation, whole-genome sequencing or laboratory developed RT-PCR methods. Results from the multiplex RT-PCR validation showed accurate speciation of MDH *Blastomyces* isolates and correctly identified *H. capsulatum*. Implementation of a *B. dermatitidis*, *B. gilchristii* and *H. capsulatum* multiplexed RT-PCR assay enabled a rapid turn-around-time and will allow for a better understanding of the epidemiology of these organisms in Minnesota.

Presenter: Eliot Stanton, Minnesota Department of Health, eliot.stanton@state.mn.us

Laboratory Investigation of an Outbreak of *Serratia marcescens* Infections among Incarcerated Persons at a State Prison

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Background: *Serratia marcescens* is a gram-negative bacterium that naturally occurs in soil and water but can survive in manmade environments, including disinfectant solutions, and has been the cause of nosocomial outbreaks. In 2020–21, an outbreak of invasive *S. marcescens* infections (e.g., osteomyelitis, endocarditis, etc.) at a state prison was investigated. From March 2020 through August 2021, 22 patients with *S. marcescens* infection were identified; most reported recent injection drug use (IDU). This investigation included a robust laboratory component to identify the source of the infections.

Methods: MC PHL collected and tested large volumes of water from various sources, including holding tanks, wells and faucets from case-patient cells and a variety of environmental samples from case-patient cells, such as sinks, re-used bottles and containers used to store CB64, a dilutable quaternary ammonium-based cleaner that is the primary disinfectant for the prison, scrub pads used for cleaning ("scrubbies") and communal showers. Two needle/syringes were also collected for testing. Samples were collected from devices and containers used for mixing, storing or applying disinfectant and cleaners, such as dilution machines, re-used containers and a mop bucket.

Whole genome sequencing (WGS) was completed on *S. marcescens* isolates from a subset of environmental samples and available patient specimens. Results: Of 152 environmental samples analyzed, 24 (15.8%) were positive for *S. marcescens*, including a used needle/syringe surrendered by an inmate, used nasal spray bottle containing methamphetamine, inmate hand rinsate, unmaintained disinfection dilution machine, bottles, mop bucket and trash cans used for diluting, storing and dispensing CB64, used bottles containing drinking water, floor surfaces and scrubbies. *S. marcescens* was not detected in water samples, rinsates from sharps containers with used needles, showers, walls and unopened bottles of CB64 concentrate. WGS identified a cluster of genetically similar *S. marcescens* strains from clinical specimens from five case-patients, the used needle and the nasal spray bottle; strains from other patients and environmental sources were highly diverse and not related to strains in this cluster. A lab experiment demonstrated persistence and growth of *S. marcescens* inoculated into CB64 diluted at 1:1000 for up to seven days. *S. marcescens* failed to survive and grow in concentrated and properly diluted (1:64) CB64.

Conclusions: An unusual outbreak of invasive *S. marcescens* occurred at a prison in 2020–21. Epidemiologic investigation suggested that the pathogen was likely introduced primarily through IDU. Lab investigation suggested widespread contamination; notably, improper preparation, storage and use of CB64 likely reduced its activity against *S. marcescens* leading to persistence and further spread in the prison environment.

Presenter: Donna Ferguson, Monterey County Public Health Laboratory, fergusond@co.monterey.ca.us

Rapid, Economical qPCR testing for Detection of Waterborne Pathogens Onsite and in the Laboratory

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Every year, there are roughly 7.2 million infections, 120,000 hospitalizations and 7,000 deaths caused by waterborne pathogens in the United States. With the increase in population size and aging infrastructure, these number are likely to rise. Healthcare and commercial facilities often have large, complex plumbing systems that promote growth of biofilms. Biofilms can harbor pathogenic microorganisms, such as *Legionella pneumophila*, which can lead to Legionnaires disease in immune-compromised patients. Routine testing for *Legionella* bacteria is primarily in compliance with ISO 11731:2017, which adopts a culture-based method for environmental water testing. However, culturing *Legionella* is time consuming and labor intensive. In addition, not all *Legionella* species are culturable. Organizations such as the EPA and CDC have researched for other analytical methods that can provide quicker turnaround time for environmental waterborne pathogen testing. Quantitative polymerase chain reaction or qPCR is the primary molecular method for waterborne pathogen testing. It is cost effective and can provide results in a relatively short amount of time. Real-time PCR is capable of positive detection of viable but non-culturable (VBNCs) pathogens by targeting the specific genetic sequence of the organism of interest. In addition, with the use of multiplexing in qPCR, it introduces the ability to detect multiple waterborne pathogens in one single run. This can be a

great screening tool for routine testing. At Nephros, we use a rapid waterborne pathogen detection method that can be deployed onsite or in a laboratory. Overall workflow consists of filter concentrating one liter of water using a 5-nanometer hollow fiber membrane to capture microbes ranging in size from protozoans to viruses. After our 1-step lysis step, we perform qPCR using our *Legionella pneumophila* and species Taqman primer/probe mix, which complies with ISO 12869:2019. This method allows us to provide a negative or positive screening result with a turnaround time of less than two hours. Real-time PCR can be an essential tool used for negative screening of waterborne pathogens and adds an extra layer of surveillance for VBNC waterborne pathogens.

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Feasibility of the Commercially Available ChromaCode® Tick-Borne Pathogen HDPCR Assay for Use in Vector Screening of Field-Collected Ticks in New Jersey

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Background: New Jersey is among the highest-incidence states for multiple tick-borne diseases, including Lyme disease, anaplasmosis, babesiosis and ehrlichiosis and is home to a variety of tick species capable of transmitting pathogens, including *Ixodes scapularis*, *Amblyomma americanum*, *Dermacentor variabilis* and the invasive *Haemaphysalis longicornis*. The ranges and abundance of some of these tick species are increasing, leading to increased disease risk. New Jersey already has a comprehensive mosquito-borne vector screening program encompassing all 21 counties and collaboration between the Departments of Health and Environmental Protection. Expanding this initiative to include surveillance of tick populations is expected to bring valuable data on vectors and human pathogens to better inform public health campaigns and evaluate the effectiveness of disease prevention strategies implemented in this high-incidence state.

Objectives: This study evaluated a commercially available tick-borne pathogen (TBP) HDPCR assay from ChromaCode® for use in screening relevant pathogens in field-collected ticks. This assay is a multiplex method capable of detecting nine unique tick-borne pathogens in a single reaction, however, it is designed primarily as a clinical assay. We evaluated the performance of the assay in four tick species to determine the feasibility of using the assay as a part of future statewide surveillance initiatives.

Results: The Chromacode® assay initially proved to be robust in detecting pathogens in ticks. Preliminary accuracy and precision validation studies demonstrated the successful detection of pathogens in simulated samples, including co-infected *I. scapularis*. However, several important procedural limitations were observed, including the potential for misidentification with variations in concentrations of target DNA, and non-specificity of the *Rickettsia* spp. primer set to differentiate between pathogenic or commensal *Rickettsia* species. Furthermore, PHEL has recently observed dramatically reduced internal consistency with this assay, which is believed to be attributed to changes made when the assay updated

to version RUO 2.0. While the Chromacode® assay has potential for applications in vector surveillance, PHEL has currently decided to move forward with an in-house developed multiplex assay for pathogen screening in all species of ticks relevant to the state of New Jersey.

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A Public Health Laboratory Response to a Recent Legionnaires' Disease Outbreak

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In August 2021, there was an outbreak of Legionnaires' disease with 18 confirmed cases in a Central Harlem neighborhood of New York City (NYC). At the start of the outbreak investigation, the Bureau of Communicable Disease (BCD) received a proximity analysis report and a SaTScan signal identifying a cluster of *Legionella*-positive patients residing in Harlem. Previous outbreaks of Legionnaires' disease in NYC were associated with cooling towers. Therefore, 19 cooling tower water samples were collected by the New York City Department of Health and Mental Hygiene (DOHMH) Bureau of Public Health Engineering (PHE). These samples were sent to DOHMH's Public Health Laboratory (PHL) for detection and isolation of *Legionella*. Two isolates of *Legionella pneumophila* serogroup 1 (Lp1) from affected patients were also obtained by PHL for potential source matching and confirmation. Using real time multiplex PCR, PHL found that 10 out of 19 water samples contained *Legionella pneumophila* DNA within 30 hours from time of collection. This rapid response allowed remedial actions to be taken, the first following the PCR results was to hyperhaloginate (water associated with) the positive sites. PCR positive cooling tower water samples were concurrently tested using ISO 11731:2017(E) and Legiolert (IDEXX, USA) culture-based methods. The culture results subsequently triggered the issuing of Commissioner's Orders to fully clean and disinfect the cooling towers found to have viable *Legionella pneumophila* present. Isolates recovered from environmental samples and patient specimens were subjected to pulsed field gel electrophoresis (PFGE) and whole genome single nucleotide polymorphism (SNP) analysis. PFGE and SNP based comparisons linked one patient with a cooling tower that was under investigation. The Lp1 strain isolated as the source of this outbreak was closely related to a reoccurring Lp1 strain that has been implicated in other Legionnaires' diseases outbreaks in New York City. This investigation and confirmatory laboratory testing by the PHL allowed the Health Department to rapidly identify a source of the Legionnaires' disease outbreak and effect remedial actions, preventing the further spread of disease.

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Validation and Implementation of Real Time PCR for Rapid Identification of *Candida auris* from Clinical Surveillance Samples in a Local Public Health Laboratory Setting

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Background: *Candida auris* is a worldwide emerging pathogen known for causing infections and outbreaks in health care settings. In Orange County, CA, *C. auris* has been circulating and causing outbreaks in long term care facilities since 2019, with a case count of 1,017 from February 2019 to December 2021. Starting October 2021, additional surveillance samples were tested from new patient admissions and potential events/outbreaks in subacute units (SAU).

Objective: To evaluate a real-time PCR assay for rapid identification of *C. auris* from patient surveillance samples at the Orange County Public Health Laboratory.

Method: To achieve this goal, an extraction protocol using the MasterPure kit (Biosearch Technologies) and automated EZ1 Advanced XL (Qiagen) instrument followed by real-time PCR was conducted using PerfeCTa Multiplex qPCR TaqMan (Quanta Biosciences) and the 7500 Fast Dx (Applied Biosystems). The assay was initially evaluated using 131 previously tested and frozen/refrigerated patient surveillance samples as well as 123 prospective fresh surveillance samples collected from different body sites (nares, axilla, groin, perirectal, fingers/hands and axilla/groin).

Results: The assay was highly reproducible at a limit of detection of 230 CFU/ml. The analytical sensitivity and specificity of the assay was 100% for both. The performance from the 131 previously tested/frozen samples was 90 % for analytical sensitivity and 86 % for analytical specificity. The clinical sensitivity and clinical specificity of the 123 prospective samples was 93% and 90% respectively. Based on receiver operating characteristic (ROC) curve analysis, a cycle threshold (CT) value of ≤ 36 as positive and >36 as negative were reached for the previously tested samples. A revised ROC curve analysis was calculated for the prospective samples yielding a cutoff of ≤ 37 which was determined to be the ideal diagnostic value for patient surveillance samples.

Conclusion: The successful implementation of the real-time PCR assay for *C. auris* in a county public health laboratory setting allows for accurate and rapid screening and identification of *C. auris* and contributes to enhanced surveillance and control of *C. auris* spread in local health care facilities.

Presenter: Lydia Mikhail, Orange County Public Health Laboratory, lmikhail@ochca.com

Clinical and Analytical Performance of the VITROS® Immunodiagnostic Products HIV Combo Assay

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Background: This study was designed to assess the clinical and analytical performance of the VITROS Immunodiagnostic Products HIV Combo Assay (VITROS HIV Combo Assay) on the VITROS® family of systems. The assay is capable of simultaneous detection of HIV antibodies (Ab) and HIV p24 antigen (Ag) to help enable earlier diagnosis of HIV infection.

