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Report on the DHS/NIST Workshop on Standards for an Enduring Capability in Wastewater Surveillance for Public Health (SWWS Workshop)

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Abstract

Wastewater surveillance is a promising approach to monitor biological and chemical contaminants on a community level in support of public health and safety response. Wastewater surveillance has recently been shown highly effective for the early detection of SARS-CoV-2 outbreaks within specific populations such as communities or buildings. As such, significant advances have been made in wastewater surveillance since the start of the COVID-19 pandemic. Nevertheless, many challenges remain to be addressed to establish an enduring capability for wastewater surveillance poised to respond to new targets as they emerge. The Standards for an Enduring Capability in Wastewater Surveillance for Public Health Workshop, co-sponsored by the National Institute of Standards and Technology and the Department of Homeland Security, Science and Technology Directorate, aimed to identify these challenges and identify potential standards-based solutions to address them. This free, three-day, virtual workshop convened over 500 participants representing over 20 countries who are involved in all aspects of wastewater surveillance from sampling and testing methods to results/reporting and use of data, and represented organizations including government, public health, testing and manufacturing, academia, and non-profits. The presentations, panel discussions, and polling led to the emergence of three themes for standards for wastewater surveillance: concordance without constraints, nationally consistent, and building for the next pandemic.

Keywords

COVID-19; documentary standards; public health; reference materials; quality control; SARS-CoV-2; standards; wastewater; wastewater-based epidemiology; wastewater surveillance.

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- Mr. Philip J. Mattson, Standards Executive, Department of Homeland Security (DHS), Science and Technology Directorate (S&T) | USA

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- Ms. Sally C. Gutierrez, Senior Advisor for Water, Environmental Protection Agency (EPA) | USA
- Dr. Scott A. Jackson, Leader of Complex Microbial Systems Group, Material Measurement Laboratory, NIST | USA
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1. Introduction

1.1. Workshop Goal and Organization

With the ongoing coronavirus disease 2019 (COVID-19) pandemic, wastewater surveillance (WWS) has emerged as an effective tool for the early detection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) outbreaks to help inform the public health response. This has built upon a wastewater-based epidemiology (WBE) approach to monitor biological and chemical contaminants on a community level by detecting and normalizing specific targets in the wastewater stream to population levels. As a result, significant efforts and initial investments within the academic, local municipality, government agency, and public health sectors across the nation have been made to improve WWS capabilities to support a WBE approach to facilitate assessment of population-level SARS-CoV-2 infection rates. Nevertheless, much work remains to transition the current WWS capabilities and underlying infrastructure into an enduring capability for reproducible, comparable WWS that is poised to address the ongoing threat from SARS-CoV-2, its variants, as well as any new pathogens or other public health relevant targets that may emerge.

The goal of the Standards for an Enduring Capability in Wastewater Surveillance for Public Health Workshop was to identify and prioritize standards needs and technology/measurement gaps and to propose a potential path forward for developing standards that enable a robust, comparable WWS capability. Topics discussed included lessons learned during the COVID-19 pandemic, the state of the art in measurement science and technology for the entire WWS workflow, and challenges in achieving comparable WWS results across locations. Stakeholder input obtained during the workshop will help inform future standards development activities, such as consensus-based documentary standards focused on best practices as well as reference materials, and ensure that any standards developed are fit for purpose and aligned with the needs of the community. Ultimately, these standards will be designed to help provide a foundation for communities and organizations to have increased confidence in WWS results to better inform public health and safety response across the nation.

To accomplish this goal, the workshop was divided into three days. Days 1 and 2 focused on identifying key challenges that hinder a comparable, reproducible, quantitative WWS capability and potential standards to address some of these challenges. Day 3 was forward looking and focused on the vision of an enduring capability and practical input into potential next steps toward reference materials, documentary standards, and improved detection methods for WWS. This input can be used to inform the drafting of a path forward for the stakeholder community to develop standards that enable an enduring capability in WWS. Days 1 and 2 were conducted as live, virtual meetings. Day 3 was asynchronous with talks posted online for viewing and virtual polls to collect information on potential next steps for standards-based solutions. The Workshop Organizing Committee consisted of broad group of experts representing the Department of Homeland Security (DHS), Science and Technology Directorate (S&T); the National Institute of Standards and Technology (NIST); the Environmental Protection Agency (EPA); the Centers for Disease Control and Prevention (CDC); the Association of Public Health Laboratories (APHL); U.S. Geological Survey (USGS); Army Public Health Center; University of Louisville School of Medicine; and AECOM.

The full workshop agenda and program are provided in Appendix A and Appendix B, respectively. Brief overview slides that helped guide workshop content are provided in Appendix C. Presentation slide decks and workshop recordings are available online.[1] Recordings include presentations and panel sessions from Days 1 and 2 as well as pre-recorded presentations for Day 3.

1.2. Demographics of Registrants

Over 600 people registered for the workshop with over 500 people attending. Figure 1 summarizes the workshop demographics and shows the reach of the workshop on an international scale, which included representation from the following: Australia, Bangladesh, Canada, China, Colombia, Cyprus, Denmark, France, Germany, India, Israel, Italy, Japan, Malaysia, Nepal, Netherlands, Poland, Singapore, South Africa, Spain, Sri Lanka, Sweden, Switzerland, Taiwan, Turkey, United Arab Emirates, United Kingdom, and United States (Figure 1A). This workshop also drew individuals from numerous sectors (Figure 1B), with varied focus areas (Figure 1C) and levels of expertise (Figure 1D).

Fig. 1. Demographics for Workshop Registrants

(A) Individuals represented 6 different continents. (B) Representatives from a wide variety of organizations were interested in standards for WWS. (C) Individuals were asked to select their primary area of focus in WWS, with the option to indicate involvement in multiple areas. (D) Individuals self-reported their level of expertise in WWS.

[1] https://www.nist.gov/news-events/events/dhsnist-workshop-standards-support-enduring-capability-wastewater-surveillance

2. Days 1 and 2

2.1. Overview

Days 1 and 2 consisted of presentations and panel discussions to identify measurement challenges in WWS and potential standards-based solutions to address these challenges. After an introductory session, workshop sessions focused on the major steps within the WWS workflow: sample collection, testing methods (including sample processing and detection), data reporting and analytics, and interpretation and use of data. Chat and Q&A from Days 1 and 2 are provided in Appendix I.

Learning Objectives: Days 1 and 2 were designed to enable participants to:

- give examples of how WWS has been used in the past and in the current pandemic,
- recall critical challenges that hinder comparable, high-quality data for the various steps in WWS, and
- summarize potential roles for standards in WWS.

2.2. Welcome Remarks

The SWWS workshop was opened with remarks from several federal government representatives. The first speaker was U.S. Senator Gary Peters (MI), Chair of the Homeland Security & Governmental Affairs Committee. Senator Peters highlighted bills funding programs related to wastewater infrastructure. He spoke of the ability to utilize wastewater infrastructure to safeguard public health and the need to harness the innovation and creativity used during the COVID-19 pandemic to bolster response to future pandemics and improve public health. Next, Mr. Robert B. Newman, Jr., Director of Engagement and Partnerships, Office of Strategy and Policy at DHS Science and Technology Directorate, reminded attendees that WBE has been used by law enforcement and other communities for illegal drug and pathogen monitoring. This capability was utilized during the current pandemic to monitor SARS-CoV-2 in a demonstration of the great promise for new ways to utilize this technology for unknown pathogens. The third speaker was Dr. Eric K. Lin, Acting Associate Director for Laboratory Programs at NIST. Dr. Lin highlighted the roll NIST can play in supporting the development and advancement of new technologies. Specifically, he noted NIST expertise in community driven standards and supplying reference materials and data, as well as the ability to convene complex stakeholder groups and supply impartial guidance to other parts of the government. He also expressed his hope for the workshop to identify and prioritize standards needs, technology, and measurement gaps as well as to help outline the path(s) forward to enable robust, comparable, WWS capabilities for staying safe in the future. The final welcome was offered by Mr. Joseph Hamel, Director of the Assistant Secretary for Preparedness and Response (ASPR) Program Office for Innovation and Industrial Base Expansion (IBx) at the Department of Health and Human Services (HHS). Mr. Hamel spoke on the promise of linking environmental and clinical data to help the community understand disease and environmental exposure. He highlighted WBE as one of the only tools available that is scalable to the population level, with the ability to track relative levels of infection and viral variants and expandability to other applications such as large-scale environmental toxin exposures. As a final point, Mr. Hamel noted the interest in development of platform technologies that are agnostic and multi-modal to enhance community-wide disease detection and support rapid deployment of lifesaving countermeasures where they are needed most.

As workshop co-chairs, Mr. Phillip Mattson (DHS) provided opening remarks, and Dr. Nancy Lin (NIST) presented an overview of the workshop logistics and goals, highlighting the guiding questions for speakers, panelists, and participants to consider during the workshop:

- What methods/techniques/measurement technologies are currently being used in your area of WWS?
    - How do they successfully contribute toward comparable, high-quality data, results, and/or decisions?
    - How do they compromise efficiency and reduce confidence in data, results, and/or decisions?
- What is needed to improve comparability and confidence in data and results?
- What are the barriers to filling these needs?
- How could standards help address the needs and overcome the barriers? What standards would you recommend?

2.3. SWWS 101: Introduction to Wastewater Surveillance

As a primer and overview of the topics to be discussed during the workshop, five international subject matter experts from government, academia, and the private sector spoke in the introductory session. The speakers brought a broad range of legacy and recent experiences to the session, and discussions provided information on progress in existing WWS activities and their implementation, insights and lessons learned in WWS, and inspiration for sustaining WWS capabilities for SARS-CoV-2 and other applications.

This session showed success in the utility and applicability of WWS from many locations across the globe. Lessons learned helped to focus the dialogue in subsequent sessions and will become a useful resource as efforts continue toward standards for an enduring WWS capability.

2.3.1. Keynote – National Wastewater Surveillance System: Implementation for COVID and Beyond

Dr. Amy E. Kirby, National Wastewater Surveillance System Program Lead, Waterborne Disease Prevention Branch, Division of Foodborne, Waterborne and Environmental Diseases, CDC

The keynote presentation was given by Dr. Amy Kirby. Dr. Kirby detailed progress made toward the use of WWS as a component within a broader public health toolbox. The talk highlighted CDC's coordination, oversight, and technical role as the lead federal agency in implementing the national wastewater surveillance system (NWSS) for SARS-CoV-2. The number of jurisdictions participating in the NWSS has grown rapidly during the pandemic, and as of June 2021, 36 jurisdictions were involved in the network with more expected to join as funding and other logistics allow. The NWSS, a collaboration among CDC, the Department of Health and Human Services (DHHS), and other federal agencies, provides coordinating expertise, laboratory capabilities, and supporting infrastructure including a centralized database (Data Collation and Integration for Public Health Event Responses; DCIPHER) where all participants' laboratory results are stored, analyzed, and visualized for public health applications. Using examples of recent findings, Dr. Kirby showed how WWS can serve as a leading indicator of community infections of SARS-CoV-2 several days before individual cases are reported. This capability is particularly valuable in targeted use cases for early warning at locations such as prisons, schools, and long-term health care facilities. Challenges to building a nationally sustainable network include hesitancy to participate, the need for more laboratory testing capacities, and the need to capture decentralized wastewater sources when approximately 25 % of the U.S. population is not connected to municipal sewers. Technical uncertainties in data interpretation, particularly where incidence is low, remain a challenge as do other aspects of data interpretation and risk communication. Dr. Kirby's presentation concluded by pointing out the value in a flexible surveillance platform provided by the NWSS that can be expanded to provide data on multiple health targets such as antibiotic resistance, foodborne pathogens, and emerging infections.

2.3.2. Australia's ColoSSoS Project: Inter-laboratory Study for SARS-CoV-2 in Wastewater

Dr. Kate R. Griffiths, National Measurement Institute of Australia

Dr. Kate Griffiths detailed recent results from an extensive interlaboratory comparison, Collaboration on Sewage Surveillance of SARS-CoV-2 [1], for detection and quantification of SARS-CoV-2 in wastewater. The comparative research, which examined method performance including limit of detection, reproducibility, and quantitative accuracy, was a collaboration with 11 representatives from water utilities, government, commercial, and academic laboratories in Australia and New Zealand. Her talk included recommendations for the use of calibrants and concentration factors to support comparable results and observations of the challenges for this type of surveillance in lower-income regions.

2.3.3. Singapore's National Wastewater Based Surveillance Programme for COVID-19: Analytical Standards, Controls and Reference Materials; Monitoring of SARS-CoV-2 and Related Markers in Singapore

Dr. Judith Wong, National Environment Agency, Singapore Wastewater Based Epidemiology; Monitoring of SARS-CoV-2 and Related Markers in Singapore, Dr. Shane Snyder, Nanyang Environment & Water Research Institute (NEWRI) at Nanyang Technological University (NTU), Singapore

Recent experiences in WWS in Singapore were presented in tandem by Dr. Judith Wong and Dr. Shane Snyder. Dr. Wong summarized the National Environment Agency's experience monitoring 100 sites in high density population centers including workers' dormitories, nursing homes, student hostels, and other residential sites. This effort successfully processed 1600 to 2000 samples per week and resulted in public health actions and individual testing in areas where viral fragments indicated community-level infections. This effort also investigated corrective and preventive actions to maintain the integrity of polymerase chain reaction (PCR) analyses in laboratories. Mitigation strategies included: appropriate cleaning of affected laboratories; assessments of laboratory environments and potential sources of sample contamination; and room segregation for reagent preparation, sample extraction, standard preparation, and product amplification. Dr. Wong also discussed lessons learned and development of validation criteria.