Methods: Antibody detection in the VITROS HIV Combo Assay is achieved using recombinant transmembrane antigens specific to HIV-1 (group M and O) and to HIV-2. The p24 antigen detection is accomplished using monoclonal antibodies (mAb). The antigens and p24 mAb are coated onto the well. Sample is added to the wells in the first stage of the reaction and HIV Ag/Ab from the sample is captured. After washing, HRP conjugated antigens and p24 mAb are added. Following a final wash, bound HRP conjugates are detected using the VITROS signal reagent. Specificity was assessed using 6003 samples from low-risk populations. Sensitivity was evaluated using 2074 antibody positive samples (1845 HIV-1, including 413 from various Group M and O subtypes and 229 HIV-2). In addition, 52 samples with various HIV-1 group M antigen genotypes were tested. Seroconversion sensitivity was assessed by testing 32 commercially available panels on both the VITROS HIV Combo assay and a commercially available Ag/Ab assay. Assay reproducibility was assessed at three sites using three reagent lots with a 14-member panel. Antigen sensitivity was determined by testing serial dilutions of the NIBSC and the AFSSAPS HIV-1 p24 Ag standards across two reagent lots.

Results: The specificity of the VITROS HIV Combo Assay for the low-risk population was 99.6% (5934/5959) [95% exact CI (99.4-99.7%)] (44 reactive samples were confirmed positive by supplemental testing). The sensitivity for HIV-1 and HIV-2 antibody positive samples was 100.0% (2074/2074) [exact 95% CI (99.8-100.0%)]. All 413 HIV-1 group M and O antibody positive subtypes were reactive. 50 of 52 HIV-1 group M antigen genotypes were reactive with the VITROS HIV Combo assay with 49 of 52 reactive on the commercially available Ag/Ab assay. For seroconversion panels, the VITROS HIV Combo assay was reactive at the same panel member as the commercially available Ag/Ab assay for 25 of the 32 panels, was reactive one panel member earlier for 6 panels, and was reactive later for 1 panel. For the reproducibility study the observed precision for the 12 panel members positioned near the assay cutoff ranged from 9.1 to 17.8 %CV. The overall sensitivity of the VITROS HIV Combo test for the NIBSC HIV-1 p24 Antigen Standard (90/636) was ≤ 0.46 IU/mL and for the AFSSAPS HIV-1 p24 Antigen Standard was ≤ 12.8 pg/mL.

Conclusion: The VITROS HIV Combo Assay demonstrates acceptable clinical and analytical performance in the simultaneous detection of antibodies to HIV-1 (group M and O), HIV-2 and HIV p24 antigen.

Presenter: Paul Contestable, Ortho Clinical Diagnostics, paul.contestable@orthoclinicaldiagnostics.com

A Streamlined Workflow For High-Throughput, Multiplexed HiFi Sequencing Of Microbial Genomes

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The SARS-CoV-2 global pandemic has highlighted the utility of pathogen surveillance pipelines that provide comprehensive genomic information, giving public health scientists a more complete view of the spread and characteristics of circulating pathogens. Beyond COVID-19, there is great interest in public health to expand high resolution surveillance to other infectious diseases. Highly accurate, long HiFi reads produced by the PacBio Sequel IIe System have brought new levels of contiguity,

completeness and accuracy to large genome assembly. HiFi reads are similarly beneficial for microbial genome assembly, as higher quality assemblies enhance our ability to investigate foodborne illnesses and monitor antimicrobial resistance. However, obstacles in library preparation workflow, cost and recovery of small plasmids have limited use in public health. Here, we introduce a new library prep workflow and assembly algorithm based on HiFi reads that enables a high throughput, end-to-end solution for microbial genome assembly. The new workflow combines steps, eliminates the need for strict size selection, shortens the total time to six hours and enables library prep automation. The assembly algorithm uses strict read-to-read overlaps enabled by HiFi read accuracy to resolve repeats. It uses a two-stage approach to first assemble chromosomes and then recover short, high-coverage plasmids. To evaluate the method, a pool of HiFi libraries with 96 microbial samples and total genome size of 375 Mb was generated. The protocol was evaluated with microbes relevant to pathogen surveillance including common foodborne pathogens (*Listeria*, *Salmonella*) and species often seen in hospital settings (*Klebsiella*, *Staphylococcus*). The microbes represent a range of genome sizes, assembly complexity, GC content, chromosome counts and plasmid content. DNA samples were sheared to 7 kb - 10 kb, prepared as barcoded libraries, pooled and sequenced on one SMRT Cell 8M on the Sequel IIe System. Reference quality de novo microbial assemblies with 5 contigs or fewer were achieved for all samples. Typical chromosome assembly quality was Q50, measured as concordance to reference assemblies. Nearly all plasmids were recovered, including those shorter than 5 kb which are often lost in workflows with strict size selection. Taken together, the new method provides a high-throughput, cost-effective approach suitable to routinely generate reference quality microbial genomes in a public health environment as part of a pathogen surveillance program.

Presenter: Meredith Ashby, Pacific Biosciences, mashby@pacificbiosciences.com

Rapid and Quantitative Enumeration of Live *Legionella* using Multiplexed Viability qPCR

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PCR-based bacterial enumeration often leads to an overestimation of viable cells due to the amplification of DNA from dead cells. To overcome this disadvantage, DNA-intercalating crosslinking dyes such as ethidium monoazide (EMA) or propidium monoazide (PMA) have been used in combination with qPCR to discriminate between viable and non-viable cells. Drawbacks of using PMA and EMA include inhibition of photocrosslinking in turbid samples, lack of consistent photocrosslinking protocols and a requirement of large amplicon sizes for robust results. The need for large amplicon size has encouraged the use of SYBR green dye-based qPCR which is not amenable to multiplexing or the inclusion of appropriate qPCR controls. To overcome these challenges and simplify the PCR workflow we synthesized a novel viability PCR crosslinker, which is composed of a live cell-impermeable DNA crosslinking dye that spontaneously crosslinks and prevents dead cell DNA amplification without the need for photoactivation. We optimized probe-hydrolysis qPCR assays for large amplicon sizes, allowing multiplexing for *Legionella pneumophila* and *Legionella* spp. detection. We also

developed quantitative calibration controls for the targets to convert the qPCR data to viable genomic units (vGU, a surrogate for CFU). In addition, an internal positive control template was included in the qPCR master mixes, controlling for the presence of PCR inhibitors that are commonly present in water samples, often causing false-negative results. With the combined workflow including filtration of water samples, treatment with crosslinker, automated DNA purification and probe-hydrolysis qPCR we were able to rapidly enumerate viable *L. pneumophila* and *Legionella* spp. from water samples in a culture-independent manner. The results were confirmed using the standard culture-based method utilizing BCYE (Buffered Charcoal Yeast Extract) agar that detects *Legionella* species in 7-10 days.

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

MacConkey Agar with Meropenem for the Detection of Carbapenemase Producing Enterobacteriaceae from Rectal Swabs. A Proof-of-concept Study

M Nelson, R Jepson and G Lester, State Hygienic Laboratory at the University of Iowa

The State Hygienic Laboratory at The University of Iowa completed a proof-of-concept study utilizing MacConkey agar supplemented with Meropenem for the recovery of carbapenem-resistant Enterobacteriaceae (CRE) from rectal swabs. Evaluation of agar dilution media was completed by prospectively testing 16 rectal swabs from two separate (CRE) outbreaks in long-term care facilities in Iowa. Colonization screening was conducted at the first long-term care facility in July 2019 (n=7). Colonization screening was conducted at a different long-term care facility in December 2020 (n=9). Using the combined results from both screening events, the MacConkey w/ Meropenem agar had a sensitivity of 86%, a specificity of 88%, positive predictive value of 78% and negative predictive value of 88%. This method does not require broth enrichment or PCR for the detection of CRE. PCR is only required for the identification of the CRE mutation after the culture has been allowed to grow for 24-48 hours. The use of agar dilution media for colonization screening reduces turnaround time to detection of CRE, reduces cost and is not limited by gene targets like PCR based assays.

Presenter: Geoffrey Lester, State Hygienic Laboratory at the University of Iowa, geoffrey-lester@uiowa.edu

Prevalidation of Bioinformatics Pipeline for Healthcare-Associated Infections (HAI) Testing at State Hygienic Laboratory (SHL), University of Iowa

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This study represents the initial prevalidation phase of NGS bioinformatics pipelines to detect and report HAI occurring in facilities across the State of Iowa. We surveyed available NGS bioinformatics pipelines for microbial characterization such as bacterial genotyping, SNP detection, species identification and phylogenetic analyses. HAI and other bacterial reference material sequences were collected from public databases such as ATCC and NCBI. Using these reference materials, we optimized a SHL

NGS bioinformatics pipeline to meet the needs for identification, characterization and reporting of current HAI in Iowa. Furthermore, we are working on i) validation plan to implement bioinformatics pipelines, ii) workflows to automate the data analysis, iii) and a preliminary results template layout to report HAI results. Based on our observations in optimizing NGS bioinformatics pipelines at public health laboratory and the complexity of NGS assays for clinical validation, the prevalidation phase is important in assessing bioinformatics pipeline performance for validation prior to wet lab sequencing. The prevalidation phase is cost effective, it reduces sequencing cost, while provides an opportunity to utilize informatics resources using known reference material sequences.

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Use of Molecular Diagnostic Testing and Traditional Virology Techniques to Surveil for Mosquito-Borne Arboviruses in Texas

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The State of Texas is vulnerable to mosquito-borne arboviral diseases for numerous reasons including unique ecosystems that support high densities of vector mosquito populations, areas with significant poverty, and periodic climatic disturbances such as hurricanes, floods and droughts. In addition, the Texas-Mexico border is a frequently crossed border, increasing the risk of emerging mosquito-borne diseases being introduced, like chikungunya, Zika and yellow fever. The primary objective of the Texas Department of State Health Services-Laboratory Services Section (TDSHS-LSS) Arbovirus/Entomology Laboratory is to detect arboviruses in mosquito populations before human cases occur. This information is used by local jurisdictions to initiate disease-prevention strategies and to inform mosquito control activities. The TDSHS-LSS Arbovirus/Entomology Laboratory currently provides mosquito species identification, population counts, arbovirus testing and insecticide resistance testing. Mosquitoes are collected throughout the state by various city and county health departments, health service regions, military installations, universities and local mosquito control programs in Texas and submitted to the Arbovirus/Entomology Laboratory. Historically, all arbovirus testing was conducted using virus isolation in cell culture. Recently, molecular diagnostic tools were incorporated into the program to increase capacity and decrease turnaround time for result reporting. Currently, these two testing workflows are used to screen the mosquitoes for endemic viruses as well as newly emerging or reemerging viruses. Molecular testing capabilities include real-time RT-PCR assays for detecting West Nile, St. Louis encephalitis, western equine encephalitis, Zika, chikungunya, dengue and eastern equine encephalitis viruses. Traditional virus isolation techniques using cell culture are conducted on approximately 20% of mosquito trap submissions to screen for new arboviruses. The combination of these two workflows has allowed the Arbovirus/Entomology Laboratory to provide quick turnaround times for reporting the detection of endemic viruses and to provide broad surveillance testing for unexpected arboviral introductions. Data will be presented from the last 5 years, demonstrating how the Arbovirus/Entomology Laboratory has played a critical role in the

response to endemic and emerging arboviruses, including West Nile virus outbreaks and local transmission of Zika virus.

Presenter: Bethany Bolling, Texas Department of State Health Services, bethany.bolling@dshs.texas.gov

Establishment of Methods for the Detection of Antimicrobial Resistant Organisms in Wastewater Surveillance Samples

J Chavez, E Young, M Vowles, N La Crosse, K Oakeson and A Rossi, Utah Public Health Laboratory

Infections with antimicrobial resistant organisms are an important cause of morbidity and mortality worldwide and an ever-growing public health concern. Tracking the geographical distribution and spread of antimicrobial resistance (AR) allows the implementation of infection prevention strategies such as targeted admission screenings. Localization of specific threats is also useful in raising awareness in the healthcare community. Here we present preliminary data on the use of wastewater samples to create endemicity maps of established AR threats such as Carbapenem-resistant Enterobacterales (CREs) and to facilitate early detection of non-autochthonous pathogens such as *Candida auris*. Untreated influent samples from 32 wastewater treatment plants (WTPs) throughout Utah were collected twice weekly for COVID-19 surveillance and aliquots used for the isolation of CREs and yeasts. CREs were selected on CHROMagar™ mSuperCARBA™ plates containing meropenem. Presumptive CRE colonies were evaluated for oxidase activity to screen out *Aeromonas sp.* and other non-fermenters abundant in wastewater. Species-level identification of oxidase-negative colonies was performed by MALDI-TOF (Bruker). Carbapenemase production was evaluated by the modified carbapenemase inactivation method (mCIM). mCIM-positive organisms were analyzed by whole genome sequencing (WGS) on an Illumina MiSeq platform. Antibiotic resistance markers were identified by using the NCBI National Database of Antibiotic Resistant Organisms. Yeasts sedimented from wastewater were selected on HardyCHROM™ *Candida* agar after incubation at 40°C. Colonies with a phenotype compatible with *C. auris* were subjected to MALDI-TOF. 300 carbapenemase-producing CREs were recovered from 19 of the 33 WTPs and included the following genera: *Escherichia*, *Enterobacter*, *Citrobacter*, *Klebsiella*, *Serratia*, *Pseudocitrobacter* and *Raoultella*. CREs seem to be concentrated in urban rather than rural areas. WGS was obtained from 268 isolates recovered from 25 WTPs and revealed the presence of the following carbapenem resistance genes: KPC-2, KPC-3, NDM-1, NDM-5, VIM-2, VIM-4, OXA-181, GES-5, GES-2. Interestingly, the detected KPC, NDM and VIM-2 subtypes have also been identified in State resident colonized individuals. Yeasts could be recovered at a concentration of about 80 CFU per 100 mL of wastewater. The most abundant *Candida* species isolated from wastewater was *C. glabrata*. We analyzed samples from 32 WTPs and we were not able to detect any *C. auris* isolates, a pathogen not currently detected in Utah based on clinical sampling. This preliminary work forms the basis for drawing complete CRE endemicity maps in Utah, comparing of environmental and clinical sampling and the exploration of wastewater surveillance for antibiotic resistant organisms at the healthcare facility-level.

Presenter: Jorge Chavez, Utah Public Health Laboratory, aphlidbjc1@utah.gov

Improving Surveillance and Cluster Detection of Carbapenem-resistant Organisms Using Whole Genome Sequencing

K Gali and R St. Jacques, Virginia Division of Consolidated Laboratory Services

According to the Centers for Disease Control and Prevention Antibiotic Resistance Threats in the United States 2019 report, Gram-negative Carbapenem-resistant organisms (CRO) are two of the top five most urgent antimicrobial resistance threats. Improving the surveillance of antibiotic resistance genes within the population and identifying disease clusters is a vital part of the continued effort to control and prevent these infections. Whole-genome sequencing (WGS) enables the comparison of pathogens at the genome-level to determine how closely related isolates are to one another. Identifying disease clusters through genomic comparison enhances the public health response through targeted intervention and infection control measures. DCLS has performed WGS of select CRO isolates for several years, but the process for utilizing that data has needed additional development. By examining the workflow and bioinformatics tools, we developed a method to enhance the analysis of our WGS data to detect resistance genes and disease clusters. Submission to the National Center for Biotechnology Information (NCBI) Pathogen Detection Project provides the initial phylogenetic and resistance gene analysis. Additional bioinformatics analysis using the Dryad pipeline and Gamma software generates data on antimicrobial resistance and hypervirulence genes, as well as resistance to disinfectants. If clusters of illness are indicated in NCBI Pathogen Detection, then the Dryad pipeline is used to assess SNP distances between the isolates. Knowing the SNP distance between different isolates allows us to determine if the cases are closely linked. Linking disease cases improves epidemiologic investigations by providing evidence that two infections are more likely to be caused by the same source or spread from one individual to another. While improving the CRO cluster detection process, DCLS began an investigation of a possible outbreak at the request of the Virginia Department of Health. Through WGS and bioinformatics analysis, the requested isolates proved to be closely related to one another. In addition, we identified genetically similar isolates from three additional cases that were previously unknown to the health department. In another investigation, several isolates with similar phenotypic characteristics to the disease case were identified. Through WGS, it was determined that some of these cases had genomically distinct bacterial strains, suggesting that a common source of infection was unlikely. Additionally, two isolates of the same multiple locus sequence type were not closely related by a pairwise SNP comparison. Our analysis enabled us to show that these cases were not linked to the isolate under investigation. We have developed a workflow that has enhanced surveillance of disease clusters and identification of resistance genes.

Presenter: Katelin Gali, Virginia Division of Consolidated Laboratory Services, katelin.gali@dgs.virginia.gov

Can Antimicrobial-resistant *Mycobacterium tuberculosis* be Diagnosed Directly from Respiratory Specimens using Targeted Next Generation Sequencing?

S Murphy, C Smith, T Halse, MC Rowlinson, V Escuyer, P LaPierre and K Musser, Wadsworth Center, New York State Department of Health

Mycobacterium tuberculosis (MTB) infections are treated with combination antimicrobial therapy; however, inappropriate therapy with antimicrobials that are not efficacious against multi- and extensively drug-resistant MTB can result in treatment failure. Phenotypic antimicrobial susceptibility testing on slow growing bacteria like MTB requires many weeks to months to complete, whereas sequencing-based approaches can predict antimicrobial resistance (AR) with reduced turnaround time and can guide antimicrobial therapy in a timelier manner. The Wadsworth Center currently uses two sequencing-based approaches to predict AR in all NYS TB cases: whole genome sequencing (WGS) of MTB-positive cultures and direct pyrosequencing of select loci. We sought to develop a multiplexed, targeted next generation sequencing (tNGS) assay that can predict AR directly from respiratory specimens in two days for under \$100. PCR pools were designed to amplify thirteen full-length genes (*katG*, *rpoB*, *embB*, *gyrA/B*, *ethA*, *eis*, *rrs*, *rpsL*) or loci (*mabA-inhA*, *oxyR-ahpC*, *pncA*, *embCA*) with mutations known to confer AR to first line antibiotics (isoniazid, rifampin, pyrazinamide, ethambutol) or alternatives (fluoroquinolones, ethionamide, streptomycin, kanamycin, amikacin). PCR conditions were optimized and resulted in two PCR pools later combined for NGS. For assay validation, DNA was extracted (EZ1, Qiagen) from MTB-positive or spiked (H37Rv type strain) respiratory specimens. Following PCR, amplicon libraries were prepared and sequenced using Oxford Nanopore MinION and analyzed with an in-house developed bioinformatics pipeline. The tNGS assay amplified targets from concentrations as low as 0.1 colony forming unit (CFU) per reaction and was determined to be specific for the MTB complex. Intra- and inter-assay reproducibility testing reliably detected pan-susceptible and multidrug-resistant MTB from clinical specimens (n=3). A blinded and randomized panel of 35 smear-positive retrospective specimens were used to assess tNGS accuracy; most tests yielded sufficient coverage of all 13 targets (71%) or 11 to 12 targets (11%). All sequenced samples showed 100% concordance with previous WGS test results. The tNGS samples that did not yield enough material for sequencing (17%) were often from low positive smears (1+) and all had higher Ct values by real-time PCR (ExtRD9 target, Ct >34). These data indicate that tNGS is highly sensitive and specific and can predict AR in MTB, similar to WGS assays, in the majority of smear-positive primary specimens.

Presenter: Shannon Murphy, Wadsworth Center, New York State Department of Health, shgmurphy@gmail.com

Antifungal Susceptibility Profiles of Common and Rare Clinical *Candida* Species Collected 2017-2021, New York

M Vaidya, V Chaturvedi and S Chaturvedi, Wadsworth Center, New York State Department of Health

Background: Pathogenic yeasts cause serious healthcare-associated infections (HAIs). *Candida* species are the fourth most common cause of HAIs in US hospitals. Currently, three different classes of antifungal agents are used to treat *Candida* infections. Antifungal susceptibility profiles and breakpoints for azoles and echinocandins are available for the common *Candida* species. However, the susceptibility breakpoints are not available for rare *Candida* species. Similarly, the geographical variations in the antifungal susceptibility profiles of common and rare pathogenic yeasts are less understood. Passive laboratory surveillance is an important tool to identify the prevalence of common and rare yeasts and their resistance patterns. In this study, we report antifungal susceptibility profiles of approximately 540 common and 330 rare clinical *Candida* isolates submitted by New York City and New York State hospitals for 2017-2021.

Methods: Identification of all clinical isolates was performed by standard mycology procedures and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS). Rare yeasts were also confirmed with Sanger Sequencing of ribosomal RNA. Antifungal susceptibility testing was done using CLSI microbroth dilution method and E-tests.

Results: We analyzed 6,900 clinical *Candida* isolates between 2017-2021. Of these, about 330 isolates were rare *Candida* species and 1,200 isolates were common *Candida* species. Antifungal susceptibility testing was performed on all 330 rare *Candida* isolates comprising 16 species and 540 of 1,200 isolates from seven common *Candida* species as per requests from the submitting facilities. Nearly one-fifth of rare *Candida* isolates comprised *Candida fermentati* with modest resistance to azoles and rare resistance to anidulafungin. *Candida haemulonii* and *Candida duobushaemulonii* constituted 10% of rare yeast species with 100% resistance to amphotericin B. *Candida metapsilosis*, *Candida nivarensis* and *Candida orthopsilosis* made up one-fourth of rare yeasts without any resistant isolates. Among 10 *Candida blankii*, eight isolates were azole-resistant and three isolates were echinocandin-resistant. Three of four *Candida famata* isolates were resistant to amphotericin B. Among the common *Candida* species, 49% of the common yeasts were identified as *Candida albicans* and nearly 9% were resistant to at least one of the azoles. *Candida parapsilosis* comprised 22% of the common yeast isolates with 5% resistance to azoles.

Conclusion: The antifungal susceptibility patterns of common and rare clinical *Candida* species in New York are consistent with published studies from a global collection of yeasts. Azole resistance was notable in common and rare *Candida* species. Amphotericin B and echinocandin resistance were rare and limited to a few rare *Candida* species.

Presenter: Mayuri Vaidya, Wadsworth Center, New York State Department of Health, mayuri.vaidya@health.ny.gov

Rapid Molecular Detection of Echinocandin-Resistance in *Candida auris*

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Background: *Candida auris* is a multidrug-resistant yeast pathogen causing outbreaks in healthcare facilities worldwide and emergence of echinocandin-resistant *C. auris* is a concern. Currently used CLSI and commercial antifungal susceptibility tests are phenotype-based, slow and not scalable, limiting their effectiveness in the surveillance of *C. auris*. There is an urgent need for accurate and rapid assessment of resistance to echinocandin-class antifungals, which are the current first line drugs of choice. We report the development of a TaqMan chemistry probe-based fluorescence melt curve analysis (FMCA) following asymmetric PCR to assess mutations within the hot spot one (HS1) region of FKS1, encoding 1,3-beta-D-glucan synthase, a target for echinocandins.

Methods: *C. auris* isolates exhibiting echinocandin resistance by broth microdilution method were obtained from our repository. These were cultured, followed by DNA extraction, PCR amplification, and Sanger sequencing of FKS1. Multiple alignments of FKS1 were done using Geneious 9.1.8 software. Primers and probes were designed from HS1 for FMCA on LightCycler® 480. Assay validation was performed using pure *C. auris* cultures, crude DNA extraction in PBS-BSA, and testing five microliters of extracted DNA in duplicate for assay reproducibility, limit of detection, specificity, blinded verification and melting temperature (Tm) range.