Dr. Snyder explained how experiences at Nanyang Technological University reinforced the need for detailed planning in an effective WWS program. This process began with planning based on surveillance goals in association with quality assurance/quality control plans. These plans included considerations and consistent protocols for sampling, transport, and storage of samples; testing methods (including sample preparation, extraction, and analysis); data reporting and analytics (qualitative/quantitative); as well as interpretation and use of data. Based on his experiences, Dr. Snyder provided a roadmap that included data and results as examples of what worked and why it worked for each critical step in the process.

2.3.4. Wastewater Infrastructure & Operations for an Enduring Public Health Partnership

Dr. Beverley Stinson, AECOM

Dr. Beverley Stinson provided a broad overview and historical context for infectious disease surveillance in wastewater. Drawing from a case study in Israel, Dr. Stinson showed how a sewage surveillance system implemented in 1989 provided advanced warning of a poliovirus outbreak at that time and again in 2013 after weekly sampling. Two recent case studies in Bergen County, New Jersey, and correctional facilities in Kentucky were presented in detail including a description of effective personal protective equipment (PPE) for sample collectors, wastewater sampling protocols, data interpretation, and lessons learned. Results of these case studies reinforced the findings of the previous presentations that showed the effectiveness of WWS as a leading indicator (days to weeks) of community infection before individual clinical infections are recorded. Among the results and conclusions drawn from these case studies:

- WWS is cost effective and fast compared to individual clinical testing.
- Viral dynamic data in wastewater can capture multiple COVID-19 waves.
- Wastewater viral trends lead clinical data by approximately one week and as much as approximately two weeks.
- WWS can provide valuable time for actions to protect human health (testing, quarantine, other measures).
- Very good correlations were found between viral RNA and protein concentration in wastewater and number of infected individuals in the sewershed of the community.
- WWS provides a measure of health for the overall community in advance of clinical testing.
- WWS can be used to determine and verify efficacy of vaccination programs.
- Monitoring can expand to other sites with complementary analysis.

2.4. SWWS 201: Sampling

The objective of this session was to discuss the current methodologies for wastewater sampling for surveillance purposes, including key challenges in sampling and corresponding standards-based solutions. The session consisted of several presentations and closed with a panel discussion, all featuring subject matter experts from government, industry and academia discussing their experience with multifaceted approaches to wastewater sampling for the detection and to monitor the spread of SARS-CoV-2.

2.4.1. Utilities Perspectives and Needs

Mr. Claudio Ternieden, Senior Director of Government Affairs, Water Environment Federation

Mr. Claudio Ternieden provided an overview and background on needs in WWS from a utility's perspective, especially those identified by individuals heavily engaged in WBE and wastewater sampling collection and processing. Based on those discussions, the areas in which the utilities would benefit from increased standardization and support were identified as: guidance and published methods for knowledge exchange; collaboration between public and private laboratories; effective use of metadata collected by the utilities; improved training on new or adjusted standard safety practices, especially in laboratories; uninhibited access to necessary materials, products and services from vendors for acquisition and/or procurement; and sampling, packaging and transportation protocols. According to Mr. Ternieden, the connection between laboratories and laboratory partners has been good during the pandemic but can be improved moving forward.

2.4.2. USGS Experience in Wastewater Sampling: 3 Issues to Guide Wastewater Surveillance for Public Health

Mr. Patrick Phillips, Quality Assurance Specialist, U.S. Geological Survey

Mr. Patrick Philips discussed the USGS experience in wastewater sampling, especially as it pertains to the conversation of WWS for public health. The briefing focused on three key questions: (1) is the sample representative, (2) how do we sample small systems, and (3) how do we integrate wastewater data with public health?

The USGS experience has largely consisted of understanding how to take a representative sample to meet the data quality objectives of the study (question one). Specifically, Mr. Philips and his colleagues work was in the context of coordinating scientists with very specific yet different objectives that included: assessing demographics and disposal impacts, the occurrence of pharmaceuticals, and the impact of weather events and variable flows on analysis. A case study completed by USGS and the University of Vermont (UVM) on "Wastewater Monitoring as Students Leave University" found that because students at UVM made up about one-third of the population in the local community, their departure (e.g., during school breaks) had a significant effect on local demographics. In the published study, USGS/UVM found increases in pharmaceutical concentrations observed at the wastewater treatment plant that were tied to the demographic shift to a year-round older population and use of medications (e.g., seasonal allergy, diabetes, and heart medications) [2]. The study of the relationship between demographics and pharmaceuticals in wastewater sampling was not common in the U.S. prior to the COVID-19 pandemic. Informed by this case study and further research, Mr. Philips presented several conclusions: data needs should drive sampling methods; previous approaches to sampling and collection may not be appropriate, which may mean that lessons learned from past mistakes need to be better communicated in the future; better documentation is crucial; and continued improvement in coordination among utilities, wastewater monitoring scientists and public health scientists is key.

2.4.3. CASE STUDY – Wastewater Monitoring for COVID-19 in Louisville

Dr. Aruni Bhatnagar, Professor of Medicine, University of Louisville

Dr. Aruni Bhatnagar presented a case study on the use of wastewater monitoring for SARS-CoV-2 in Louisville, Kentucky. This ongoing research effort, also known as the "Co-Immunity Project," focuses on understanding the incidence and prevalence of SARS-CoV-2 infection in Jefferson County, the community surrounding University of Louisville [3]. Dr. Bhatnagar and team broke down their studies into two main research objectives, to understand: (1) the actual rate and spread of infection within the community, and (2) how both WWS and community testing can be used to produce a comprehensive assessment of the incidence of disease. To evaluate both objectives, Jefferson County (KY) was sectioned into zones based on socio-demographic factors and placement of watersheds to avoid lumping the entire county and to identify specific changes. Randomized sampling was used to obtain samples representative of people living in each particular location.

For objective (1), the team concluded that wastewater results must correspond to community prevalence. The idea behind that conclusion is if the wastewater samples give a true or valid representation of infection rates in the community, then there must be correspondence between the rate of SARS-CoV-2 measured in the wastewater and the rate measured in the community. When the team performed antibody testing with their samples, however, they noticed a discrepancy between the number of positive antibody results and the number of positive cases reported in the county. The difference could potentially be explained by the lack of county tracking of asymptomatic individuals, as those not presenting symptoms were less likely to seek a county test; however, it is difficult to connect these two factors and truly account for the inconsistency.

For objective (2), the overall aim was to associate the prevalence of infection in the community to the wastewater estimate by random sampling. One major limitation was the low degree of reliability of the data collected as part of the convenience sampling, especially between infection spikes. Any number of sociological factors could interfere with sampling and thus make it difficult to attach a level of certainty to publicly available data. Random sampling, Dr. Bhatnagar noted, could help researchers obtain less-biased samples in the future. Dr. Bhatnagar also highlighted a few important issues regarding wastewater estimates: researchers must be rigorous in their evaluation protocols and procedures, and there is a need for better determination on how much to normalize the signal when conducting sampling.

2.4.4. Rapid Fire Presentations

2.4.4.1. Wastewater Sampling for WBE Surveillance

Mr. Kaushal Trivedi, Business Development Manager, Teledyne ISCO

In the first of two rapid fire presentations, Mr. Kaushal Trivedi, provided an overview of his research efforts on wastewater sampling. The objective of the research was to find prevalence and trends through wastewater analysis. Sampling was conducted at several different locations including a wastewater treatment plant and different sewer spots as well as building outlets, such as those at dorms, hospitals, nursing homes, and industry facilities. Sampling methods at these locations included manual sampling with a "dipper" and automatic sampling with an automatic sampler. Sample collection modes included composite mode, where all samples over the sample period are collected in a single bottle, and sequential mode, where each sample or multiple samples go in different bottles. Mr. Trivedi highlighted several lessons learned from this research: samples should be collected close to the source as it helps in the implementation of local measures and controlling the spread of the virus; composite sampling was adequate (for the most part), but sequential sampling was helpful in certain cases; flow paced sampling was found to be better for varying flow conditions; and samples should be stored at 4 °C. Mr. Trivedi identified several future needs for WBE sampling: the development of location-based sampling standards and guidelines, the use of real time sensors to detect the presence of a virus and trigger the sampler to collect samples for further analysis and qualification, remote communication from the field to alert personnel and promote quick and proactive action, and the quick implementation of data-driven results in public health initiatives.

2.4.4.2. Building-Level Wastewater Monitoring for COVID-19 Using Tampon Swabs and RT-LAMP for Rapid SARS-CoV-2 RNA Detection

Dr. Aaron Bivins, Post-Doctoral Research Fellow, University of Notre Dame

In the second rapid fire presentation of this session, Dr. Aaron Bivins presented his team's approach to sampling driven by an interest in accessible and scalable WWS methods. The passive tampon swabs had ≈ 4 % inhibition rate, and the team found that solids exhibited lower inhibition and greater SARS-CoV-2 positivity than the liquid fraction of the sample; therefore, most of the processing focused on solids. Reverse transcription - loop-mediated isothermal amplification (RT-LAMP) was used as the method for viral detection in samples. The research team monitored nine residence halls at the University of Notre Dame for six weeks, collecting samples once a week using the passive tampon method. There were some false positive results likely from convalescent cases (recovering individuals), but the success of the study was that the team was able to generate same day results using the tampon swab approach [4].

2.4.5. Panel Discussion: Sampling

Moderator: Ms. Sarah Wright, Environmental Laboratories Manager at the Association of Public Health Laboratories

Panelists:

- Mr. John Birkner, Manager of Technical Services, Bergen County Utilities Authority
- Dr. Raul A. Gonzalez, Environmental Scientist at Hampton Roads Sanitation District
- Dr. Rochelle Holm, Associate Professor, Mzuzu University, Malawi
- Mr. Greg Kester, Director of Renewable Resource Programs at the California Association of Sanitation Agencies (CASA)
- Mr. Bruce Smith, Environmental Engineer at USEPA
- Mr. David Swain, Associate Vice President at AECOM

The panel discussion focused on the topic of sampling and continued to address the main workshop goals: to identify and prioritize standards needs and technology/measurement gaps. An initial poll of workshop participants found that 98 % of respondents thought standards were needed for WWS sampling. The panel discussion led to several insights regarding standards needs, with the initial standards needs identified as guidance documents and process control materials.

First, the majority of well-established wastewater sampling protocols are for samples collected for regulatory purposes, and these protocols are accessible through published documents. Several general quality control/quality assurance measures from those protocols are likely applicable to WWS sampling for WBE. For example, it is essential to continue collecting metadata during the sample collection process (e.g., flow rates, weather). Sample controls should exist throughout the process, including positive and negative controls, field replicates, trip controls, field blanks, and a duplicate or blind sample for every 1 in 20 samples. Samples should be stored and/or shipped on ice or in a refrigerator at 4 ºC for a maximum of 72 hours prior to laboratory sample analysis. One specific standard suggested for WWS indicates that sample container lids should be sealed with film when transporting biological samples.

Additionally, there could be guidance specific to how samples are collected. For example, standard personal protective equipment used to protect staff from expected pathogen exposure during routine sample collection of untreated wastewater are equally protective for SARS-CoV-2 sample collection. Developing a sampling guidance that is adaptable to both SARS-CoV-2 and future targets would clarify the extent to which established and valid sampling protocols are applicable to this process, while also specifying WWS needs. Aspects to include are guidance on how to incorporate site- and population-specific factors into the sampling plan, and potentially establishing standard operating protocols (SOPs) that ensure continuity of collection processes across different countries. Panelists were cautious on the extent that sampling protocols could be standardized and emphasized the importance of developing sampling plans that are specific to a sewershed's health, population, and site limitations and needs. Only once objectives and questions are defined can the necessary standards be developed.

2.5. SWWS 202: Testing Methods

The session focused on analytical methods to detect SARS-CoV-2 in wastewater, including implementation experiences, research, and technologies. Speakers provided perspectives on current laboratory testing methods in use, challenges and complexities in obtaining comparable results, and the needed standards to improve testing. A variety of analytical methods have been developed and adopted for immediate use over the last year and a half. Due to the rapid need for methods and lack of benchmarks or widely accepted standardized methods, much of the methods development work has utilized a "learning by doing" approach. Issues surrounding the methods include limits of detection, recovery efficiency, and interference, among other factors. This development process continues to change as more is learned. Most recently, the methods for WWS are evolving to determine if the mutations of the virus can be detected via sequencing. In response to the increased use of WWS nationally and internationally, the private sector has recognized the need to provide products that will improve the efficiency of the methods and the quality of the results.

Confidence in analytical results enabled by future tools such as standardized analytical methods, NIST developed standards, and appropriate reference materials will have a direct impact on the overall integrity of wastewater surveillance programs at the local, regional, and national levels both in the short and long term.

2.5.1. Global Update on Wastewater Surveillance for SARS-CoV-2

Dr. Christobel M. Ferguson, Chief Innovation Officer at The Water Research Foundation

Dr. Christobel Ferguson presented ongoing efforts by the Water Research Foundation (WRF). In the United States the WRF has been convening experts and leading efforts to identify research needs, share best practices, and address research gaps related to WWS. Notably, WRF completed and published an interlaboratory study in 2020 that compared wastewater analytical methods for SARS-CoV-2 across 36 laboratories [5]. In April of 2021, WRF organized a virtual symposium to review the progress in implementing WWS programs globally [6]. As a part of the symposium, a number of programs were highlighted including the states of Colorado, Nevada, Utah, and Oregon in the U.S., and other countries including Spain, France, South Africa, Netherlands, Australia, Canada, and Switzerland. Ghana and Bangladesh, lower resourced countries, were also highlighted. These countries have used WWS in areas without wastewater services or with limited centralized and on-site wastewater systems.

The highlighted programs have addressed multiple issues such as: how to best recover viral RNA, how to best map and characterize the collection system, how to relate the wastewater data to clinical testing results, and how to improve measurement of very low levels of virus. The overall goal of the programs is to provide early warning to assist in guiding deployment of public health measures such as masking and individual testing.