Results: Twenty-five *C. auris* isolates were sequenced using ten primer pairs to produce full-length FKS1 (~5600-bp). Multiple alignments revealed several mutations within the ~590-bp of FKS1 comprising HS1 but not HS2 region. Additional 39 isolates were sequenced for HS1 with three primer sets. Of 64 isolates, 25 were wildtype and 39 were mutants. Of mutants, one each was F635del and D642H/R645T, 4 were F635Y, 9 were F635C, 3 were S639P, 7 were S639F and 14 were S639Y. All wildtype isolates were susceptible while all mutants were resistant to echinocandins except D642H/R645T by broth microdilution. FMCA correctly identified wildtype, F635C, F635Y, S639P and S639F mutations with distinct Tm. FMCA also produced unique but identical Tm for S639F and S639Y but could not identify F635del or D642H/R645T. Results showed FMCA is highly specific and reproducible for identifying mutations in FKS1 for *C. auris* isolates, conferring echinocandin resistance. Additionally, we found excellent concordance between FMCA, Sanger sequencing and antifungal susceptibility testing.

Conclusions: Echinocandin-resistant *C. auris* pose a high risk for outbreaks worldwide. There is an urgent need for accurate and rapid assessment of echinocandin resistance as that is the drug of choice for patient management, infection control and prevention programs. The FMCA assay developed in this study will aid in the shortcomings of the turnaround time faced with current phenotypic assays used for resistant detection.

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WSLH Response to the Measles Outbreak in Afghan Refugees at Ft. McCoy in September 2021

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On August 29, 2021, the United States government started an effort to resettle Afghan refugees from Afghanistan to the US. Many of the refugees were temporarily housed at military bases, where they were given medical care and screenings before being resettled in communities across country. Roughly 13,000 refugees were housed at Ft. McCoy in Wisconsin. A 17-year-old Afghan evacuee arrived at Ft. McCoy on September 4 and shortly after presented with a fever of 107.6°F and a maculopapular rash. Specimens were collected for measles testing and sent to the Wisconsin State Laboratory of Hygiene (WSLH) for testing. Active, wild-type measles was confirmed by real-time reverse-transcriptase polymerase chain reaction (rRT-PCR) on September 5 and molecular genotyping using Sanger sequencing revealed a genotype of B3. WSLH immediately started working to validate a higher throughput extraction platform to better respond to a potential large scale measles outbreak. Over the course of the outbreak, 50 measles specimens were tested with both the CDC-developed pan-measles PCR and the CDC-developed measles vaccine PCR assay, which is able to differentiate wild-type from vaccine-related measles infections. A total of 28 specimens were positive for pan-measles PCR and 22 of those specimens were wild-type. All of these specimens genotyped using Sanger sequencing as B3, which is the genotype most common in Afghanistan. In response to the measles positives at Ft. McCoy and other bases, incoming flights of refugees were temporarily paused on September 10th and a mass vaccination campaign was undertaken. By September 24, >99% of eligible evacuees were vaccinated with MMR and Varicella vaccines. The last positive measles case tested at WSLH from Ft. McCoy was on October 5. In addition to measles specimens, Ft. McCoy also sent specimens to WSLH for a variety of other viral detection assays including 1 rubella, 1 mumps and 7 Varicella Zoster Virus (VZV). Three of the VZV specimens were positive for wild-type VZV using the CDC VZV Bi-allelic Strain Typing assay; all three specimens were further genotyped using Sanger sequencing as Clade 5. In summary, the availability of PCR testing for measles at a Wisconsin laboratory allowed for the rapid identification and typing of a measles specimen over Labor Day weekend. The confirmation of wild-type measles in this Afghan refugee was instrumental in launching a swift public health response and led to the eventual containment of the measles outbreak at Ft. McCoy.

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Identifying Carbapenemase Genes using Whole Genome Sequencing

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The rise in carbapenem-resistant organisms (CRO) and carbapenemase-producing organisms (CPO) necessitates new detection and intervention strategies to maintain the effectiveness of these critical antibiotics. Currently, detection of CRO/CPO in clinical laboratories involves a combination of antimicrobial susceptibility testing (AST), phenotypic or biochemical assays

(mCIM/eCIM or CARBA-NP, respectively) and/or molecular tests (Xpert Carba-R, lab developed PCR). With the increased adoption of whole genome sequencing (WGS) in clinical and public health labs, we evaluated the use of WGS to identify the presence of antimicrobial resistance genes in bacterial isolates. To accommodate this effort, we developed a new bioinformatics pipeline, Spriggen, to assemble bacterial WGS data and identify antimicrobial resistance genes. Using a dataset of 220 bacterial isolates sequenced at the Wisconsin State Laboratory of Hygiene (WSLH) between 2019 and 2021, we evaluated the performance of this pipeline by comparing the wet lab and genomic results. A carbapenemase gene was detected by CARBA-R and/or custom PCR panel in 210 of the 220 isolates analyzed (95%). WGS results were in agreement for 208 of these isolates (99%). Of the two discrepant isolates, one was the result of an improperly labeled sample, which was resolved through repeat testing. The other discrepant isolate tested positive for two carbapenemase genes by molecular methods, but WGS only identified one of them. An additional 10 isolates that were mCIM+/PCR- during lab testing were determined by WGS to have carbapenemase genes not covered by the molecular assays available at WSLH. This highlights the ability of WGS to detect uncommon or novel resistance mechanisms. Herein, our results demonstrate the feasibility of using WGS to detect antimicrobial resistance genes in bacterial isolates that could complement, or potentially replace, traditional methods.

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INFORMATICS

Rapid Expansion of Bioinformatics Capabilities at the Texas Department of State Health Services

S Marcellus, K Bobier, J Lu and R Lee, Texas Department of State Health Services

Through the addition of dedicated bioinformaticians and the acquisition and establishment of bioinformatics infrastructure, the integration and usage of genetic and genomic data has grown rapidly. The Bioinformatics Group at the Texas Department of State Health Services (BIGTex) grew from one APHL-CDC Bioinformatics Fellow and one APHL Covid-19 Associate to four full-time bioinformaticians over the course of six months. The BIGTex staff have launched projects across infectious disease and newborn screening. Infectious disease efforts to analyze SARS-CoV-2 DNA sequences, automate sequence submission to public data repositories such as NCBI-SRA and GISAID, sequence SARS-CoV-2 in community wastewater and establish an antibiotic resistance laboratory network pipeline have provided much needed genetic and genomic information for epidemiologic surveillance and decision making. The newborn screening group is in development of a variant interpretation pipeline and whole-exome sequencing data analysis pipeline in addition to utilizing the bioinformatics infrastructure for epidemiologically based projects such as cut-off value evaluation for screening of metabolic conditions. To facilitate the rapid expansion and staff development, connecting and communicating with other bioinformaticians and state laboratories was vital. Configuration of existing pipelines and projects in use at other states expedited the

implementation of genomic analysis, specifically for SARS-CoV-2 and newborn screening. The vast web of bioinformatics growth allows our public health laboratory to better serve Texans through genetic and genomic analysis and provides a foundation for future expansion of genomic surveillance and testing in Texas.

Presenter: Samantha Marcellus, Texas Department of State Health Services Laboratory, smarcellus@live.com

Laboratory Reporting Technical Assistance: A Model for Improving Electronic Laboratory Reporting Systems in State Public Health Agencies

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Background: Given the progression and urgency of the COVID-19 pandemic, the Centers for Disease Control and Prevention (CDC) has worked to improve the timeliness, completeness and accuracy of laboratory COVID-19 data reported to CDC via the provision of technical assistance (TA) to public health agencies (PHAs). The TA aims to apply subject matter expertise in onboarding processing and reporting data when PHAs face reporting challenges and may need technical help. TA expertise encompasses electronic laboratory reporting (ELR), PHA electronic information exchange, and interoperability of surveillance systems, with an end goal of providing CDC with national surveillance data and enabling a data-driven response to COVID-19.

Methods: The Booz Allen Hamilton Laboratory Reporting Technical Assistance (LRTA) project has supported the CDC's Laboratory Reporting Working Group, within the CDC COVID-19 emergency response, since July 2020. States can submit requests for TA to the Association for Public Health Laboratories (APHL) via a Smartsheet form. The team composition, tools and processes developed vary by PHA needs.

Results: The LRTA team has worked with 10 PHAs: Arizona, Florida, Louisiana, Oklahoma, Oregon, South Carolina, Texas, Vermont, Virginia and Washington. The LRTA contract team has participated in various ELR-related tasks, such as onboarding facilities which provide ELR records into state health surveillance systems; improving HL7 message handling in Rhapsody, BizTalk and Mirth; creating automatic data validation/output tools for facilities, using Python and VBA, to manually audit and QA spreadsheet data; configuring the National HL7 Generator tool, created by APHL, for PHAs to convert flat file data into HL7 messages; and creating automated process improvement tools and dashboards in R Studio and PowerBI to identify reporting anomalies. Recently, the team has developed a data-scraping tool from faxed PDF files using optical character recognition and creating RestAPI calls to automate data transformations from point-of-care forms into state surveillance system standards.

Conclusion: The LRTA project has addressed various challenges in laboratory data reporting at the PHA-level: providing solutions for a lack of qualified HL7 resources and informaticians to onboard and implement large number of facilities for ELR; manual and time-consuming validation processes; manual data entry due to paper reporting; data quality issues due to CSV/flat file reporting; and inefficient message processing and manual error handling for PHA systems. Each process has improved the timeliness, accuracy

and completeness of ELR data collection and the processing from healthcare facilities and reporting jurisdictions to PHAs, ultimately resulting in enhanced data reporting to both the CDC and to HHS.

Presenter: Michelle Schmitz, Booz Allen Hamilton, Schmitz_Michelle@bah.com

Office of Regulatory Affairs Data Exchange: Sample Data Sharing

O Fennell, US Food and Drug Administration

The ORA Data Exchange (DX) enables FDA and regulatory partners to securely share sample collection, receipt and analysis data on selected microbiological, chemical and radiological analytes. This real-time data sharing initiative allows for rapid review of analysis data to improve food safety testing efforts, assist in risk assessment analysis, increase efficiencies and aid in timely and accurate regulatory decision making. The ORA DX allows for multiple regulatory information sharing capabilities via the ORA Partners Portal (ORAPP) and the System-to-System Services (a.k.a. NFSDX). ORAPP is a web portal that provides a user interface for data sharing between FDA and regulatory partners. The System-to-System Services provide an interface to submit data directly from the LIMS/regulatory partner system to FDA system, which reduces manual data entry. Also, the ORA DX includes the Enhanced DX Client, a desktop client application, which also supports the submission of sample data to FDA. There are labs that have come onboard for all available sample data sharing options (ORAPP, System-to-System Services and Enhanced DX Client). Maryland Department of Health (MDH) Laboratories Administration is a noteworthy example of System-to-System Services onboarding funded by the Laboratory Flexible Funding Model (LFFM). The agency completed system integration development in only three weeks. Such fast-paced system integration was the result of a long-standing partnership between the agency and their IT vendor on a series of projects including a largescale LIMS upgrade that preceded System-to-System integration. ORA Office of Regulatory Science has initiated state lab production usage of sample data sharing capabilities. So far, Connecticut Agricultural Experiment Station, Michigan Department of Agriculture and Rural Development and MDH have electronically sent sample data via ORA DX.