Multiple analytical methods have been used successfully. As a result, a **single** standardized method does not seem warranted. There is an opportunity to standardize certain aspects of the analytical workflow processes. Many of the use cases are focused on detection of trends over time. Most groups are using N1 and N2 genes and reporting in gene copies per liter. Use of WWS to track the emergence of variants through identification of mutations is a new aspect of the work.

2.5.2. CASE STUDY – Overview of the Ohio Wastewater Monitoring Network (OWMN)

Dr. Nichole E. Brinkman, Biologist at U.S. Environmental Protection Agency and Ms. Rebecca Fugitt, Assistant Chief, Bureau of Environmental Health and Radiation Protection at Ohio Department of Health

Dr. Nichole Brinkman and Ms. Rebecca Fugitt presented a case study from a joint effort between the Ohio Department of Health and the EPA. The OWMN was established to provide early warning of infection rates in communities across Ohio. The system was developed early in the pandemic, and currently 68 locations are being monitored twice per week. Eight individual laboratories were recruited to conduct the analysis. Recently, OWMN established a system to test for mutations to help provide information on variants circulating in a community. A statewide dashboard has been developed and is available publicly [7]. The data, by sewershed, have been made available to the communities and utilities. Since each laboratory uses different workflows (no specific methods are prescribed), there is considerable variation from lab to lab, and thus communities are advised to not compare themselves to other communities. The trend data are intended to guide and prioritize actions by the local health district or wastewater utilities. Guidance on interpretation of the data and tool kits for community response have been developed and are available. OWMN is also developing vaccination data by sewershed.

USEPA's Office of Research and Development laboratory in Cincinnati, Ohio is one of the laboratories that has been participating in the OWMN since its inception. As a result of this experience and lessons learned, recommendations that would help improve the program include mechanisms to reduce measurement error, standardized methods and quality control procedures, frequent communication among the laboratories, reliable supply chains, and robust statistical models to quantify uncertainty. Standards that would assist in improving methods comparability include matrix spikes, extraction controls, reverse transcription (RT)- quantitative PCR (qPCR) standards, RT-droplet digital PCR (ddPCR) controls, and inhibition and sequencing controls.

2.5.3. Scalable, Sensitive, Representative, and Comparable Approach for High Throughput SARS-CoV-2 RNA Analysis in Settled Solids

Dr. Alexandria Boehm, Professor of Civil and Environmental Engineering at Stanford University

Dr. Boehm presented data on measurement of SARS-CoV-2 in settled solids as an alternative to testing the liquid influent wastewater at the head of the wastewater treatment facility [8]. The SARS-CoV-2 RNA present in primary solids is 1000 times higher in concentration than in influent on a per-mass basis, meaning the solid matrix may be the most sensitive for WBE target monitoring. Results from the analyses of the solids across major cities in the United States suggest that solids appear to be representative of COVID-19 incidence rates in communities. Furthermore, by using pepper mild mottle virus (PMMoV) as an internal control to normalize SARS-CoV-2 data, results were found to be comparable from community to community. This approach appears to be sensitive, representative, scalable, and comparable.

2.5.4. Rapid Fire Presentations from Technology Providers

2.5.4.1. Reducing Erosion: Using a Rapid Deployable Testing Solution to Reduce Time Between Sampling and Action

Dr. Jordan J. Schmidt, Director, Product Applications at LuminUltra Technologies

Dr. Jordan Schmidt described the goal of LuminUltra technology to reduce sampling time to action by providing a field deployable testing kit. This approach maximizes the time to provide early warning of possible increases in coming clinical cases and is adaptable to areas that may not have access to universities with advanced analytical capability, laboratories, or other analytical measurement infrastructure. These settings include remote areas of the globe, such as, worksites, mining sites, and cruise ships. The LuminUltra *Genecount* SARS-CoV-2 wastewater test kit is reported to allow sampling and analysis in the field with results within 24 hours. Recommendations for future wastewater surveillance standards include considering non-traditional testing environments and various sample matrices such as solids. In addition, consistency in aqueous versus solid-bound viral particles and equal consideration of recovery, sensitivity, and useability were discussed.

2.5.4.2. The Importance of Validation for Wastewater Surveillance

Dr. Brian Swalla, Staff Scientist, IDEXX Laboratories, Inc.

Dr. Brian Swalla identified improving WWS method consistency and comparability as critical needs. During the rapid development of WWS methods, there was minimal time for experimentation and optimization. Routine monitoring on the other hand requires stability, consistency, repeatability, proper and detailed record keeping, quality control and standard operating procedures as well as standardized training. IDEXX has extensive experience in wastewater RT-qPCR, including validation testing, establishing procedures, and identifying constraints on stability of sample and reagents. To this end, IDEXX has developed a RT-qPCR test kit for use in WWS. A use case implemented by the Houston Health Department has shown that the support and test kits from IDEXX assisted in the production of consistent results across a panel of different wastewater samples when compared with another external partner laboratory.

2.5.4.3. Nano Plate Digital PCR for Wastewater Surveillance

Dr. Michael Bussmann, Associate Director, Global Product Management – dPCR, QIAGEN

Dr. Michael Bussmann stated that standardization of WWS analysis requires reduction of variability along the entire workflow. Digital PCR (dPCR) for detection offers an advantage in reducing variability due to its absolute quantification (no standard curve needed). However, there remain sources of variability in dPCR applications that are important to address. These include process complexity, staff skill set, and partition droplet volume and partition count. QIAGEN offers the Nano Plate Digital PCR that aims to (1) reduce variability and enhance inter-laboratory comparability, and (2) provide higher precision, detection in two hours, and simplification of workflow. The use of this dPCR system is also scalable and comparable.

2.5.4.4. SARS-CoV-2 Variant Profiling in Wastewater by Sequencing

Dr. Ellen M. Beasley, Chief Science Officer at Pangolin Health

Pangolin Health recently began providing WWS services to customers who need to characterize SARS-CoV-2 levels and detect variant SAR-CoV-2 strains for buildings, dormitories, and other facilities. There has been considerable effort to develop reports that are useful, can be coupled with clinical data, and are actionable in a public health context. In addition, standards are needed to accelerate the impact of WWS testing. Dr. Beasley presented two case scenarios for standards: 1) those for "wet" variant detection in actual samples and 2) "dry" variants or variant information that is obtained from available data sets. Testing and identification of "wet" variants can be enabled by strain-specific assay development and validation; standardization of protocols; and reporting data with a standardized unit of measure, inclusion of endemic biological controls, improving strategies to estimate human contribution, and use of spike controls. "Dry" variant analysis could be supported by adoption of shared, structured annotation sources, standards for variant classification, rapid update of information, and community standardized practices for reporting new observations and identifying new variants of interest. This effort should leverage existing clinical annotation and analytical standards.

2.5.4.5. BioFire Defense

Mr. Ryan Gregerson, Product Marketing Manager, BioFire Defense

Mr. Ryan Gregerson presented for BioFire Defense, which provides an automated and quick (45 minutes to one hour) PCR-based test that runs multiplexed panels targeting a variety of genetic targets. The majority of this broader line of *FilmArray* panels are used in a clinical setting with a set-up time of approximately two minutes. The tests can also be used for environmental sampling including wastewater. Clinical tests are grouped based on the patient symptoms for example, gastrointestinal. This approach increases the likelihood that the pathogen will be correctly identified the first time. The company has also developed software for data and trends tracking. The tool anonymizes data that are collected. The technology was used in Marseille, France for wastewater analysis, and the tests showed a similar sensitivity as the clinical test samples (300 gene copies/ml) [9].

2.5.4.6. Viral Concentration with Nanotrap Magnetic Virus Particles

Dr. Roberto J. Barbero, Chief Business Officer at Ceres Nanosciences

Dr. Roberto Barbero stated that Ceres Nanosciences has developed a technology to sequester the SARS-CoV-2 virus and other viruses using a Nanotrap magnetic virus capture particles system. The company manufactures hydrogel particles that can capture a broad range of different types of analytes, in this case, viruses in a wastewater sample. The nanoparticles can be added directly into raw sewage, eliminating the need for filtration to concentrate the virus. Capture can then be followed by direct RNA extraction. The Nanotrap can be incorporated into any workflow. The technology has been used at the University of California San Diego the University of California Davis, and University of Connecticut [10, 11] . As a result of the gained efficiency in wastewater processing, each university has been able to expand their surveillance efforts to other local sites. Ceres Nanosciences has recently been awarded a grant from the NIH RADx program to establish 15 sites with automated, high-throughput wastewater processing methods and is seeking partners for this program.

2.5.5. Panel Discussion: Need for Standards to Support Testing Methods

Moderator: Dr. Jay Garland, Associate Director for Research, EPA Office of Research and Development

Panelists:

- Dr. Kartik Chandran, Professor of Environmental Engineering at Columbia University
- Mr. Kahlil Lawless, Microbiology Segment Manager - Americas at Illumina
- Dr. Mia Mattioli, Environmental Engineer at the Centers for Disease Control and Prevention
- Dr. Gertjan Medema, Principal Microbiologist at KWR

- Dr. Martin Shafer, Senior Scientist at University of Wisconsin-Madison, State Laboratory of Hygiene

The panel focused on analytical methods to measure SARS-CoV-2 in wastewater and the role of standards and controls to support these approaches. The panelists discussed sources of variability in the WWS workflow ranging from sample receipt to detection. While the panelists generally agreed there is variability in each step, they identified the sample processing stage, which might include concentration and extraction, as being particularly prone to variability and in critical need of standards. The detection step was considered to be a less critical need for standards development. Therefore, the sample processing stage would benefit the most from standards to improve WWS. It was suggested that there is also a need to contextualize or normalize the fecal strength (amount of human waste) in a wastewater sample.

Laboratories need to recognize that it is a challenge to apply molecular methods to highly variable sewage matrices, and it is currently not possible to compare results from different laboratories because the analytical methods used are not standardized. Laboratories are encouraged to have strong quality assurance programs including individual laboratory proficiency testing, defined limits of detection, and internal laboratory reproducibility metrics. Due to differences among laboratories, individual laboratories should rely on internal trends and document any issues that may impact sample results. A recommendation was made to address the inherent variability by carefully documenting protocols with an emphasis on steps that can lead to variability within and between laboratories. It was also noted that a single standard analytical method may not be feasible nor suitable across the broad range of facility and sample types. Although there is considerable research in progress, studies to determine why there is so much variability in analytical methods would be particularly helpful. There is also a need to conduct research to assist in development of standards.

The panel suggested that there is a need for reference methods, method comparability guidance, interlaboratory method validation, and standardization of multiple methods. Specifically, there were comments that matrix issues have not been solved and that recovery spike controls are important. A reference material for synthetic wastewater was suggested as well as standards to help identify potential bias in measurements due to amplification inhibition. Additionally, there are needs in data interpretation and to the extent appropriate, to adapt lessons learned from bacterial surveillance. Regarding sequencing, there is a need for bioinformatics standards and robust quality assurance schemes. A comment was made to consider the sequencing approach used for cancer as a possible model. For sequencing, it may also be useful to develop a spike material to enhance quality assurance. The panelists emphasized that we need to be clear about the concentration of the target we are seeking to detect and for that to be clearly articulated in the quality assurance data quality objectives. It was emphasized that we must determine what standards can and cannot do.

The panelists also addressed the potential future of the WWS field. The sector should seek to develop, as much as possible, generic frameworks because we may not be able to predict what the next target of concern will be. There was support for generic (not "bug" specific) approaches, because we need to prepare for future pandemics and other situations where the pathogen or target may not be known at the onset. Regarding the pandemic cycle, there is a need to use WWS to predict shifts in community clinical test results and use this information as early warning signal of potential rapid disease transmission, understand how long the incidence of disease remains high, and determine when it reaches a stage where pathogen levels are very low.

This information would allow communities to adapt quickly from one phase of the pandemic to another and adopt appropriate public health management practices. It was also suggested that a framework for method comparability would be useful that identifies a set of emergent targets for research, incorporates standards development, evaluates analytical methods development, and establishes a framework for each phase of pandemic where potential bottlenecks are anticipated, such as supply chain issues, and how to overcome them. Also noted was the need for a process to facilitate comparable methods.

2.6. SWWS 203: Data Reporting and Analytics

The focus of this session was the need for standardization of data reporting and analytical methods. The importance of data utility and integrity for different stakeholders, including policy makers, public health officials and the public at large, is paramount to the success of a WWS system. In addition, timely turnaround, preferably within 24 h to 36 h, for test results and reporting to a standard data visualization platform is needed to provide the early warning system that is wanted and needed to support an enduring WWS capability. This session addressed these needs.

2.6.1. National Water Wastewater Surveillance System (NWSS)

Dr. Wiley Jennings, Health Scientist, Data Lead for the Water Wastewater Surveillance Program (WWSP), CDC

Dr. Wiley provided an overview of data flow through the CDC NWSS and an explanation of the data platform. Participation in NWSS is growing, with 36 Epidemiology and Laboratory Capacity (ELC) funded jurisdictions utilizing $223M in funding. An additional $34M is being allocated to further increase participation. Currently data from six states are being entered into the NWSS platform amounting to approximately 10,000 samples from 199 sample locations. Results from sampling by commercial entities, Aquavitas and Biobot, are also being submitted to NWSS through their HHS contracts. Dr. Wiley explained that data collected include wastewater data for SARS-CoV-2 RNA concentrations; sewershed spatial boundaries; sewershed COVID-19 case data; and laboratory protocols. Dr. Wiley discussed how NWSS is addressing challenges to centralized reporting and standardization including:

- DCIPHER as a single platform that hosts NWWS and other COVID-19 surveillance programs. Having a single platform makes it much easier to synthesize data and report results.
- Timely data reporting and rapid submission. NWSS has a very tightly controlled vocabulary that facilitates standard submission from a variety of laboratories.
- The need for standardization of wastewater normalization practices to facilitate inter-laboratory comparison. This includes endogenous controls, matrix spike recovery controls, and PCR test types used by laboratories.
- Data visualization that shows the time series trends of concentrations plotted against actual case data, sample locations color coded for range of concentrations (low, medium, high, etc.), and other metrics.

2.6.2. CASE STUDY – SARS-CoV-2 Wastewater Surveillance and Public Health Applications in Houston, TX

Dr. Lauren Stadler, Rice University AND Dr. Loren Hopkins, City of Houston

Dr. Stadler provided an overview of the sampling framework in Houston which involved 24-hour composite influent/wastewater samples collected from 39 wastewater treatment plants (serving 2.3M+), 51 schools, 25 high risk congregate living facilities (nursing homes, homeless shelters, jail), and 60 smaller catchment areas each week. Samples were sent to two labs that used slightly different methods, which were resolved in subsequent statistical analysis. Levels of SARS-CoV-2 were reported as:

- Percent of the July 6, 2020 viral load
- Percent one-week change of estimated levels
- Associated with zip code

Their results suggest that these adjusted levels of SARS-CoV-2 were comparable across catchment area size and that changes in wastewater viral levels preceded clinical reporting by one to two weeks. The data were transformed into a color-coded heatmap and shared with the Houston Health Department. Dr. Stadler shared some results of their recent work using two different methods to monitor SARS-CoV-2 variants. The first using the PCR platform to detect and quantify the "UK" variant and its spread, and the second applied full sequencing to reveal the complete range of mutations present in the wastewater.

Dr. Hopkins provided an overview of how the health department used the wastewater data by zip code to compare with other data from those zip codes to inform interventions. The health department creates a "cluster analysis" report each week with the following criteria:

- Top 15 zip codes by overall positivity rate
- Cluster defined as an address with 10 or more cases in the past 5 weeks
- WWS findings
- Vaccination rates
- Demographic data

These reports are used by "strike teams" that are dispatched to these hotspot locations. Dr. Hopkins discussed their success with continued focus on hotspots. She also indicated that wastewater has been more important as clinical testing rates decrease, becoming the primary source of infection data in that circumstance. The health department also performs manhole level testing at some congregate living facilities, and results are interpreted relative to past results at those facilities. Outreach is targeted to facilities that have high levels until 10 days after wastewater levels become negative.

2.6.3. CASE STUDY – Translating Wastewater Data for Policymaking

Ms. Aparna Keshaviah, Mathematica

Ms. Keshaviah described the role Mathematica has played in convening stakeholder groups to inform public policy. Their work in wastewater started in 2017 with a convening on the role of WWS for opioid surveillance and more recently, has shifted to COVID. Ms. Keshaviah presented three areas of focus:

- Impact of standardization on wastewater trends and alerts
- Contextualizing wastewater data for policymakers
- Triangulating information through data synthesis

Results from Haywood and Jackson Counties, North Carolina were presented unadjusted and adjusted for parameters such as Bovine coronavirus (BCov) recovery and PMMoV. The variation in results suggested that no single standard metric would be appropriate but rather that the reporting approach should include a standard framework. This matter is further complicated by multiple arbitrary criteria used to define an increase. For example:

- Levels crossing a threshold? Based on absolute levels or percent change?
- Sustained increase for X days? How many days? Ignore large spikes?
- Statistically significant increase? What type I error rate is appropriate?
- Regression modeling of trends? Ability to handle non-linear effects?

Contextualizing the data with other complementary sources of information is useful for supporting public officials with their task of situation determination. Ms. Keshaviah shared examples of wastewater dashboards from several communities and emphasized the importance of integrating multiple data elements that could include clinical case data, social media survey tracking and population risk factors. The Curated COVID-19 repository was introduced as an example of a clearinghouse of COVID-19 data sources [12].

Finally, Ms. Keshaviah shared a summary of the role of data triangulation and synthesis from work done in Montana for opioid surveillance. Wastewater data were compared with prescription methamphetamine data and with Emergency Medical Services (EMS) runs for heroin overdose and law enforcement drug seizures. The presentation ended with some recommended readings of Mathematica publications relevant to the topic.

2.6.4. CASE STUDY – Biobot Analytics: Building Early Warning Health Analytics from Data Available in Our Sewers

Dr. Mariana Matus, Biobot Analytics, Inc.

Dr. Mariana Matus provided an overview of the genesis of their company dedicated to wastwater analytics. Wastewater analytics has three features that make it valuable to public health monitoring:

- Predictive - Wastewater data are a leading indicator for new infectious disease cases.
- Inclusive - Everyone has a voice in the sewer. Data include everyone, not just people who access clinical care.
- Versatile - Wastewater is a rich source of health data, including Covid-19, influenza, opioids, diet, stress, and others.

The company started with a focus on opioid surveillance. Data were shared that showed how manhole-level monitoring was credited with interventions that reduced overdose rates in several neighborhoods. For SARS-CoV-2, the company provides kits that are shipped to municipalities and returned to Biobot for analysis. Composite data were shared from over 500 communities in 46 states since the beginning of the pandemic [13]. Biobot was awarded a 10-week contract from HHS to monitor 320 communities in 50 states with a total of 6,000 samples. In conclusion, the three main advantages of WWS were highlighted: this platform can inform clinical data trends, outbreak detection for early intervention, and SARS-CoV-2 rankings amongst a nationwide database.

2.6.5. RAPID FIRE – Global COVID-19 Wastewater Monitoring Efforts: Development of the COVIDPoops19 Dashboard

Ms. Ana Grace Alvarado, University of California Merced

Ms. Grace Alvarado presented the UC Merced COVIDPoops19 Dashboard as an aggregation of all publicly documented wastewater dashboards covering 86 dashboards from 54 countries and 256 universities at the time of the workshop [14]. Forty-nine percent of the dashboards present their data in the form of graphs while 48 % present maps. A third of dashboards use colors to indicate trends. Several dashboards were shown to illustrate the variation on dashboard design and suggestions for 'best practices' included use of colors, downloadable data, video explanations, and options for further technical understanding.

2.6.6. Panel Discussion – Need for Standards to Support Data and Analytics

Moderator: Mr. Paul Storella, AECOM

Panelists:

- Dr. Ellie Graeden, CEO at Talus Analytics
- Mr. Robert Greenberg, CEO at G&H International Services, Inc.
- Dr. Wiley Jennings, Health Scientist at the Centers for Disease Control and Prevention
- Mrs. Stacie Reckling, Geographic Information Systems (GIS) Analyst at North Carolina Department of Health and Human Services, Division of Public Health
- Dr. Rachel R. Spurbeck, Senior Genomics Research Scientist at Battelle Memorial Institute

The focus of this panel discussion was an end-user perspective on the potential role standards could play in supporting the public health response using WWS data, and to identify potential challenges in implementing standards during data analysis and reporting. Issues that were discussed included the interoperability and utility of data across different end-users with various interests in the results.

To facilitate interoperability, the panel emphasized the need for system platform integration and data integrity to ensure quality and consistency. The key driver is data standardization, not necessarily that the actual dashboard visualization be standardized. On a related note, in order to provide actionable WWS data to different end users, including policy makers and public health officials, there should also be standardization in the reporting units of the data. The panel pointed out the difficulty of aggregating data across laboratories and the challenge of providing all the levels of aggregation that researchers request. NWSS has addressed this by standardizing the data input and the metadata vocabulary that accompanies it. Usage of the data will continue to improve as public health labs become more familiar with the new data types needed and how to communicate sequencing data. The CDC also works to make sure their system is secure, data are well protected, and all the ethics related to new data types are considered. These data protection methods include user vetting through legal agreements and memorandums of understanding to help protect the integrity of the data.

The panel discussed some of the challenges related to geographic sampling and linking wastewater data to the appropriate clinical data. Data must be usable by multiple agencies and should also be identifiable by "hospital shed" in order to understand the demographics that are being more severely impacted. One challenge is how to collect these metadata and report them back to public health officials and combine them with other wastewater data. One panelist is currently working on this by creating geographic information system (GIS) sewer maps of the sewersheds and overlaying those with US census data to provide a real count of the population being sampled. Although many wastewater utilities are currently using GIS maps for their sewer systems, a standard approach is needed to obtain the required granularity and to inform how the census data are incorporated. An idea for standardization is to include sewershed mapping in the wastewater utility permit. One remaining challenge relates to users on septic systems and how to include them in the analyses.

Lastly, the panel discussed how lessons learned thus far can be applied going forward. One panelist described how work began with qPCR for viral load but has now evolved to encompass sequencing across entire states. This leads to new considerations such as the importance of nucleotide versus amino acid differences. Given the utility of sequencing during the pandemic, they suggest it could add value in many other areas such as surveillance of other pathogens. This would be supported by more qualitative or trend analysis because population-level data need to be reported differently than clinical data.

2.7. SWWS 204: Role of Standards in Supporting the Use of WWS Data

Just as sampling and testing methods have been diverse, so too has been data reporting. The workshop brought together a wide range of experts and practitioners who shared a number of common themes about data interpretation and application. It was generally agreed that the public health community is the primary audience for data reporting and usage, thus their information needs should be clearly elaborated if WWS is going to be successfully adopted and used for public health response. Overall, these kinds of data communications and implementations were viewed as an area where guidance would be the first step in the journey to codifying standards on data usage that might follow after field and lab standards development.

2.7.1. Barriers to Public Health Use of Data

Dr. Sandra McLellan, Professor at the University of Wisconsin School of Freshwater Sciences

Dr. Sandra McLellan reviewed the results of her study on the information needs of the public health community conducted in 2020. She assembled an expert panel of wastewater researchers to determine the state of knowledge regarding SARS-CoV-2 field and laboratory science. This was followed by a more expansive dialogue that included public health and wastewater industry professionals. The importance of "fit" between what was known by researchers and what is needed by public health and wastewater experts was central to the study.

The study identified barriers to the adoption of these data by public health professionals.

Barrier 1:

- Public health practitioners did not have adequate personnel and resources to review and incorporate WWS data into their established workflow and decision-making frameworks
- Unfamiliar units of measure (e.g., copies of virus versus case counts)
- Sources of uncertainty and variability not well characterized
- Lack of methodological standardization leading to uncertainty regarding interpretation

Barrier 2:

- Public health agencies wanted to see their data, in terms of how wastewater viral concentrations and case counts corresponded, in their own communities to gain confidence in the application of WWS

Broad insights from the study include that the wastewater data were not at a point to be used as a definitive description of the public health situation, but rather that these data may be useful in combination with other public health data sources to inform decision making. Further work is needed to simplify the presentation of the data with a focus on interpretation. Also, sample-to-sample variation can be high, which contributes to confusion and reduces confidence among the public health community. Last, the sampling interval for wastewater can directly affect data utility. If the sample frequency is low (e.g., weekly), it will take longer to determine if a trend is present which translates into lost response time for public health.

The current shift in focus from virus concentrations to genomic variant detection brings many of the same challenges in communication. The lessons of this study, particularly around engaging early with public health officials, should inform the field as it moves forward.

2.7.2. Panel Discussion

Moderator: Dr. Ted Smith, University of Louisville School of Medicine

Panelists:

- Major Michael Dietrich, PhD, Bioenvironmental Engineering Flight Commander at United States Air Force
- Ms. Rosa Inchausti, Deputy City Manager at City of Tempe
- Dr. Nathan LaCross, Wastewater Surveillance Program Manager at Utah Department of Health
- Dr. Sandra McLellan, Professor at the University of Wisconsin School of Freshwater Sciences
- Mrs. Halley Reeves, Vice President of Community Health Impact for Oklahoma University Medicine

Dr. Ted Smith hosted a panel discussion with experts on public health use of data from various communities across the country. The panel discussion started by acknowledging that we are dealing with a new kind of data, and introducing new data is hard. As Dr. McLellan put it, we are "building the boat as we sail;" there are technicalities to work out including understanding variation in the data and how to interpret results that are discordant from other public health data. Literature that has emerged during the pandemic has shown that trend data can be useful, and everyone's data (at this point) are noisy. Right now, WWS data may not be able to stand-alone during determination of the public health response but have proven useful when used alongside other data sources.

The topic then switched to the importance of understanding the population being monitored in data interpretation, specifically as it relates to the size of the population. Dr. LaCross pointed out that in Utah, community sampling can be enough to answer public health questions, sub-community can be harder because the maps don't necessarily tell you the source you are sampling. One exception is university sampling efforts that have precise information about how wastewater samples are connected to the population being monitored, thus contributing to the success in this area. Mrs. Reeves observed from her experience in Oklahoma that variation does become a factor in the smaller areas, and it is important when considering when to deploy resources to achieve smaller scale. The group emphasized the need to understand population dynamics, including the contributions from temporary residents (e.g., tourists, commuters) for proper data interpretation. One panelist suggested the potential to incorporate cell phone mobility data to account for human movement. In addition to understanding the population, inclusion of metadata was also discussed. Specifically, the utility of flow and temperature data was mentioned, and some groups have already internally developed protocols related to these variables. However, the importance of finding a balance between sufficient data to interpret the results and keeping the reporting timely and understandable was noted. One panelist recognized that standards would be helpful and are needed for public health buy-in; however, the specific nature of the standards was not discussed.

3. Day 3 - Standards to Help Build an Enduring WWS Capability

3.1. Overview

Day 3 focused on collecting input and ideas for a potential path forward for participants and the stakeholder community to develop standards that enable an enduring capability for WWS. Participants who participated in this asynchronous session should be able to:

- describe key characteristics of an enduring capability from various perspectives,
- list potential next steps toward standards to support this capability, and
- determine where they would like to participate in standards development activities.

A set of presentations provided relevant examples from related fields and perspectives on what an enduring capability might look like. Three breakout sessions provided the opportunity for respondents to share focused feedback on Methods and Data Comparability, Reference Materials, and Documentary Standards/Guidance Documents. In observance of Juneteenth as a new Federal Holiday in 2021, Day 3 transitioned from a live, virtual format to asynchronous activities conducted on each participant's own time. In the asynchronous format, pre-recorded presentations and slides from presentation sessions were made available through the workshop website,[2] and breakout sections were conducted via focused feedback polls that were live for approximately 2 weeks following the workshop.