Presenter: Omari Fennell, US Food and Drug Administration, omari.fennell@fda.hhs.gov

A Standardized Approach to Electronic Test Orders and Results (ETOR)

J Kondamuri, . Park, T Hardin, E Jordanov and S Downer, J Michael Consulting, LLC

Background: The ability to exchange electronic test orders and results (ETOR) streamlines a laboratory's workflow, improves data quality and completeness, reduces turnaround time and enhances forecasting. Yet due to the challenges of implementation, ETOR adoption remains low among public health laboratories (PHLs). J Michael Consulting (JMC) has developed standardized and scalable tools and processes to help PHLs efficiently onboard trading partners and maintain production-level ETOR interfaces with many clients with minimal overhead. This poster describes JMC's ETOR solution and toolkit.

Methods: JMC leverages a LIMS-agnostic, table-driven Rhapsody route as the cornerstone of its ETOR solution. As many PHLs already use the Rhapsody integration engine for other use cases, this solution builds on existing infrastructure and expertise within the PHL. The ETOR solution connects all clients via web service, rather than onerous point-to-point connections. JMC recommends basing the ETOR message specification on the HL7 LOI and LRI Implementation Guides and has developed tools to facilitate the gap analysis and validation process for both the hospital and the PHL. JMC has a suite of test scenarios for communicable disease testing and newborn screening that can be modified based on use case and the PHL's test catalogue.

Results: JMC has partnered with multiple PHLs, hospitals and other trading partners to implement an agile ETOR solution that is easy to maintain and reuse across laboratories and laboratory systems.

Conclusions: The JMC ETOR solution is driving adoption of ETOR between PHLs and their clients.

Presenter: Jyothi Kondamuri, J Michael Consulting, jkondamuri@jmmichael-consulting.com

LIMS Lite: A Public Health Informatics Ecosystem for Implementing Sequencing Workflows, Bioinformatics Analyses and Data Sharing in Public Health Laboratories

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Public health agencies are expanding genomic sequencing capacity to improve surveillance and epidemiological investigations. While sequencing data adds valuable information to aid in metabolic disorders, outbreak detection, transmission chain tracing and understanding large-scale dynamics of pathogen evolution, it also poses new challenges for public health laboratories (PHLs). For example, performing sequencing workflows requires new laboratory processes and new biostatistical expertise. Presently, a lack of standardization in sample processing into sequencing libraries, implementation of bioinformatics pipelines and result reporting makes it difficult to reliably reproduce and share results. This presents an urgent need for a unified informatic ecosystem to support standardized pathogen sequencing workflows and analyses and to facilitate efficient data sharing for public health. This session will introduce participants to the innovative LIMS Lite platform that CDC and J Michael Consulting are building to bridge this informatics gap. LIMS Lite is an open source unified informatic ecosystem designed to facilitate the execution of standardized laboratory workflows and bioinformatics analyses, to ease reporting of genomic attributes and to simplify efficient data sharing among public health agencies. Currently being built to support multiple pathogens, including Influenza, SARS-CoV-2 and Anthrax, LIMS Lite aims to unify the sequencing methodologies used among public health laboratories. Uniting laboratory analysis and reporting will improve process validation, reproducibility and traceability of sequencing data, advancing our ability to monitor and understand outbreaks. Several key features of LIMS Lite are designed to increase accessibility and auditability of sequencing to public health laboratories. A user-friendly interface implements click-through protocols for conducting sample workflow, execution of bioinformatics pipelines, viewing of results and data sharing. This

decreases the advanced technical computing expertise typically required to set up and perform advanced bioinformatics analyses of sequencing data. LIMS Lite contains a partner communication portal that facilitates secure sharing of sample metadata, input files and results to appropriate partners. LIMS Lite is built with a set of containerized open-source modules enabling local- or cloud-based deployments that can be rapidly scaled for an efficient response to emerging outbreaks. The LIMS Lite platform will ensure that the CDC and other public health partners have the software and infrastructure required to establish consistent end-to-end sample processing from specimen preparation through in-silico bioinformatics analysis and data reporting to facilitate near-real time surveillance activities.

Presenter: Bonny Van, J Michael Consulting, LLC, blewisvan@jmmichael-consulting.com

Using Rhapsody to Support Instrument Interfaces for Public Health Laboratories

J Kondamuri, MK Yost-Daljev, J Park, C Pasfield and T Hardin, J Michael Consulting, LLC

Public health laboratories (PHLs) use instruments with a diverse set of capabilities and complexity that integrate with laboratory information management system (LIMS) applications. Existing instrument interfaces are rigid, often proprietary and tightly coupled. Development and maintenance of such interfaces is complex, time consuming and dependent on third-party software, as well as unnecessarily expensive. These factors can deter PHLs from implementing instrument interfacing. An architecture that is loosely coupled, reusable and able to support agile implementations can be a cost-effective and sustainable alternative for any laboratory. J Michael Consulting (JMC) is using Rhapsody to integrate laboratory instruments with LIMS. Rhapsody has built-in standard communication adaptors to connect with instruments. The Rhapsody components are portable, modular and reusable. These features give the flexibility of a plug-and-play model to integrate different instruments with minimal development effort. Using an iterative approach of instrument connectivity, results code mapping, Rhapsody route development and user acceptable testing, the team was able to interface a wide variety of instruments, saving the laboratory significant time.

Presenter: Mary Kate Yost-Daljev, J Michael Consulting, myostdaljev@jmmichael-consulting.com

Modernizing Data Exchange in Public Health Laboratories

MK Yost-Daljev, J Kondamuri, J Park, C Pasfield, T Hardin, L Parlier and E Jordanov, J Michael Consulting, LLC

In order to perform their mission to surveil, protect and improve the health of populations, public health laboratories and agencies rely on a diversity of applications and tracking systems. These systems must be able to send and receive data with many different data sharing partners, including systems used by healthcare providers; by public health analysts at the local, state and federal level; and by different programs within the agency. This data sharing can present an enormous challenge, requiring the translation and transformation of data from various standards and formats

to exchange, ingest and use the data in a meaningful way. The pandemic has placed increased strain on these overtaxed systems, as well as a new urgency to improving public health data exchange. Integration engines such as Rhapsody present an agile way to integrate systems, share data and report information to public health. J Michael Consulting (JMC) is partnering with multiple state and federal partners to leverage Rhapsody, Mirth and other tools to create robust designs that are scalable and loosely coupled to support ever-changing public health needs. The solutions that we are implementing use a combination of existing architecture and new technology to modernize, consolidate and automate existing systems to improve the processes of data exchange for surveillance and reporting. Use cases include Electronic Laboratory Reporting (ELR), Electronic Laboratory Surveillances Messages (ELSM), Electronic Test Orders and Results (ETOR), electronic case notifications, electronic case reports (eCR) and billing. JMC has also automated yearly fiscal years, monthly overdue reports and daily statistical reports. We are developing a PowerBI dashboard to present messaging activity to agency leadership in an actionable format. Many of the solutions that we built responded to the strains of COVID reporting and testing volumes, but our goal is to build tools that are system and workflow agnostic. Agencies and laboratories can quickly adapt and deploy these solutions within their own systems.

Presenter: Jyothi Kondamuri, J Michael Consulting, jkondamuri@jmmichael-consulting.com

Leveraging Strategic Consultation for Informatics Modernization

B Lewis Van, A Prada and MK Yost-Daljev, J Michael Consulting

Laboratory informatics systems selection support services is a need for public health laboratories on an infrequent basis. The typical laboratory software system is used for a decade or longer. This means when replacement is needed and financially possible, the expertise to guide the selection process is often not available internally. The purpose of this presentation is to present a method to leverage independent consultants to make strategic decisions on laboratory informatics. Over a period of 10 years, we have created tools, documented themes and commonalities, and delivered approaches for informatics creation and implementation in public health laboratories. We have served as an extension of the laboratory leadership and staffing and as a go-between from software vendors to the laboratory. We will present a general approach to systematically determine informatics needs, evaluate existing software options, formulate system requirements and manage the implementation. Tools, tips and tricks will be shared. This approach can form the basis to identify the right consulting partners for strategic planning and implementation purposes.

Public health laboratory leadership will be provided with an approach to methodically identify resources to augment IT and laboratory staffing to support laboratory informatics infrastructure.

Presenter: Bonny Lewis Van, J Michael Consulting, blewisvan@jmmichael-consulting.com

Utilization of a Project Management Planning Model for Client Enrollment in an Electronic Test Ordering and Reporting System (ETOR) in the New Jersey Public Health Laboratory

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Orchard Outreach, an Orchard electronic test ordering and reporting (ETOR) LIMS component, was purchased and installed using Centers for Disease Control and Prevention (CDC) Epidemiology and Laboratory Capacity (ELC) Enhanced Detection funding. Fully implemented, Orchard Outreach will enable elimination of all order forms for Virology and Microbiology and an antiquated fax/email reporting process. On October 6, 2021, a goal was set for enrollment of all clinical laboratories, COVID testing sites, STD clinics, TB clinics, veterinarians and local health departments by January 1, 2022. The Outreach Program (Manager and Program Specialist) were recruited to coordinate the project. A Project Management Planning (PMP) approach was employed to reach the goal of 100% enrollment in three months for all clients excepting rabies submitters. This approach consisted of eight specific elements: Stakeholder Needs, Project Objectives, Deliverables and Due Dates, Project Schedule, Roles and Responsibilities, Project Budget, Communication Plan, Tracking and Management Tools. In the initial planning, four phases with defined deliverables and due dates were identified: Preparation, Planning, Implementation, Evaluation. By identifying phases, setting priorities and defining roles and responsibilities, the rollout was managed successfully.

Over the course of the three month rollout, the team conducted 12+ live group trainings, 10+ individual facility trainings and onboarded 1,300+ new users representing 91% of clinical laboratories, 82% of STD Clinics and 86% of TB Clinics. Efforts are now underway to enroll the outliers within these client groups. Beginning January 2022, the team will utilize the PMP model with the assistance of a Rutgers Masters in Health Administration (MHA) intern to enroll and train all rabies clients. This project management model appears promising for short term projects that impact overall operations and may be of more widespread value for effecting operational change.

Presenter: Hannah Hergert, New Jersey Public Health and Environmental Laboratories, hannah.hergert@doh.nj.gov

Data Storage Implementation on SQL Server for COVID-19 Lab Data

A Rahat, M Alexander, A Devito, S Suleiman, J Wang and S Hughes, New York City Department of Health and Mental Hygiene

One major challenge in public health informatics is to integrate data from various sources, which often do not have matching identifiers. The goal was to create an efficient centralized storage implementation for the laboratory data that could be accessed by data scientists and other laboratory informatics personnel with minimal time spent on matching data. Additionally, the data needs to be accurate and up to date. A key focus at NYC Public Health Laboratory (PHL) was maintaining updated pangolin lineage results associated with the sequenced samples which needs to

be linked to its corresponding metadata. The data we received included COVID-19 patient metadata from the Electronic Clinical Laboratory Reporting System (ECLRS) as well as SARS-CoV-2 laboratory sequence data from internal and external laboratories. We automated raw patient metadata input to the SQL Server using python scripts and created SQL queries to filter and merge relevant data depending on the needs of different analysis procedures. The data storage implementation contains global SARS-CoV-2 sequencing data from Global Initiative on Sharing Avian Influenza Data (GISAID), data submitted by external laboratories, and local data from NYC PHL and affiliated lab sequencing results. To match this data accurately, we cleaned the data by removing incorrect or duplicate values, then matched based on a combination of sequence ID, accession number, and fuzzy matching using patient identifiers. After matching the data from various sources, data scientists were able to filter relevant data using methods created in the R programming language, bash scripting, and SQL queries. As of January 10, 2022, there were 51,689 matched records from NYC PHL and NYC Pandemic Response Laboratory (PRL). The pangolin lineage results reported, however, are fixed values at the time of data submission. Using the SQL database we developed, we were able to retrieve the sequences, reanalyze with the latest pangolin tool, link to the clinical records, and share up-to-date lineage results to agency laboratory and epidemiology staff to perform COVID-19 surveillance work. We highlight the advantages of automating the data integration and share the challenges in data matching. The implementation of a centralized storage system on SQL Server has automated data matching and eliminated manual data cleaning while providing all users with the most updated data.

Presenter: Ahmed Rahat, New York City Department of Health and Mental Hygiene, arahat@health.nyc.gov

A Collaborative Model for Streamlining Public Health Data

J Napoli, S Premutico, O Joshi and E Malaj, Palantir Technologies Inc.

At pandemic outset, the US Government had an urgent need for high-quality national-level data for data-driven decision-making. To minimize burden on already overwhelmed public health institutions, it required a simple approach for organizations to share granular data. The US Government solved these issues by standing up a robust data platform within days, enabling HHS/CDC to make decisions based on up-to-date data. Known as Protect, the solution has offered insight into what is required of a technology platform to transform a decentralized lab network into a coordinated effort to detect public health threats. Protect serves as both a central, low-burden, and flexible reporting mechanism and a secure analytics and decision-making platform. It is backed by open APIs and flexible data sync, pipelining, and protection capabilities. These facilitate the generation of high-quality, usable data without burdening data sharing institutions—and enable rapid integration of new data (e.g., new tests). Impacts include:

- 1,900+ hospital labs can report data that they previously had no means of reporting
- Nearly 7,000 hospitals can report data at the national level, as compared to less than 1,000 hospitals at pandemic outset
- The CDC can widely share transparent, near real-time data on

testing based on authoritative data in Protect (see <https://bit.ly/3rvapq7>)

- The CDC can dynamically map new LOINC codes for new COVID-19 tests to capture the full range of COVID-19 testing data, such that changing LOINC codes do not result in data gaps
- The lessons the US Government has learned, which can be applied to modernize laboratory systems and data and analytics for public health response, include:
- Standard pipelines (including connectors and health checks) significantly reduce reporting burden by making it easy for new labs to onboard and share data with the US Government
- With granular-level permissions, data at the facility, local, state, and national level can be made available to authorized users (e.g., federal users) within the bounds of a rigorous privacy framework
- Feedback loops and direct engagement with individual labs is critical to maintaining data quality and trust over time
- Out-of-the-box analytics and dashboards directly on top of high-quality shared data enable rapid reporting of information to senior leaders and other stakeholders

In the future, the US Government and its partners can use such commercial tools to collect new types of laboratory and other data with minimal effort and in a timely manner to accelerate cross-government collaboration on emerging threats.

Presenter: John Napoli, Palantir Technologies, jnapoli@palantir.com

Anomaly Detection for Public Health Electronic Lab Reporting (ELR)

J Hightower, Ruvos

A major issue with any source of data is an awareness of quality issues, how they may impact models, and the subsequent impact on decision-making strategies. Under the stress of COVID-19 Public Health Agencies are experiencing a rise in data quality issues including batch sending records, delays in ELR delivery, and missing records. The key problem is that public health agencies are trying to prioritize very limited resources in containing the COVID-19 pandemic, while maintaining regular epidemiological day-to-day operations, on poor quality data. One of the solutions Ruvos has developed through the Integrity Platform™ is Anomaly Detection notification and reporting for data ingestion of ELR to state departments of health. Through this cloud-based solution pipeline data are ingested directly from labs, filtered through a validation protocol, and are exposed to a real-time analytics model to predict if the inbound ELR message count exceeds safe thresholds determined by the anomaly detection engine. Several Anomaly Detection algorithms were tested and weighted based on identifying crucial historical anomaly markers, flexibility, scalability, and computation cost. The chosen model is an AutoRegressive Integrated Moving Average (ARIMA) trained uniquely per data sender (i.e., lab, hospital, clinic) and updated daily. The training mechanism has been automated, including hyperparameter fitting, such that the users of Anomaly Detection never have to manually adjust settings as the model auto updates daily. Unique models are trained per data sender with an output of several threshold bands that indicate high or low quantity volume anomalies. As these thresholds are

crossed, notifications and visualizations are immediately generated to allow end users to identify problematic senders and irregular data volume. One of the major advantages of this system is how anomaly detection is allowing for a paradigm shift from reactive to proactive response to poor data quality. We have found a strong positive correlation between a critically low daily ELR volume preceding an extremely high volume in the subsequent days. This Anomaly Detection identification and reporting mechanism allows users to get ahead of the error before it impacts the community as a whole in the form of false spikes or dips in daily cases and the subsequent response from policy makers. The second advantage of Anomaly Detection is the ability to be an early detection mechanism for true, unexpected surges in cases.

Presenter: Jake Hightower, Ruvos, jhightower@ruvos.com

Expanding Participant Engagement and Equity in Clinical Trials through Improved Communication, Consent, and Patient-reported Data

D Bhadra¹, M Ravelo²; ¹The MITRE Corporation, ²Ruvos

The purpose of the Alliance Participant Engagement Portal (PEP) initiative is to enable clinical trial teams to better engage with patients who participate in oncology clinical trials, with the aim to improve outreach and longitudinal connection to trial participants independent of participant or trial site resources. The initiative will adapt the capabilities of the Sara Alert™ system to enhance the clinical trial experience for trial participants by 1) connecting participants to trial details, consent, and trial updates; collecting patient-reported demographic data and social determinants of health to give researchers insight into equity in trials and allow adaptation to improve equity and inclusion; and 3) enabling future collection of additional patient-reported information. The goal of the effort is for participants to have a unified, more informed experience during the trial and to inform broader and more equitable participation in current and future trials. The PEP initiative is a partnership, led by Alliance Data Innovation Lab with technology and infrastructure support by MITRE and Ruvos.

PEP's key components include the following.

- Anteroom – Website that contains information on the trial that is relevant to trial participants. Anteroom data includes but is not limited to trial purpose, description of required elements, research team, trial updates, initial results, etc.
- Participant Engagement Management – Web-based tool that allows clinical trial teams to communicate directly with the participant community and provides tools to manage patient responses.
- Participant Surveys – Surveys via text, email, web, phone that enable participants to share self-reported data.
- Dashboard and Reporting – Capability to view dashboards at the site or trial level and report/export data for further analysis.

This project is in the early stages of planning and development, and with launch targeted for 2022.

Presenter: Diane Bhadra, The MITRE Corporation and Manny Ravelo, Ruvos, dbhadra@mitre.org & mravelo@ruvos.com

NEWBORN SCREENING & GENETICS

Development of an Interactive Tennessee Newborn Dried Blood Spots Screening Performance Indicators Dashboard

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Problem: Since 2015, the Tennessee Newborn Screening Program has published monthly reports for key performance indicators on its webpage. However, these reports are static and convey statistics for the state and individual birthing facilities for one specific month. A need has been identified for a reporting tool that promotes data transparency while being dynamic, interactive and easily accessible.

Objective: Our goal was to develop an interactive Tennessee Newborn Dried Blood Spots (DBS) Screening Performance Indicators Dashboard that would include time trends for key performance indicators at the state and facility level. This would be used to monitor program performance and identify areas for improvement.

Methodology: Collaborating with the APHL Newborn Screening Bioinformatics Fellowship Program, a full-time fellow was placed with the program to spearhead the project. A team was formed including members from the newborn screening laboratory and follow-up program. The team met weekly for six months to design and develop this dashboard using Tableau (v2021.2.7) software. Inspiration was drawn from existing public health dashboards, and the evolutionary prototyping model, where prototypes are iteratively created with additional functionality to address project needs and prior feedback, was utilized to develop this dashboard. Thirty hospital personnel across Tennessee gave feedback on the dashboard's design during this process. The final iteration of the dashboard is in the process of becoming a publicly available resource.

Results: Performance indicators include screening rate, unsatisfactory specimen rate, and the timeliness of blood spot collection, transportation and reporting of screening results. The dashboard main page visualizes the three most recent months' worth of data for these statistics across Tennessee or for a user-selected facility. Users can select each indicator and adjust the facilities, date range and date granularity (monthly, quarterly, yearly) of interest in separate views that are linked to the main page. The dashboard is automatically updated monthly to minimize the time and resources required to maintain it.

Conclusions: The Tennessee Newborn DBS Screening Performance Indicators Dashboard supplements the existing online performance reports. Once it is published, it can be accessed interactively by newborn screening personnel, hospital personnel, and the public. This dashboard will drive introspection at the state and facility level and make it possible to identify potential issues and necessary corrective actions earlier, therefore improving the completeness and timeliness of newborn screening in Tennessee. We hope that our experiences can be applied not only to the development of other dashboards within Tennessee newborn screening, but to other areas of public health in Tennessee and across other states.

Presenter: Charles Lechner, Tennessee Newborn Screening Laboratory, Charles.Lechner@tn.gov

Prenatal Screening and Interventions for Lead and Mercury: Reducing Exposure

E Bind, A Steffens, V Chandra, CH Yu, D Haltmeier and Z Fan, New Jersey Department of Health

Lead and mercury readily cross the placental barrier and can severely harm developing babies' brains and bodies, change behavior, and cause a multitude of other issues. Taking steps early to reduce mothers' exposure helps reduce babies' in utero exposure. NJ Biomonitoring partnered with University Hospital (UH) in Newark, NJ to offer prenatal lead and mercury testing as standard-of-care to the patient population. Expectant mothers receive educational materials on minimizing exposure and micronutrient supplements at the first prenatal visit. A tube of blood is also collected at that visit, as is cord blood for the baby at birth. The specimens are sent to the NJ Biomonitoring for testing and the results are sent back to help guide doctors in making medical decisions. Patients with elevated lead ($\geq 3.5 \mu\text{g}/\text{dL}$) or mercury ($\geq 5 \mu\text{g}/\text{L}$) receive care through UH and other resources through Program partners. Elevated mercury samples are speciated and those data are combined with questionnaires to identify the source. Follow up testing is conducted to ensure that interventions are successful and compared to the cord blood at birth. To date, NJ Biomonitoring has analyzed specimens from over 9000 individual patients. We are conducting retrospective chart reviews to gain an in-depth understanding of the exposure risks faced by the patient population and to gauge how well the Program is improving measurable outcomes. We identified a surprising trend for mercury with 50% of patients having levels at which health effects can be expected in babies. Chi-squared analysis indicates a significant trend in reduced exposure in babies during Phase 2 as compared to Phase 1. Data analysis has also identified that patients of immigrant status as particularly vulnerable with exposure leading to statistically significant resulting adverse health outcomes (lower Agpar scores, greater risk of abnormal newborn screen, etc.). Data analysis is still on-going and additional findings will be added. NJ Biomonitoring is concurrently looking to expand to other hospitals to 1) test the transferability of the model and 2) gather data from other geographic areas in the state.

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

Modernizing Newborn Screening (NBS) Through Partnerships/Collaboration, Leveraging Funding and Technology Related to Electronic Testing Orders and Results

J Vasquez, Ruvos

Developing and fostering partnerships and collaborations that lend to closing gaps in funding and leveraging technology can assist state newborn screening programs in their efforts to develop and implement a fully interoperable NBS HL7 electronic orders and results process. Such partnerships include federal agencies, interstate agency partnerships, and programmatic partnerships within state Departments of Health. Ruvos has been working with state public health departments to help them build their infrastructure needed to modernize NBS processes for electronic orders and results with the long term goal of identifying future

funding for pilots and implementation of NBS HL7 electronic testing orders and results. Key components to this work include:

- Online Validation, allowing HL7 providers a process to test new NBS orders or changes to their orders using an online validation tool.
- Inline Validation, validating data sent from NBS order providers, and rejecting data if the data does not meet syntactical or when possible, data quality standards.
- Dashboard, enabling the ability to capture metrics that can be used to gain information about the system's users, throughput, reporting, etc. The Dashboard is used to monitor the health of the NBS processes, and to provide alerts and notifications.
- Alerts and Notifications, assessing and implementing alerts and notifications when infrastructure supporting the HL7 processing workflow fails.
- Long-term benefits of this work include leveraging technology and interoperability to increase efficiency, reduced manual intervention and costs, improved data quality and reliability of our processing
- Infrastructure, and better management and oversight of both the system's usage and performance.