[2] https://www.nist.gov/news-events/events/2021/06/dhsnist-workshop-standards-support-enduring-capability-wastewater

3.2. SWWS 301: Building an Enduring Capability

3.2.1. Session Overview

As suggested by the title of the workshop and this session, a major goal of this workshop was to lay the groundwork for standards to support WWS as an enduring capability. In this session, several experts in other fields were invited to share their perspectives on how applying standards in other areas of biosurveillance supported their needs and advanced their capabilities. Then, speakers representing both federal and local entities provided insights into the current use of WWS for SARS-CoV-2 and what is necessary to transition this current effort to an enduring capability that goes beyond SARS-CoV-2 detection.

3.2.2. Examples from Related Fields

3.2.2.1. Example Surveillance Program: Making It Count - Keeping It Available: Wildlife Disease Situational Awareness

Dr. Kimberli Miller, Wildlife Disease Specialist, US Geological Survey National Wildlife Health Center

Dr. Kimberli Miller discussed three recent projects at the USGS to standardize and provide consistent data. One common goal of the projects was to address challenges pertaining to data collection and sharing relevant to wildlife diseases. In the first example, Dr. Miller described an effort to develop disease case definitions, which helped improve the ability for data comparability among different pathologist's diagnoses. Having definitions derived from scientifically based criteria for determining presence of specific disease, syndrome, or pathogen provided clarity and consistency to the diagnostic process, which improved clarity in reporting.

In the second example, Dr. Miller described the development of an online platform, WHISPers (https://whispers.usgs.gov/home), that enables communal data sharing between multiple partners including representatives from state, federal, and tribal agencies. The current version provides near real-time reporting of event and two tiers of information: public and private. A public facing map displays morbidity and mortality data down to the county level, and a private portal enables partners to enter data and have access to additional metadata. Two considerations highlighted by Dr. Miller were the decision not to disclose specific locations to the public to maintain privacy and to restrict permissions by event. Other key features of the development plan included: crafting metadata and business rules from the beginning to provide a framework for development, including existing standards such as the Integrated Taxonomic Information System (ITIS; https://www.itis.gov/)) for species name and Geographic Names Information System (GNIS; https://www.usgs.gov/us-board-on-geographic-names) for location; use of drop-down menus for entry fields whenever possible; and user review and usability assessments. Additionally, Dr. Miller described two resources that were instrumental in development of this platform: Agile development training and the Science Gateway Community Institute.

In the final example, Dr. Miller detailed the effort towards white nose syndrome (WNS) diagnostic harmonization. WNS is a disease devasting bats across the country, and the response effort has exploded to over 100 agencies working under one collaborative plan. As a rapidly evolving situation, inconsistencies developed in diagnostic testing, interpretation, and reporting leading to potentially contentious situations. One main issue identified was the interpretation of low-level detection of the fungus: was it an issue with the assay or interpretation? In response a set of standard samples was used in interlaboratory testing to evaluate how assays performed under different conditions. The testing data resulted in guidelines to improve reporting consistency, as well as two manuscripts and a lab handbook that are currently under review. As a final thought Dr. Miller emphasized the need for enduring data. Knowledge builds on previous knowledge, therefore considerations for data discoverability and accessibility need to be suitable for both now and the future.

3.2.2.2. Example Tool: Documentary Standards for Biological Response

Dr. Jayne B. Morrow, Assistant Vice President for Research and Economic Development at Montana State University

Dr. Jayne Morrow's slides provide a breakdown of the development of policy frameworks and associated documentary standards to support enduring capabilities in biological response. Dr. Morrow's insights were gathered from her tenure at NIST and as Executive Director for the National Science and Technology Council (NSTC). Dr. Morrow shared her experience developing policy, strategic science and technology (S&T) roadmaps, and consensus standards in the fields of biosurveillance and biothreat response in order to define the mission space, jurisdictional authority, and regulatory frameworks for new technology emergence. Specifically, interagency efforts such as a joint DHS, NIST, CDC, Federal Bureau of Investigation (FBI) and EPA Framework for a Biothreat Field Response Mission Capability released by The Department of Homeland Security in April 2011 coupled with the 2013 publication of the national Biological Response and Recovery Science and Technology Roadmap provided the foundation for biological detection used in a biological threat response [15, 16]. These efforts, alongside interagency and stakeholder engagement, helped lay the groundwork for biological detection and offered guidance to first responders for the assessment of suspected biological incidents. ASTM E2270-17 provides operational guidelines (Concept of Operations or CONOPS) for the initial response to a suspected biothreat agent, including planning for communication and coordination, minimum training and PPE for field personnel, and guidance for the risk assessment [17]. ASTM E2458-17 provides guidance for collection of suspicious powders and details two sample collection methods[18].

A description of the critical elements for a mission capability in field biological response (2011 Framework document) serves to indicate how available documents and efforts support this capability and where remaining gaps exist, which could be addressed by additional standards. The elements to a field response mission capability included the concept of operations (CONOPS) that defines guidance including how an assay, method, or detector must be operated by a trained and proficient user; training; proficiency testing; sampling and sample handling; and assay design (performance specification, testing and certification). NIST and DHS worked with standards development organizations to develop the standard guidance, methods, and test data to help realize the core elements of the Framework. In addition, DHS developed an interagency partnership with NIST to develop a standard material for responder training and proficiency testing. This effort resulted in NIST Reference Material 8230: Lyophilized *Saccharomyces cerevisiae* NE095, anticipated to be available for purchase in 2022. Based on interlaboratory studies and field testing, this material has been demonstrated to successfully challenge and evaluate the biological response workflow including sampling procedures and detection via mobile and public health lab qPCR assays. Finally, biological detection assay performance specifications, testing, and certifications are guided by Association of Official Analytical Chemists (AOAC) International Standard Method Performance Requirements (SMPRs) developed by the Stakeholder Panel for Agent Detection Assays (SPADA). Together, this suite of multiple types of standards has helped to increase confidence in the field biothreat response and biosurveillance.

3.2.2.3. Example Tool: Development and Performance of NIST SRM 2917 for Molecular Recreational Water Quality Testing

Dr. Orin C. Shanks, Senior Scientist at the U.S. Environmental Protection Agency

Dr. Orin Shanks provided slides on the development and performance of a new standard reference material (SRM) for recreational water quality molecular testing. Surface water fecal pollution remains a national challenge with both public health and ecological impacts. In response, the EPA maintains an active research program focusing on the development, performance assessment, and implementation of fecal characterization molecular methods. To date, EPA has successfully developed tools allowing for same day recreational water quality results as well as the identification of multiple fecal pollution sources. Several methods are nationally validated with standardized protocols available to the public (https://www.epa.gov/cwa-methods/other-clean-water-act-test-methods-microbiological). Rapidly growing interest in these methods has led to the need for a standard control material to support national implementation.

To address this need, EPA partnered with the NIST to produce and distribute a standard control material. The team used a composite DNA target strategy in which a single DNA construct containing 13 qPCR assay targets was cloned into a plasmid vector. The linearized plasmid will be available as a dilution series (10, 10^2, 10^3, 10^4, 10^5, and 10^6 copies/2µL) eliminating the need for laboratories to prepare their own dilutions thus reducing error in qPCR measurements. The targets include 11 fecal source identification markers and two general fecal indicator bacteria genetic markers selected by the EPA [19-26]. The standard control material, Standard Reference Material 2917 (NIST SRM 2917), was produced at NIST via large-scale preparation of the material followed by dilution concentration determination, aliquot homogeneity assessment, and stability testing using ddPCR. Fitness-for-purpose was addressed by the EPA, first in a single laboratory performance study and then in a national multiple laboratory study with 16 volunteer participants. NIST SRM 2917 is now available for purchase [27].

3.2.2.4. Example Tool: Accreditation: Draft Accreditation Checklist for Wastewater COVID PCR Testing

Ms. Patsy Root, Regulatory Affairs Manager, IDEXX Water, Representative of the American Council of Independent Laboratories (ACIL)

Ms. Patsy Root gave the final presentation on Examples from Related Fields and discussed the collaborative development of an audit checklist for WWS methods to detect SARS-CoV-2. The implementation of WWS has several advantages including independence from healthcare-seeking behavior and the ability to identify trends at a sub-region level, which can support public health decisions. Specifically, WWS data can be used to inform decisions on public health communication, monitor the impact of mitigation strategies, and inform decisions on clinical testing. Both environmental (in this instance wastewater) and clinical testing are important in decision making; as such, environmental testing should be held to similar standards as clinical testing. When a sample is sent to the clinical lab, the assumption is that tests are performed in a certified laboratory, and results should come with a level of assurance bestowed by the laboratory certification. To this end, wastewater testing should also be performed in a certified or accredited laboratory to help assure data quality. Importantly, decision makers want to be sure that when the data demonstrate a change in the detection trend, this change is true and not just an artifact due to analyses being run by different labs. Accreditation or certification is applied in other areas of environmental testing. In some cases, these accreditations are required at the federal or state level, and in other instances laboratories have voluntary accreditation to provide result assurance. The primary goal of this accreditation/certification process is for labs to assure that the processes and procedures for analyzing wastewater for the presence of SARS-CoV-2 are defined and described in a way that assures minimum error and the best data quality.

A team composed of ACIL EES (Environmental Sciences Section - several member laboratories and assessing bodies), EPA, CDC, WRF, APHL, The NELAC Institutes (TNI, NELAC - National Environmental Laboratories Accreditation Conference) collaborated to create and promote an accreditation checklist for wastewater SARS-CoV-2 testing that promotes high quality laboratory processes and procedures and assures data are fit for purpose. A major consideration for developing the checklist was that the checklist be free and widely available for labs that want to be accredited/certified as well as for labs that do not. For example, the list may be used by academic labs to assure data quality, even though those labs may not be state accredited or certified. Another objective for the checklist was to ensure that quality assurance/quality control (QA/QC) expectations for each step in an analytical method are clear; simple and clear requirements help reduce inter- and intra-lab variability. The group broke the audit checklist into the following steps: sample checks, standard curve (RT-PCR), pretreatment, concentration, extraction of RNA, PCR (RT-PCR or ddPCR), and results interpretation and data reporting. For each step, a series of categories for QA/QC is listed: quality control measure, description, frequency (how often the QC should be invoked), the purpose, control limit, and corrective and preventive action. The checklist is currently under review by stakeholders and other external reviewers. Once the checklist is approved, it will be formatted to be easily readable and searchable, then released and promoted. The hope is that labs will seek accreditation under ISO 17025. The group is exploring expansion to TNI and state options for accreditation/certification. Future projects include coordination with similar efforts globally and/or expansion to other wastewater parameters/programs.

3.2.3. Enduring Capability Perspectives

3.2.3.1. NWSS Perspective

Dr. Amy E. Kirby, National Wastewater Surveillance System Program Lead at the Centers for Disease Control and Prevention

The first presentation was from Dr. Amy Kirby. The NWSS was developed specifically to support COVID-19 surveillance; however, the infrastructure was built to allow development and flexibility to address public health needs as they arise. A unique challenge presented by WWS are the many diverse stakeholders: utility partners, testing laboratories, and communities each with their own needs, capacities, and constraints. For a successful system, Dr. Kirby emphasized the need to keep the end-user in mind during development. The data generated by WWS are in support of public health at all levels (local, state, federal) and therefore success relies on full engagement and understanding of the applications of these data to support public health. Some keys to long term success will be: (1) A system that can address health inequities - how do we reach the 25 % of people who are not on a sewer system? (2) Financial support - utility companies cannot be expected to cover everything, this will require support from public health; (3) Complete sewershed mapping to understand the networks and community connections; and (4) Supporting health departments' and labs' transition to sustainable platforms. Additionally, expansion to a multi-target platform with the flexibility to adapt to emerging public health needs will be critical. When WWS was previously proposed, it was not clear how it would help avoid or control disease or reduce healthcare costs, and therefore the return on investment was deemed insufficient. While SARS-CoV-2 has demonstrated the utility, this single target platform is not economically viable long term and additional applications could include food-borne infections, emerging pathogen surveillance, or antimicrobial resistance genes. Sustainability will require a balance of more and better data from wastewater testing and public health to identify data that are interpretable and actionable.

3.2.3.2. One Health Perspective

Dr. Tonya Nichols, Senior Science Advisor, US Environmental Protection Agency

Dr. Tonya Nichols presented the next talk and focused on a One Health approach to WWS. The goal of wastewater monitoring is to identify disease-causing agents, use occurrence data to predict community disease prevalence, and then make decisions on how and where to allocate resources. One way to implement an enduring capability is to take a systems approach, and One Health provides the context. A One Health perspective recognizes that animals, humans, and plants are all interconnected with their environments and strives to provide a framework to connect experts in different fields to achieve optimal health. Dr. Nichols equated looking for disease agents such as SARS-CoV-2 in sewage as akin to "Finding Nemo" – a small fish in a large ocean. Not only do you have a small target, but there are many other components surrounding it that can interfere. A successful biosurveillance network will require the cooperation of human, animal, and environmental health partners (e.g., healthcare personnel, epidemiologists, veterinarians, agriculture workers, ecologists, wild-life experts, teachers, parents, utilities workers).

Dr. Nichols outlined three components necessary to build this capacity: leadership, authorities and mandates, and tools and implementation; she focused on the first two. Leadership needs to understand the utility of the predictive capability and how to use these data. They will count on scientists and engineers to provide scientifically sound tools that are feasible and economical to implement. Importantly, leadership needs to be mindful of mandates and is responsible for ensuring funding to meet these mandates. With respect to authorities and mandates, what can we use to leverage this WWS capability? The National Biodefense Strategy released in 2019 mentions biosurveillance 16 times to include drinking water and wastewater in the protection of humans, plants, animals, and the environment [28]. Goal 1 specifically states one must use a multi-sectoral approach to promote timely sharing of information among biosurveillance programs and those designed to investigate biothreats and bioincidences. There is also a call for enhancing biosurveillance laboratory operations, which is necessary for a nationwide capability. Goal 2 calls for strengthening resilience of the water sector to prevent or contain disease outbreak. Moreover, there is support for advancing emergency preparedness through the One Health Act in 2021.