Most importantly, mothers and newborn infants will benefit from the modernization and improved interoperability within a state's newborn screening program with improved data accuracy and reduction in time to report. Potential barriers for providers include IT costs and required nurse training for altered procedures or workflow necessitated by the electronic ordering. However, these concessions will ultimately result in significant, yearly recurring savings for their facilities as well as timely treatment decisions.

Presenter: Juan Vasquez, Ruvos, jvasquez@ruvos.com

PREPAREDNESS & RESPONSE

Implementing Electronic Laboratory Reporting for the Laboratory Response Network for Biological and Chemical Threats Preparedness

R Rahmat, Association of Public Health Laboratories

The reception of timely, accurate, complete and consistent laboratory data is instrumental in how effectively and efficiently public health responds to emerging threats. This is particularly important for members of the Laboratory Response Network (LRN), who respond to biological, chemical, and radiological threats with coordinated effort. Electronic data exchange systems support the LRN by ensuring that laboratory data, including test results and proficiency data, can be transmitted securely in real time. One system LRN laboratories use to transmit data is through electronic laboratory reporting (ELR). ELR is advantageous compared to other electronic data exchange systems as it reduces the manual entry burden and thus leads to fewer errors, streamlines data requirements so less data needs to be reported, and maximizes both data volume and quality during surge events. ELR utilizes Health Level 7 (HL7) messaging as it offers guidelines and standardization for laboratories to uniformly communicate with the

US Centers for Disease Control and Prevention (CDC). Furthermore, ELR is compatible with HL7 messages used by other programs. Since 2019, the Association of Public Health Laboratories (APHL) and CDC have collaborated to assist LRN laboratories with ELR implementation. This funding is open to all public health LRN member laboratories to cover laboratory staff time, vendor staff time, vendor implementation costs, and any other costs directly associated with implementation. To date, more than \$850,000 has been provided to LRN laboratories to support ELR implementation. Eight LRN for Biological Threats Preparedness (LRN-B) laboratories have completed implementation while five labs are currently implementing. Seven LRN for Chemical Threats Preparedness (LRN-C) laboratories have completed implementation while one is in-progress this fiscal year. In addition to these laboratories that were funded to complete the ELR implementation, about nine laboratories are implementing without funding assistance. Through their support of coordinated electronic data exchange systems such as ELR, APHL and CDC strive to strengthen public health laboratories' capabilities in responding to and reporting biological and chemical threats.

Presenter: Rana Rahmat, Association of Public Health Laboratories, rana.rahmat@aphl.org

Implementation of an Inventory Management System to Build Emergency Preparedness for COVID-19 and Future Outbreaks

S Patel¹, A Patel², S Ferro³, J Vora¹, J Shaji¹, J Mulligan¹, A Oyelade¹, H Hergert¹, S Mikorski¹, D Woell¹, J Ochal¹, M Ellethy¹, R Finney¹, R Siderits¹, T Kirn¹; New Jersey Public Health and Environmental Laboratories, ²Rutgers School of Public Health, ³University of Memphis School of Public Health

Objective: The NJDOH PHEL has implemented a simple, cost-effective system for the management of materials for distribution to health care facilities and congregated living settings, such as specimen collection materials, PPE, and rapid tests. During the pandemic, the PHEL initially faced challenges in acquiring adequate supplies and assuring acceptable quality of materials. Over the past two years, the funding and demand has since increased, highlighting the importance of efficient emergency preparedness, inventory control, and providing situational awareness for leadership. This same awareness and emergency management system currently used in the PHEL could also be applied to other departments within the NJDOH and providing similar gains.

Methods: Using Microsoft forms, effective outreach was performed, providing insight on the perspectives of various external organizations such as the NJ State Police, Acute Care Hospitals, Commercial Laboratories, Long term care facilities and several others. Predictive data analytics of material orders has been used to find and target regions that are hot-spot locations and distribute testing or collection materials based on need. Use of the Outreach Technology Center to disseminate educational information and materials to at-risk or vulnerable populations, ethnic enclaves, and cultural nexus points.

Results: Organizations working with vulnerable or at-risk populations have had all specimen collection and transport needs completely handled by the NJDOH for several months. Approximately 14.3 million specimen collection resources were distributed to the various

types of organizations as of January 2022. Point of care instruments for testing were also distributed, among at least 25 locations across the state. Over 375,000 rapid COVID tests have been distributed to support testing of vulnerable populations and to aid in outbreak management.

Conclusions/Future Outlook: Increased use and dissemination of point of care instruments is a powerful strategy for earlier detection/quarantine, mitigation of risk, and management of cases. Furthermore, sufficient supplies at the various facilities and organizations would both encourage and enable significantly more testing throughout the state, but this is again dependent on effective communication, efficient inventory management, and availability of resources. By having a robust system of collaborative operational effort, multiple benefits follow. Initially, by proper dissemination of materials and supplies to as many clients as possible, stronger partnership could be nurtured. In addition, the culture of preparedness would extend to situations beyond this pandemic. Once in place, the organizational system could be applied to supplies and materials for seasonal diseases, such as Influenza, TB, and STDs, but could also serve as a powerful tool for future emergencies.

Presenter: Satyam Patel, New Jersey Public Health and Environmental Laboratories, satyam.patel@doh.nj.gov

Field Deployable Laboratories for Testing in Vulnerable Populations, during Public Health Emergencies

R Siderits, S Mikorski, T Frez, D Woell, W Underwood, J Ochal, H Hergert, L Kimball, A Oyelade, S Patel, R Finney, M Ellethy, L Yeldell, J Deverell and S Delaney, New Jersey Public Health and Environmental Laboratories

Near the beginning of the Pandemic, there were some misconceptions about the definition of a mobile testing facility, a mobile command center and a field deployable laboratory. Our perspective is that laboratory testing, in any location, must provide an environment for laboratory personnel that is safe and also supports correct, timely and cost managed testing. To provide remote testing during the pandemic, we were able to configure three Field Deployable Laboratories (two BSL-2 and one BSL-3) under ELC enhanced funding. These became operational in late November 2020. Logistical considerations prior to deployment included: minor workspace revisions, vehicle SOPs, validating molecular platforms, materials management, license plates, insurance, state gas cards, start-up procedures, maintenance, certification, shore power connections, validation of molecular equipment, router selection and installation, LIMS connectivity and systems competency for driving and on-site setup. We developed a cross-operational support model for training that provided adequate staff for various deployment strategies. Deployment strategies during public health emergencies and for routine use were evaluated. These included a broad range of strategies including cultural nexus points, vulnerable populations and distributed ethnic enclaves. In the first seven months of active service the FDL team was deployed 130 times, performing over 2100 tests, including Rapid Response Testing (RRT), 60%, molecular testing 40% multiplexed testing. Deployments included back to school events, family services, vaccination

events, shelters, safe houses, outbreaks hotspots and psychiatric support centers. All testing was performed under CLIA certification (documents maintained in each FDL). The ongoing strategic plan will include utilization in TB surveillance, STD testing, education and possibly biothreat response. The platforms are integrated with the LIMS, can support advanced molecular testing and secure sample transport. The FDLs will be incorporated into the Distributed Public Health Laboratory Infrastructure (DPHLI). This will facilitate near real time Geo-Temporal surveillance efforts; "incursion" detection; predictive contact tracing; input for data rendering; inform public policy and enable mitigation strategies by providing targeted surveillance with rapid response testing, in collaboration with Communicable Disease Services and the Vulnerable Populations taskforce.

Presenter: William Underwood, New Jersey Public Health and Environmental Laboratories, Email: william.underwood@doh.nj.gov

Clinical Laboratory Network Supports Pandemic Response

A Sterkel, E Bowles, J Keuler and A Bateman, Wisconsin State Laboratory of Hygiene

Successful response to a pandemic requires strong communication amongst all partners. The Wisconsin Clinical Laboratory Network (WCLN) was crucial for rapid dissemination of information, building testing capacity, and supporting testing during worldwide shortages. The established network, with over 25 years of experience in emergency response, provided the means for rapid communication and had the breadth to reach clinical labs across the entire state within 24 hours. When the COVID-19 pandemic first hit, the Wisconsin State Laboratory of Hygiene (WSLH) hosted weekly COVID-19 webinars to provide information and to answer questions on the spread of the pandemic, the testing available at the WSLH, regulatory information, and new test methods available for clinical labs. Information about supply shortages, new test methods, and validation panels was quickly disseminated through the existing WCLN laboratory messaging system and the WCLN email list serv. The WSLH sent out more than 100 validation panels to support the network in validating new SARS-CoV-2 tests. This brought testing closer to patient care and expanded the states capacity for testing quickly and well beyond what could be supported by the public health lab alone. Through the network, labs were able to help and support each other, sharing experiences, best practices, validation panels, and testing supplies. When the Wisconsin Division of Health and Human Services (DHS) wanted to gain insight on the testing capacity and impact of supply shortages, the WCLN facilitated the means to distribute a live survey to monitor this information. This helped facilitate state level support for the clinical labs, which resulted in the creation of a test distribution center to provide free supplies around the state. The vast amount of information and speed of change around the pandemic were challenging to keep up with. Thanks to the WCLN, the Wisconsin public health system was able to support clinical labs faster and more effectively, leading to a stronger testing system and pandemic response.

Presenter: Alana Sterkel, Wisconsin State Laboratory of Hygiene, Alana.Sterkel@slh.wisc.edu

QUALITY SYSTEMS

Continuous Quality Improvement: New Public Health Laboratory Resources

B Su, Association of Public Health Laboratories

The Laboratory Systems and Standards committee, in partnership with APHL staff, federal partners and a consultant, produced multiple new quality-related resources. These documents support public health laboratories (PHLs) in their continuous quality improvement efforts through many quality pathways. This poster will cover a brief description of each resource and its practical uses.

Quality Management

- **Quality Manager Position Description:** Provides a list of standard job requirements and competencies that can be tailored in the level of detail, experience and complexity. The position description supports PHLs who want to define roles and responsibilities of existing staff or for hiring new personnel.

Accreditation

- **CLIA Checklist:** A fillable document, divided into sections that correspond to the CLIA regulatory requirements for high complexity testing. The checklist has examples of compliance, color-coded laboratory positions and related tools. The checklist guides internal self-assessments and identifies potential deficiencies and corrective actions.
- **CLIA resources on the APHL website:** an updated list of resources that cover checklists, bench aids, policy, reports and briefs, and external resources from CDC, CMS and FDA.

Quality Management System

- **Quality System Essentials (QSE) for Non-Conforming Events:** Model practices and related templates supported among member PHLs, following the CLSI guidelines. The resource supports PHLs who want to improve their implementation of a QSE.

Public Health Laboratory System

- **PHL Outreach Model Practices:** Model practices collected from PHLs with robust outreach activities within infectious diseases. Includes information on structure, funding, topics, methods and benefits. The guidance document supports PHLs who want to improve their outreach initiatives.
- **PHL Impact Tool:** Excel tool that allows PHLs to measure change in Salmonella cases and associated costs due to surveillance activities. Provides easily generated output data to produce a summary of health and economic impacts, as well as estimates of cases, hospitalizations and medical costs avoided. Outputs can be used for educating legislative staff, improving internal operations, or building relationships with clinical partners.

These resources will be shared through the APHL eUpdate, Quality Assurance Community and Quality Improvement Forum calls to ensure they are widely publicized. Utilization of the applicable resources will ensure PHLs have a standardized approach to addressing these areas, saving time to implementation while applying best practices.

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

Pilot Test of a Thirteen Module Quality Assurance Course for Scientific and Technical Personnel in the New Jersey Public Health Laboratory

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A series of quality assurance (QA) online learning modules was developed based on the results of a Training Needs Assessment (TNA) of public health laboratory staff conducted in 2020 and presented at the APHL 2021 Annual Conference. Results revealed most participants indicating a need for QA training, which launched the development of the course. This effort is being supported through the Epidemiology Laboratory Capacity (ELC) Grant. A QA Committee of subject matter experts (SMEs) in QA, clinical chemistry and education was formed to design and develop the curriculum. Other SMEs were recruited as required. The order of modules and their titles are: Introduction to Quality, What is Quality Management?, Personnel, Training and Training Assessments, Facilities and Equipment, Information Management, Regulation and Delegation of Authority, Documentation, Biosafety Risk Assessments, The Total Testing Process, Measuring Analyte Concentrations, Basic Statistics and Quality Control, and Non-Conforming Events. A pre- and post-test for each module was created to measure learning. A final draft of each was vetted by the SMEs for format, relevance, accuracy, continuity, and overall presentation. Eleven participants from the following programs are piloting the course: Clinical Services, Newborn Screening, Microbiology and Biothreat Response, and include two post-doctoral fellows and an undergraduate intern. Pilot group titles are representative of the target audience. The effectiveness of learning is being measured based on the Kirkpatrick Level 2 model: comparison of answers to questions appearing in both pre and post-tests, and overall test scores.

All thirteen modules were completed by the pilot group. Comparing the score for all modules per participant, we find the range of participant improvement to be 11.5% - 43.9%. Every participant showed an increase in knowledge from taking the course. Comparing the score for all participants per module, we find the range of improvement per module to be 9.9% - 190.6%, with the greatest improvements seen for Module 3: Personnel (190.6%), Module 5: Facilities and Equipment (83.4%) and Module 7: Regulations and Delegation of Authority (102.0%). Course completion will be required of all technical and scientific staff and be integrated into their annual performance goals as part of a Total Quality Improvement initiative. The long-term plan is to design and deliver a Kirkpatrick Level 3 evaluation to assess impact on overall work performance.

(APHL 2021 poster: *Investing in the Future of the Public Health Laboratory Workforce: Results of a Training Needs Assessment in New Jersey*)

Presenter: Tiffany Frez, New Jersey Public Health and Environmental Laboratories, tiffany.frez@doh.nj.gov

WORKFORCE DEVELOPMENT

Career Pathways: Become a Public Health Hero

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Background: Currently amid a global pandemic and the pressing need for a sustainable public health workforce, there is a lack of qualified staff available for work in public health laboratories (PHLs). Despite the attention brought to PHLs during the COVID-19 pandemic, the extensiveness of work provided by them remains unclear to many. The workforce required to support these activities must contain diverse yet highly skilled and qualified individuals from several disciplines. Though the world has begun to understand the role of PHLs in response to an outbreak, the breadth of work and the opportunities available in their field of work are widely unknown. This lack of awareness leads to PHLs struggling with recruitment of the qualified PHL professionals to meet program needs. Cohort 14 of APHL's Emerging Leader Program aims to develop a series of short videos showcasing different PHLs around the country and what they do to promote professional opportunities in PHLs.

Methods: In fall 2021, APHL's Emerging Leaders Program Cohort 14 included a diverse group of 10 public health professionals, including representatives from US state and local PHLs, the Centers for Disease Control and Prevention, Alberta Provincial Laboratory, and APHL. We propose to support the need for a sustainable public health workforce through the creation of four 20–60 second videos which will be created at PHLs across the country. These short videos will be focused on Environmental and Food Testing, Infectious Diseases, Newborn Screening, Laboratory Response Network, and Informatics and will be shot from the approach of encouraging individuals to become a “Public Health Hero.” The target audience of these videos will be Generation Z, individuals from science, technology, engineering and mathematics (STEM) programs and will focus on how PHL work affects daily lives. Fifty-four percent of Generation Z are currently in STEM degrees and have a high rate of electronic media consumption.

Results: Two of these four videos will be completed by the APHL annual conference in May 2022 and shared with attendees, who consist of current public health professionals. Release of this video during this event has the potential to improve currently declining workplace satisfaction due to stressors placed on PHLs during the pandemic. In addition, this coincides with the traditional end of the academic year, which is when the target audience is likely to begin searching for available jobs. The remaining videos will be completed by the end of 2022. The final videos will be marketed using hashtags and QR codes and are intended for upload on social media sites.

Conclusion: To build a stronger public health workforce, these videos will promote opportunities in PHLs, such as internships, fellowships and other careers, ultimately leading to an increase in valued individuals committed in their support of PHL services.

Presenter: Brian Pope, Indiana Public Health Laboratory, bpope1@isdh.in.gov

Expanding the Federal CARES Act to Require Patient Demographic Data for All Laboratory Testing to Advance Health Equity

J Sunshine, J Yuen, C Fang, S Di Lonardo and S Hughes, New York City Public Health Laboratory

Complete and comprehensive data is crucial for public health emergency response decision-making. The COVID-19 pandemic highlights the dearth of demographic data that accompanies laboratory tests and reports, hampering efforts to appropriately understand the burden of disease and allocate resources. As a result, the federal government passed Public Law 116-136, § 18115(a), as part of the Coronavirus Aid, Relief and Economic Security (CARES) Act, requiring laboratories to report on race and ethnicity in all data submissions to the US Department of Health and Human Services (HHS).

New York City's Public Health Laboratory (NYC PHL) evaluated completeness of race/ethnicity reporting for COVID 19 tests in NYC. Data from laboratory tests received by NYC PHL over two time periods from March 2, 2020 – July 31, 2020 (pre-CARES) and August 1, 2020 – October 20, 2021 (post-CARES) were assessed. PHL's COVID data completion for race and ethnicity increased from 4.3% (pre-CARES) to 59.9% (post-CARES). Including reports of “unknown” as a completed data point, the completion rate post-CARES was 63.4%. While the increase is promising, additional exploration and technical assistance will need to be conducted to understand barriers to reporting. There are various benefits to further disaggregating the data by including more granular race and ethnicity options for reporting. Within group variations can only be detected with greater level of detail. PHL, along with the NYC DOHMH's Division of Disease Control, is currently working to align data fields with a more inclusive list of races and ethnicities. Locally relevant and granular categorizations can provide additional context for why certain trends exist and drive tailored engagement and outreach efforts. Race and ethnicity fields remain voluntary for reporting results for other, non-COVID, laboratory tests. Expansion of the CARES policy on data requirements has extensive implications and should be standardized across all testing types. More accurate, complete, and comprehensive data allows for more robust analyses, deeper understanding of health inequities, and improved clinical decision-making and public policy. This exploration demonstrates the impact of requiring patient demographic fields for laboratory reporting, which has the potential to advance health equity in both emergency and non-emergency settings.

Presenter: Jessica Sunshine, New York City Public Health Laboratory, jsunshine@health.nyc.gov

What Have You Done for Me Lately? Expanding the APHL Reach

R Randolph¹, J Ramirez², C Benton³, M Cone⁴, H Blankenship⁵, A Gorzalski⁶, N Brinkman⁷, J Lee⁸, J Maitland⁹, J Danzeisen¹⁰;

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APHL's Emerging Leader Program (ELP) Cohort 15 is striving to improve awareness among public health laboratory professionals and offer easier access to APHL-developed resources, tools and information. The ELP is a 12-month leadership development program that provides public health, environmental and agricultural laboratory professionals with an opportunity to learn and enhance effective leadership capabilities. Cohort 15 is comprised of 10 individuals from state, local and federal public health laboratories. As the cohort discussed possible project ideas, a recurring theme emerged: APHL has invested significant time and resources developing materials to support every facet of PHLs, and while APHL markets these materials through various platforms such as Lab Matters and eUpdate, this information often fails to be distributed from the originally emailed recipient (often Laboratory Management) to other PHL staff. This inconsistent dissemination of information fails to provide a cohesive picture of what APHL has to offer and how to access these resources; it also has potential to negatively affect future PHL workforce training and retention in the face of the currently aging workforce. Cohort 15 aims to promote and highlight existing resources provided by APHL to PHL professionals of all levels by:

- Cataloging the offerings from APHL to both members and non-members
- Surveying employees in Cohort 15 laboratories as well as current APHL Fellows on awareness of APHL resources
- Developing a quick reference document and poster, "What is APHL and What Can it Do for You?"
- Evaluating APHL web traffic to detect an increase in resource awareness and use

Every resource developed by APHL addresses a public health challenge, and by raising awareness of and familiarizing PHL professionals with these available resources, the benefits of already developed content will be amplified, which may lead to APHL membership growth and increased training and retention of current and future PHL professionals.

Presenter: Christopher Benton, New Hampshire Public Health Laboratory, christopher.benton@dhhs.nh.gov

Understanding Public Health Laboratory Practices Across Generations

A Wright and L Kurimski, Association of Public Health Laboratories

Objective: Provide public health laboratory (PHL) members and stakeholders with data findings, highlights and next steps from the Understanding Public Health Laboratory Practices across Generations survey, launched in spring 2021. The poster content will highlight how APHL members view their laboratory practices within five different topic areas.

Background: In 2020, APHL held a full-day focus group discussion with millennial laboratorians who expressed that there were apparent generational differences in the laboratory when it came to knowledge retention and transfer, team building, communication and workplace culture. In response to this discussion, the Knowledge Management Committee, in partnership with the Workforce Development Committee and the Quality Systems and Analytics program, fielded a survey to gain a better understanding of effective generational practices across APHL member laboratories while at the same time, identifying barriers and opportunities for improvement. Survey findings will also support the development of new resources.

Conclusion: The survey was fielded to all roles within APHL member laboratories and collected 482 total responses. Forty-seven percent of respondents were technical/scientific staff, 81% worked in state or territorial public health laboratories and the generational breakdown put millennials as the highest respondents, followed by Gen X, Baby Boomers, Gen Z and the silent generation. The poster will include graphics and summary top lines in the following areas: knowledge retention and transfer, team building, communication across generations and workplace culture.

**This is work done by the APHL Knowledge Management Committee in partnership with the Workforce Development Committee.*

Presenter: Andrea Wright, Association of Public Health Laboratories, andrea.wright@aphl.org

Investing in the Future of the Public Health Laboratory Workforce: Results of a Basic Molecular Methods TNA in New Jersey

S Mikorski, T Frez, R Siderits, T Kirn, M Schachter, B Jeong and A MacMillan, New Jersey Public Health and Environmental Laboratories

A four-part Training Needs Assessment (TNA) to identify the “gap” between knowledge and skills in Quality Assurance, Basic Molecular Methods, Next Generation Sequencing and Bioinformatics was developed in 2020 with the assistance of internal and external subject matter experts and funded by the Centers for Disease Control and Prevention, (CDC) Epidemiology and Laboratory Capacity (ELC) Cooperative Agreement. The first two parts of the assessment were administered to 80 staff members in the Public Health Laboratory Services (PHLS) and Clinical Laboratory Improvement Services (CLIS) units with the titles Laboratory Technicians, Medical Technologists, Microbiologists, Research Scientists and Clinical Laboratory Evaluators; 67 staff gave complete responses to both surveys. A poster with data from the Quality Assurance TNA was presented at the 2020 APHL meeting. Subsequently a thirteen module Quality Assurance online course was developed and a pilot study is underway. The Basic Molecular Methods survey results revealed most participants perceived a need for full or refresher training on all Skills and Knowledge objectives measured. This indicates that the learning objectives identified by Subject Matter Experts for inclusion in a training program were of value to survey participants. Following the model used for development of the Quality Assurance course, a hybrid curriculum will be developed by integrating existing training resources with inhouse developed modules. As sequencing technologies evolve, a reassessment of the NGS and bioinformatics surveys created for administration to a select group within the laboratory is being conducted. A broader application is under consideration.

Presenter: Tiffany Frez, New Jersey Public Health and Environmental Laboratories, tiffany.frez@doh.nj.gov



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