The next three speakers provided a local health perspective on the implementation of WWS for SARS-CoV-2 and thoughts on how to support an enduring capability.

3.2.3.3. Public Health Decision Maker Perspective

Mr. Jeffrey A. Wenzel, Chief of the Bureau of Environmental Epidemiology at the Missouri Department of Health and Senior Services

Mr. Jeff Wenzel spoke on building the capability for SARS-CoV-2 surveillance. This includes detecting the virus, finding meaning in the results, and communicating the results to the public and decision makers. Mr. Wenzel emphasized the importance of how to communicate results. This aspect was particularly important for his group when looking at sequencing data used to monitor mutations in the viral genome. To help support this capability, Mr. Wenzel proposed the development of resources to help with communications, as well as guidelines for troubleshooting when wastewater data do not match clinical data. Additionally, use cases for sewershed testing, for example monitoring known pathogens versus searching for new or emerging pathogens, will help dictate how to set the program up for the future.

3.2.3.4. A Local Health Department Perspective

Dr. George A. Conway, Director at Deschutes County Health Services Department

Dr. George Conway gave the next local health department perspective. Testing in his area began early on in Bend, Oregon through collaborations with a start-up company and later with Oregon State University as a pilot for their TRACE program. The TRACE program looked at cross-sectional community prevalence estimations using a model based on samples of residents from randomly selected, voluntary households in 30 neighborhoods. Local sampling from manholes in some neighborhoods was added, and additional sampling points downstream from large long-term care facilities were also included when rapid spread of infection was observed in these settings. A second pilot study was conducted in the smaller city of Redmond, Oregon in January 2021. Overall wastewater data trends correlated well with prevalence estimates from the TRACE program when examining data from Bend taken early in the pandemic. Interestingly, when the Redmond data were analyzed, the SARS-CoV-2 signal was maintained in the wastewater, but the number of reported cases decreased. One possible explanation for this divergence could be increased hesitancy for people to get tested, especially in vaccinated populations. Interpreting and understanding data inconsistencies between clinical and WWS results will be key for this enduring capability. Deschutes County, Oregon has piloted and now routinely uses WWS for supplemental SARS-CoV-2 screening and has found it promising for early detection of arrival or reemergence of the pathogen and screening for the presence of variants. To expand WWS to be a more generally effective and reliable surveillance tool for early warning and detection of pathogens, Dr. Conway recommends better standardization of methods for concentrating and testing samples. Overall, improving the sensitivity, reliability, and reproducibility of the tests is needed to enable results from different labs to be directly comparable among states and local districts. One of the primary barriers to achieving an enduring capability is funding; this is a large, highly collaborative, multi-sector endeavor that involves many sectors already dealing with funding challenges and limited capacity to support new efforts. Funding and technical support should come from the federal and state level from combined areas of wastewater, environment, and health agencies.

3.2.3.5. State Perspective

Dr. Larry Madoff, Medical Director at the Bureau of Infectious Disease and Laboratory Sciences, Massachusetts Department of Public Health

The final perspective was provided by Dr. Larry Madoff. In his slides, Dr. Madoff laid out the process and use cases for their SARS-CoV-2 WWS efforts. The use cases in Massachusetts included testing one multi-city location, five city locations and eleven priority sites (nine prisons through collaboration with the Department of Corrections and two Soldier's Homes for veterans). Surveillance was a collaborative effort with the engineering firm CDM Smith and WWS company, Biobot. Three pieces of data were utilized: detection (presence/absence), raw virus concentration (per liter sewage), and normalized viral concentration (per liter sewage). This normalized value was derived by adjusting the raw virus concentration to the concentration of PMMoV, a fecal marker, which can account for fecal dilution in sewage. Wastewater data were then compared to clinical data by looking at an overlay of the Bayesian seroprevalence offset by one month. Future directions for WWS in MA include ongoing monitoring for SARS-CoV-2 and other pathogens, and support for these efforts has been requested from the CDC in the form of the ELC grant.

3.3. Focused Breakout Sessions

One of the principal goals of the SWWS workshop was to initially frame out what is needed in terms of standards to help build an enduring capability for the WWS community. Notably, standards can be developed in many forms including reference data, reference instruments, documentary standards, methods and best practices, and reference materials, in addition to other measurement services such as instrument and device calibrations and Quality Assurance Programs (QAPs). To support this goal, a series of three online, focused feedback polls were devised to collect input from all workshop participants and inform future standards development activities for WWS and ensure that any standards developed are fit for purpose and aligned with the needs of the community. The three polls focused on the topics of methods and data comparability, reference materials, and documentary standards/guidance documents.

3.3.1. SWWS 302: Methods and Data Comparability

Standards can be applied to many parts of the WWS workflow. During the workshop, sample processing/testing was identified as the workflow step most in need of standardization. Additionally, differences in methodology were highlighted as a main hindrance to data comparability. Given the identified need, the objectives for the Methods and Data Comparability Poll were to: 1) identify trusted methodologies and best practices for use in sampling and analysis for WWS, and 2) identify critical gaps within currently employed methodologies that might compromise data comparability, with applicability to areas including sampling methods, (bio)analytical methods, measurement QA/QC, and data validation. A total of 32 poll responses were received. The full poll results are available in Appendix F with highlights are provided here. Demographics of the poll respondents indicated their association with the stakeholder groups identified in Figure 2.

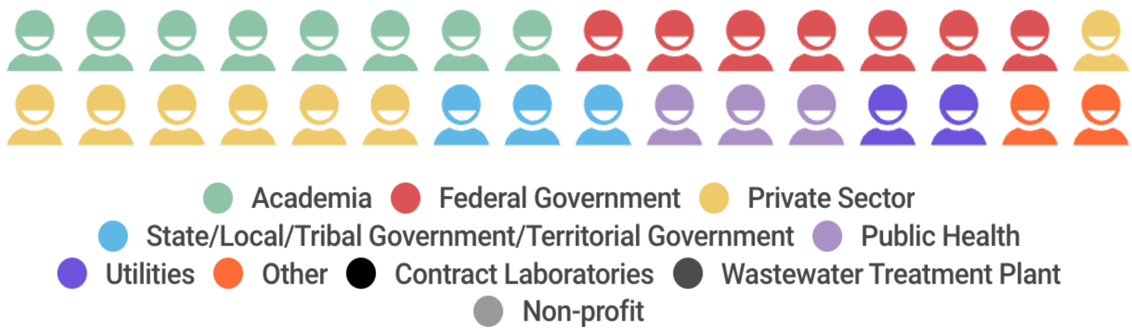

Fig. 2. Respondent Demographics for the Methods and Data Comparability Poll

In WWS, the testing methods component encompasses several steps following sample collection. When asked to identify which area had the greatest need for advances in technology, approximately 55 % of the 39 respondents identified "concentration," which refers to concentrating the wastewater sample prior to detection methods (Figure 3). When asked to identify the current state-of-the-art technologies for sample concentration, Nanotrap and ultrafiltration were the most comment responses to the free response question.

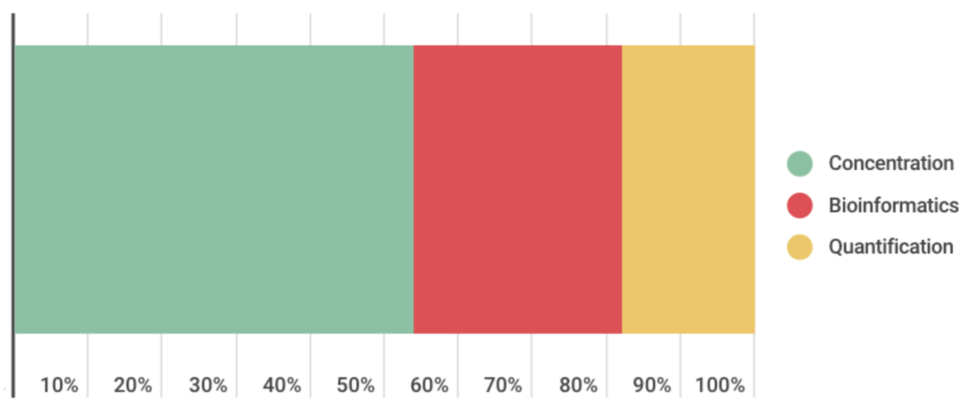

Fig. 3. Poll Response: "Which area has the greatest need for advances in technology for wastewater methods?"

When asked about the preferred, state-of-the-art, analytical technology for quantitating SARS-CoV-2 viral loads, the unanimous response was PCR technologies, including qPCR and dPCR (Figure 4). It is important to note that this question specifically asked about quantitation and not detection, identification, or variant tracking. This unanimous response is a strong indicator that PCR-based methods warrant consideration as the primary technology for quantitating SARS-CoV-2. This finding is perhaps expected given polling results from the pre-workshop poll indicated qPCR and dPCR were the most promising detection technology for biological targets (Appendix E).

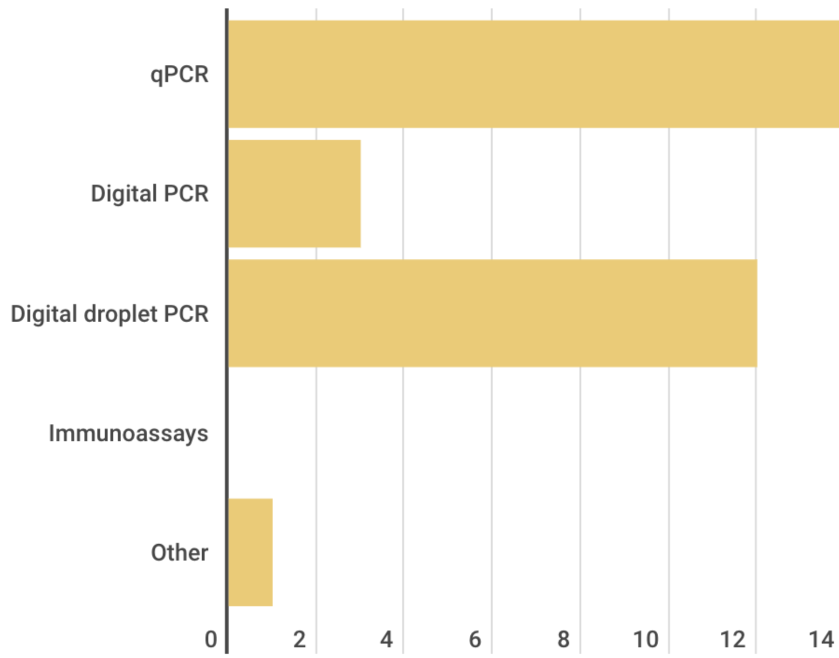

Fig. 4. Poll Response: "For SARS-CoV-2 quantitation, what is the preferred, state-of-the-art analytical technology?"

In addition to detection and/or quantification of biological targets such as SARS-CoV-2, another area of interest in WWS is variant tracking. Specifically, respondents were asked to rank the state-of-the-art analytical methods from most to least impactful. Results suggest that targeted amplicon sequencing is the preferred technology for variant tracking. Amplicon sequencing can be performed using different approaches. For instance, commercial SARS-CoV-2 tiled amplicon sequencing kits are available for variant tracking. Traditional amplicon sequencing methods can also be designed and employed using either long-read or short-read next generation sequencing (NGS) instruments.

WWS has gained popularity during the COVID-19 pandemic; however, the focus of this workshop was building an enduring capability. To this end, respondents were asked to select analytes/targets that are most critical for WWS (Figure 5). The top answer was SARS-CoV-2 followed closely by "other known viral pathogens (e.g., Hepatitis, HIV, etc.)." This response suggests that a panel of PCR-based assays that target the top viral pathogens might be an appropriate starting place for the design of an analyte reference material.

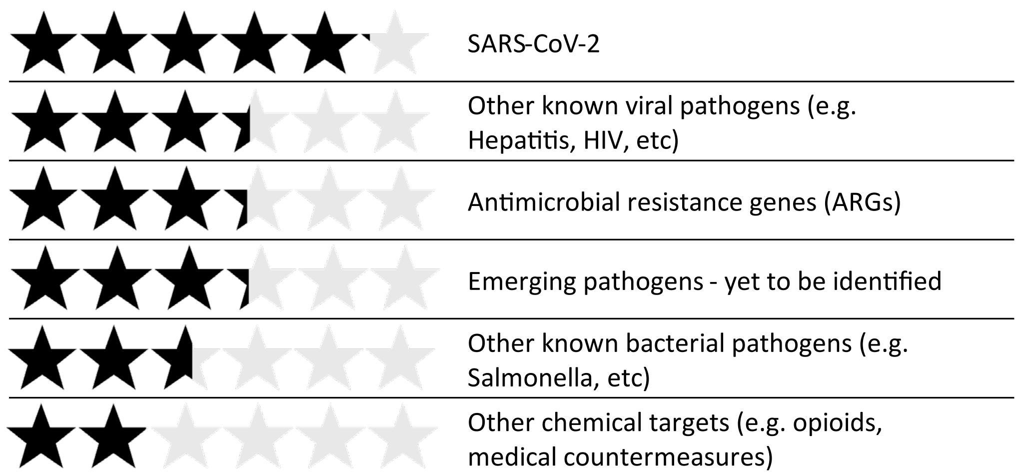

Fig. 5. Poll Response: "Rank analytes/targets associated with wastewater surveillance most critical to least critical."

In addition to gathering feedback on methods, this poll also focused on data comparability. A key component for data comparability identified during the workshop was a marker for normalization. Assays designed to detect and quantitate a specific pathogen (e.g., SARS-CoV-2) found in human fecal material will need to be normalized against the fecal load present in the wastewater. Normalization may be important to help improved comparability of data, given human fecal material represents a relatively small fraction of the contents of wastewater. Moreover, the relative fraction of human fecal material in wastewater can vary dramatically based on many factors including weather (rainfall), community demographics, and population. For example, a statement such as "the genome copy number of SARS-CoV-2 in wastewater is 10^3 copies per liter" may be much less informative than "the genome copy number of SARS-CoV-2 in wastewater is 10^3 copies per mg of human fecal material." In response to the question, "What normalization targets should be prioritized?" the top selection (35/39 respondents) was fecal indicator viruses (Figure 6).

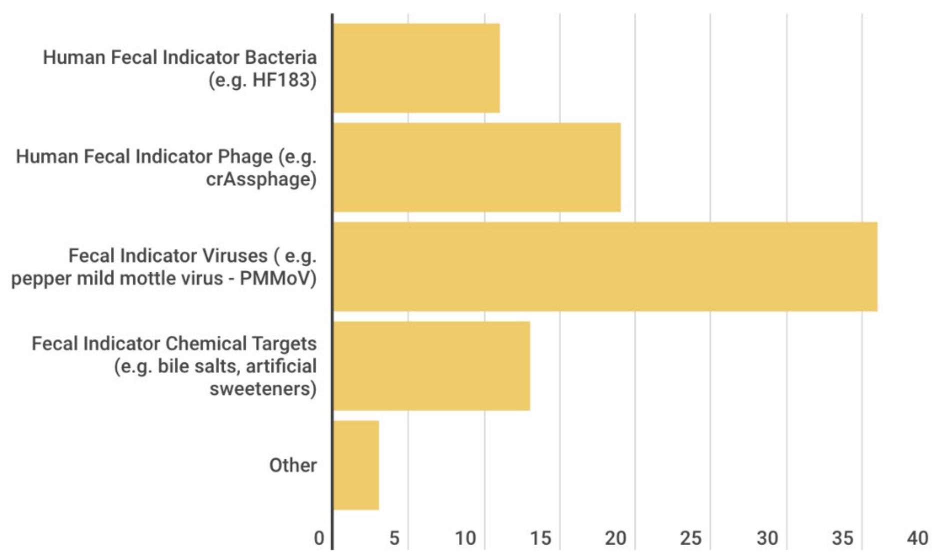

Fig. 6. Poll Response: "What normalization targets should be prioritized?"

PMMoV was listed as a specific example in the poll question. This plant virus is widespread and globally distributed. Healthy individuals can excrete up to 10^9 gene copies per gram of feces[29]. Fecal indicator phage (e.g., crAssphage) were the second highest response followed by chemical targets (e.g., bile salts or artificial sweeteners) and fecal indicator bacteria (e.g., HF183). One respondent noted, "This depends on target of interest, SARS-CoV2 or other virus, we need viral norm target. Bacteria target should have bacterial norm target."

The importance of a normalization control was highlighted by the response to the open text question: "Please provide suggestions on a universal data output for comparing data from different regions." Of the 16 recorded responses, 9 responses included the use of normalization or reference to a marker. Additional common responses included reporting gene copy per liter, and the need to include metadata and sample processing information. With respect to the type of information being reported, one respondent recommended the development of guidelines similar to the MIQE (Minimum Information for Publication of Quantitative Real-Time PCR Experiments) guidelines for qPCR.

This workshop was meant as a starting point for the development of WWS as an enduring capability with the goal of continuing these conversations through the most appropriate mechanism. Forty percent of the 39 respondents identified cooperative efforts, agreements, and/or partnerships as their favorite forum(s) or mechanism(s), with working groups and future workshops each receiving approximately one quarter of the votes. Overall, respondents prefer interactive discussions and communication for continuation of work to develop methods and improve data comparability for WWS.

3.3.2. SWWS 303: Reference Materials

Feedback from Days 1 and 2 of the SWWS workshop identified several areas where reference materials (RMs) may serve as initial standards to help build an enduring capability for the WWS community and underpin the necessary "building for the next pandemic." This includes (but is not limited to):

- synthetic wastewater materials (designed for longevity, sustainability)
- wastewater materials that are representative of each step
- standards composed of mimics for biological analytes/targets
- standards composed of distinct normalization factors (e.g., chemicals or biomarkers from human or fecal input, in contrast to other wastewater chemical or biological components)
- positive (+) standards as well as negative (-) standards

Furthermore, the concept of building wastewater or equivalent sample "banks" or archives could be an approach towards standardization. Subsequent guidance on the appropriate retrospective analysis of these banks would also be necessary.

The objective for the Reference Materials Poll was to build upon the initial feedback from Day 1 and Day 2 of the workshop and further capture input from workshop attendees. Specifically, the objectives were to: 1) identify appropriate RMs that close critical measurement and data gaps for WWS, 2) identify key characteristics of RMs to be used for most effectively for WWS, and 3) devise next steps for the WWS community and stakeholders to provide essential input towards the development of future RMs.

RMs can be generated in a variety of forms and be designed to provide value and property assignment for a wide range of chemical and physical characteristics. By definition, RMs are homogeneous and stable materials that are well-characterized for one or more chemical and/or physical properties with clear intended purposes for their usage. Matrix-based RMs are typically prepared from natural sources (e.g., biological fluids, environmental materials) and are used for method or process suitability as well as QA/QC, method validation, or accuracy controls when the properties are certified. Calibration RMs are reference standards most often provided in a neat (or pure) form or as a mixture of components in a solution form that are primarily used for providing International System of Units (SI) traceability, calibration of instruments and devices, or in the identification of the components within a test sample.

A total of 24 poll responses were received. The full poll results are available in Appendix G. Highlights are provided here. Demographics of the poll respondents indicated their association with the stakeholder groups shown in Figure 7.

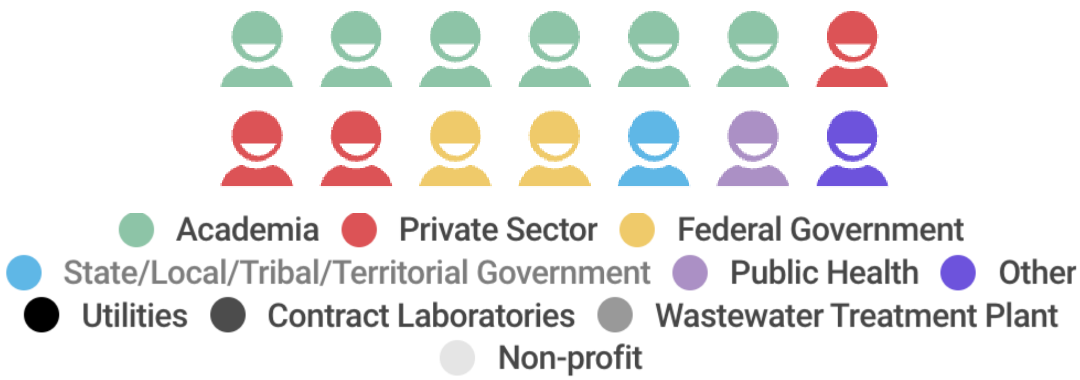

Fig. 7. Respondent Demographics for the Reference Materials Poll

Respondents were also asked about their experience in WWS. A majority (79 %) of respondents indicated involvement with testing methods, followed by a significant percentage involved with data reporting and analytics (50 %) and data implementation and decision making (36 %). A smaller portion (29 %) were focused on the sampling process, whereas 14 % of respondents were not currently active in WWS but were in attendance to learn more about the need for standards. Almost half of the respondents' test wastewater on a weekly basis (45 %), whereas a smaller percentage test daily (27 %), only as needed (18 %), or on a monthly basis (9 %).

A large majority of respondents (86 %) indicated that QC materials were included in every run of their process, and 64 % reported that reference materials were used in WWS applications, whereas the remainder indicated that either reference materials were not used (21 %) or were not applicable to their process (14 %). Among the respondents that are using QC materials, the top reported target was by far SARS-CoV-2, followed by fecal indicators (such as PMMoV) and to a lesser extent illicit drugs and/or pharmaceuticals. Nearly half of the respondents indicated that within WWS applications, the analyte measurement step has the greatest need for a RM (46 %). This is followed by sample concentration (31 %), data interpretation (15 %) and sample collection (8 %).

For SARS-CoV-2 wastewater surveillance applications, matrix-based RMs are rank ordered based on usefulness in Figure 8, and calibration type RMs are rank ordered in Figure 9.

Fig. 8. Poll Response: “For SARS-CoV-2 wastewater surveillance applications, please rank matrix-based RMs from most to least useful for future development.”

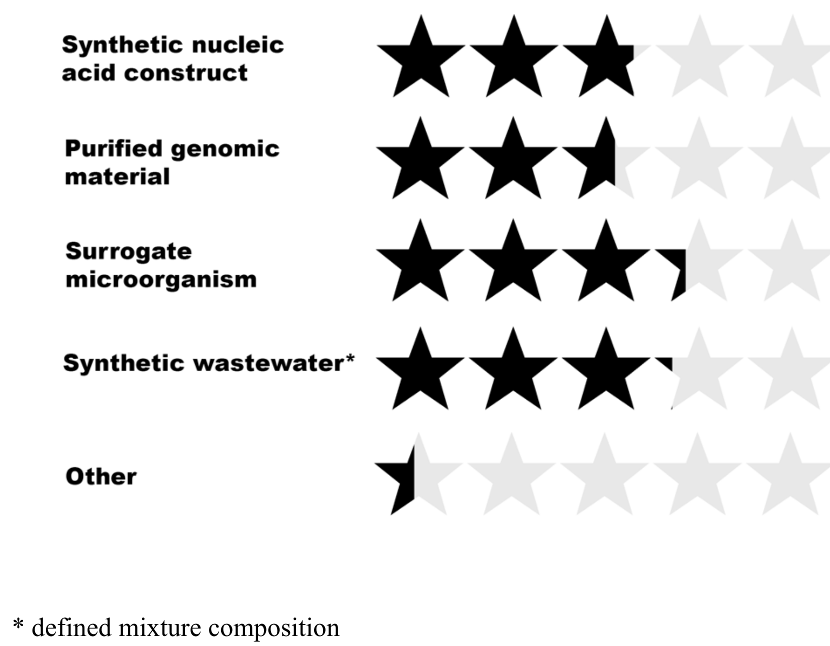

Fig. 9. Poll Response: “For SARS-CoV-2 wastewater surveillance applications, please rank calibration RMs from most to least useful for future development.”

Just over half of respondents indicated they are normalizing to a human fecal waste-associated target (such as PMMoV, HF183, crAssphage or other biochemicals) for their measurements in sewage. The other half indicated that they were not normalizing results (25 %) or that it was not applicable to their current process (20 %). Of those normalizing SARS-CoV-2 measurements, a large majority (79 %) were employing PMMoV, followed by HF183 (14 %), crAssphage (7 %) and bile salts (7 %) or other biochemicals (e.g., bilirubin, creatinine) (7 %). It was also noted that the use of biomarkers or other chemicals to normalize measurements would not be anticipated to increase confidence in the results, as both their stability and inherent levels in wastewater may be uncertain.

The currently employed surveillance technology platforms used to characterize SARS-CoV-2 in wastewater include both RT-dPCR/dd-PCR and RT-qPCR (47 % and 63 %, respectively), in addition to NGS techniques (42 %).

The respondents ranked potential components of a monitoring program in their ability to increase confidence in an enduring sewage surveillance capability (Figure 10). Poll respondents also ranked the anticipated barriers for the development and implementation of RMs as shown in Figure 11.

Fig. 10. Poll Response: “Rank the following from most to least important towards establishing confidence in a sewage surveillance monitoring program.”

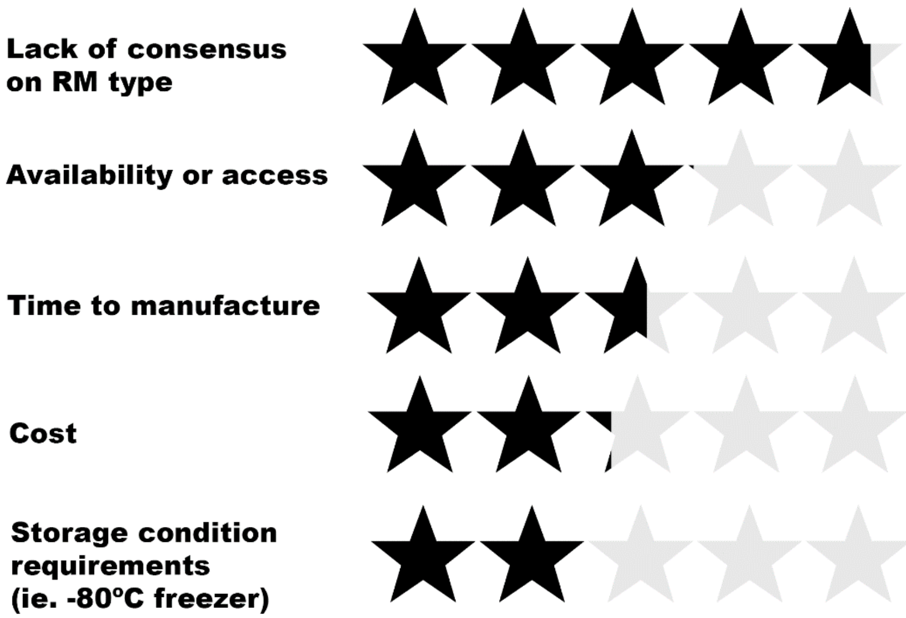

Fig. 11. Poll Response: "Rank the following potential barriers (from most to least impactful) for development and implementation of a RM."

Lastly, the best forum or mechanism to ensure continued stakeholder feedback in the development of future RMs was identified as cooperative efforts, agreements and partnerships between RM producers and stakeholders (48 %), followed by stakeholder working groups (38 %) and future workshops (14 %). Seminars, workshops and working groups associated with international conferences (such as the International Water Association, Water Environment Federation) may also be impactful.

3.3.3. SWWS 304: Documentary Standards/Guidance Documents

Documentary standards provide another arm of standards that can help support WWS. Documentary standards cover a range of document types ranging from highly prescriptive methods and performance requirements to general guidance documents. In fields such as WWS where the methods and technologies are still evolving and rapidly advancing, guidance based standard documents may represent an ideal starting point for documentary standards efforts.

The objectives for the Documentary Standards Poll were to: 1) identify steps in the WWS process in greatest need of standard/guidance documents, 2) identify and prioritize critical gaps that could be addressed by a standard or guidance document to support an enduring capability for WWS, and 3) identify volunteers and partnerships to help develop future guidance documents.

A total of 20 responses were collected, with not every respondent answering all questions. The full poll results are available in Appendix H. Highlights are provided here.

Fig. 12. Respondent demographics for the Documentary Standards/Guidance Documents Poll

Figure 12 shows the stakeholder groups represented by poll respondents. Eight out of 15 respondents currently use documentary standards and/or guidance documents in their WWS, including USGS and NWSS requirements as well as other guidelines. Respondents indicated that organizations including CDC-China, USGS, CoSeS (Communicating Sewage Surveillance), National Science Foundation Research Coordination Networks (NSF RCN), CDC NWSS, University of California San Diego (UCSD), and organizations in Spain either have available guidance or are developing guidance to support WWS.

The respondents ranked the four main steps of WWS in terms of their need for documentary standards/guidance documents (Figure 13). Laboratory methods was the top need, followed closely by both sampling and data, and then coordination/implementation/use of data.

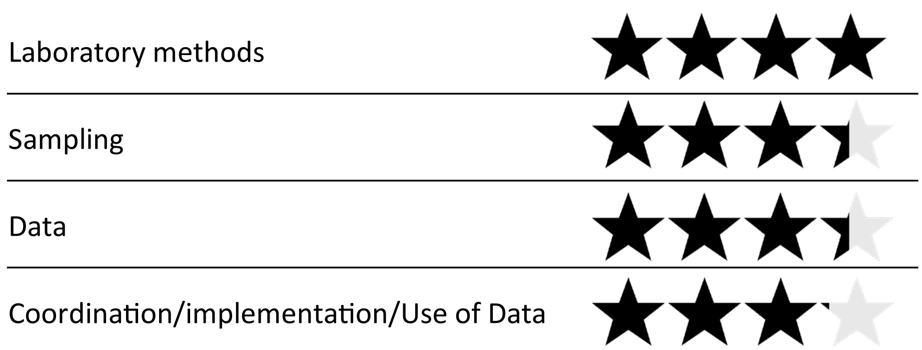

Fig. 13. Poll Response: "How would you prioritize these areas, in terms of their need for documentary standards/guidance documents?"

Areas in need of documentary standards were further expanded (Figure 14), with respondents selecting one area that would benefit most from documentary standards. The top response was methods, followed by use of data, and a tie between sampling and overall program implementation. Note that three options received no votes: documenting the justification and purpose for a WWS program, rationalization for and identification of sampling sites, and confirming the process to access, store, and report data. Reasons for prioritizing the areas with the most votes are provided in Appendix H. Examples of standards that could meet these needs, as provided by respondents, included guidance for the entire process, guidance for sampling plan and sample collection, guidance on controls, test methods, data reporting templates and requirements, and data analysis methods.

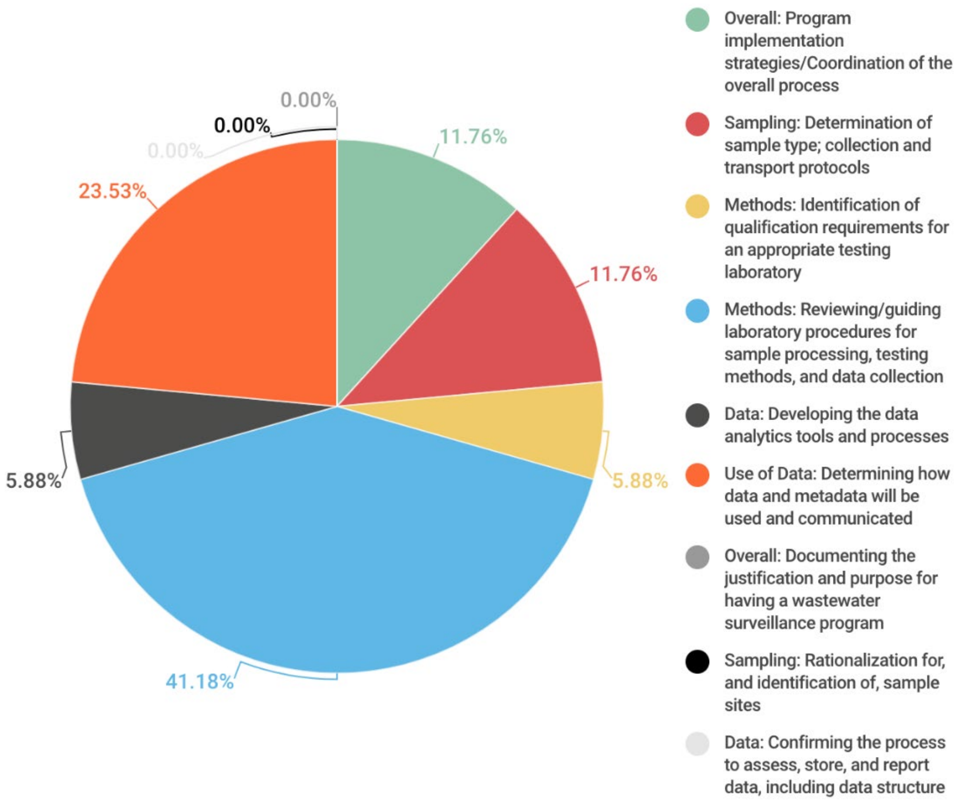

Fig. 14. Poll Question: "More specifically, what step in the wastewater surveillance process could benefit MOST from documentary standards?"

The poll also asked respondents to provide their thoughts on the documentary standards/guidance documents that would have the greatest impact within each of the four main steps of the WWS workflow. Responses included the following:

- Sample collection
    - Big picture and specific data quality objectives for various scenarios
    - Type of appropriate sample
    - Guidance on sample collection, storage, shipment, and transport
    - Use of solids
    - Where and how to sample
    - Proportional sampling best practices and sample storage
- Testing methods
    - Inhibition controls
    - SOP
    - Controls
    - Extraction methods
    - QA/QC of PCR
    - If/how to use % recovery data from a matrix spike
    - Quantification standards
    - Fecal indicator standards

- List of acceptable methods
- ddPCR

- Data reporting and analytics
  - Standard template for reporting results
  - Data standards
  - Metadata requirements, including quality assurance samples
  - Limit of detection reporting
- Implementation/Use of Data
  - Data analyses and reporting
  - Trends
  - National database/dashboard
  - Implementation

Respondents were then asked to rank factors that might hinder the development of documentary standards/guidance documents. Figure 15 lists those factors from those with most likely to least likely to hinder progress. All factors were ranked similarly, suggesting there is no one factor that stands out as a major hindrance to be addressed first.

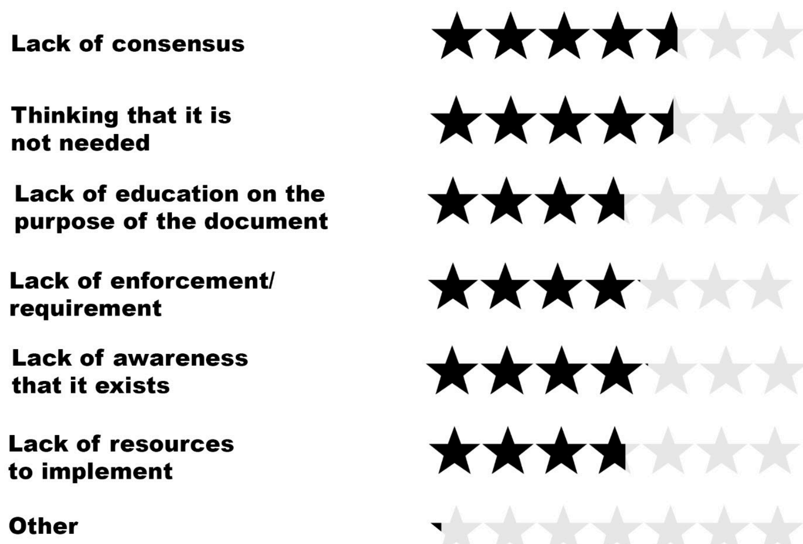

Fig. 15. Poll Question: "What might potentially hinder the development and use/ implementation of a documentary standard or guidance document related to wastewater surveillance?"

Going forward, respondents indicated that a stakeholder working group or cooperative effort/partnership would be preferred mechanism to obtain stakeholder feedback for development of documentary standards/guidance documents.

3.4. Day 3 Summary

In summary, Day 3 of the SWWS workshop was focused on building an enduring WWS capability. In observance of Juneteenth as a national holiday, this portion of the workshop was held as an asynchronous session that included presentations from subject matter experts and focused feedback polls for participants. Dr. Kimberli Miller, Dr. Jayne Morrow, Dr. Orin Shanks, and Ms. Patsy Root provided four different perspectives on elements of successful surveillance programs in the fields of wildlife disease surveillance, biological response preparedness, recreational water quality testing, and laboratory accreditation, respectively. While each presenter offered examples of tools and strategies that could be incorporated in building an enduring WWS capability, some recurring themes for success included the importance of interlaboratory testing and collaboration between multiple stakeholder groups and/or agencies. In addition to the examples from related fields, participants also had access to presentations of different perspectives for a successful WWS program. These presentations were given by representatives from federal and state/local governments and highlighted the needs at both levels for an enduring program. Support for this effort, including funding and personnel, was highlighted as a primary need by federal and local representatives. Additionally, the need for increased confidence in data generated by WWS, such as through improved sensitivity, reliability, and reproducibility of the tests, was also highlighted. The asynchronous Day 3 session also included feedback from participants via focused feedback polls in three areas: Methods and Data Comparability, Reference Materials, and Documentary Standards/Guidance Documents. The results from these polls were summarized above, and the complete poll results can be found in Appendices F-H. These poll results provided valuable insight from the stakeholder community on the challenges and potential paths forward for WWS. Notably, from the focus feedback polls, PCR based methods were identified as the preferred state of the art technology for WWS, suggesting standards geared towards this technology would be most beneficial at this point. Additionally, proficiency testing/quality assurance programs was identified as a primary way to increase confidence in the WWS data. The area most in need of documentary standards was identified as methods, including sample processing and analysis. Moving forward, the formation of cooperative efforts, agreements and partnerships was identified as the preferred mechanism for future engagement in the areas of reference material development and standards for method and data comparability. The development of stakeholder working groups was identified as the preferred mechanism for documentary standards. Together these responses provide valuable insight into standards that will be the most beneficial to support an enduring WWS capability.

4. Poster Session Summary

Thirty-eight posters (abstracts in Appendix D) were displayed using a virtual poster session platform. Posters were available for view throughout the week, and presenters had the option of hosting video chat session(s) with hours of their choice or communicating via a discussion board. A majority of posters focused on processing/testing methods (19); however, other topics included: data reporting and analytics (5), interpretation and use of data (5), sampling (3), and standards/reference materials (5). When considering the poster collection, some recurring focus areas emerged and are summarized here. While the main application was SARS-CoV-2 surveillance, there were also several posters discussing research that is more broadly applicable or focused on other targets, lending support to the workshop theme of WWS as an enduring capability beyond the current pandemic.

Within processing/testing methods, two main areas highlighted by the posters were method development and method evaluation. Several posters covered strategies for sampling and testing protocols, new technologies for capturing/concentrating targets, and development of target analyses including qPCR methods, NGS, and mass spectrometry. With respect to method evaluation, several groups presented comparison studies between commonly used technologies at various steps in the WWS workflow. Several of these studies pointed out instances where reference materials or standard practices may be helpful to support WWS.

Some posters highlighted case studies for ongoing SARS-CoV-2 surveillance from several areas at local, city, and state levels. A subset of these posters focused specifically on communication and data use strategies that were implemented to most effectively use the data to inform analyses to support the public health response. In addition, a handful of posters specifically focused on reference materials development to support WWS as well as other biosurveillance efforts.

Overall, the posters covered a broad range of topics and expanded the scope of the research presented at the workshop beyond what was possible through oral presentations over a 3-day workshop.

5. Summary of Findings

Wastewater surveillance has been the subject of much interest after reaching new levels of recognition during the COVID-19 pandemic, where it emerged as an effective tool for early detection of SARS-CoV-2 outbreaks. WWS offers several benefits as a tool in the public health toolbox. It is predictive – serving as a leading indicator for early intervention and resource allocation, it is inclusive – providing information on a population level independent of healthcare seeking behaviors or access, and it is versatile with the potential to monitor for a diverse array of potential targets. While the concepts of WWS and WBE are not new, the potential impact of these capabilities has been affirmed during the COVID-19 pandemic; however, much work remains to transition the current capabilities into an enduring capability for reproducible, comparable WWS equipped to address new or emerging targets. Through this workshop we gathered stakeholders from various communities including government, research labs, academia, and utilities to identify technology and standards gaps and outline potential standards-based steps towards developing an enduring capability.

The outcome of the three-day workshop had an emphasis on harmonization across all steps in the workflow. Additionally, throughout the sessions and focused feedback polling, multiple avenues for standards development were identified. These can be broken down into three categories of standards: reference materials, methods and data, and documents and guidance. Proposed standards development activities within each of these categories have been summarized in Figure 16. In addition, three themes emerged with respect to standards development for WWS: (1) Concordance without Constraints, (2) Nationally Consistent, and (3) Building for the Next Pandemic.

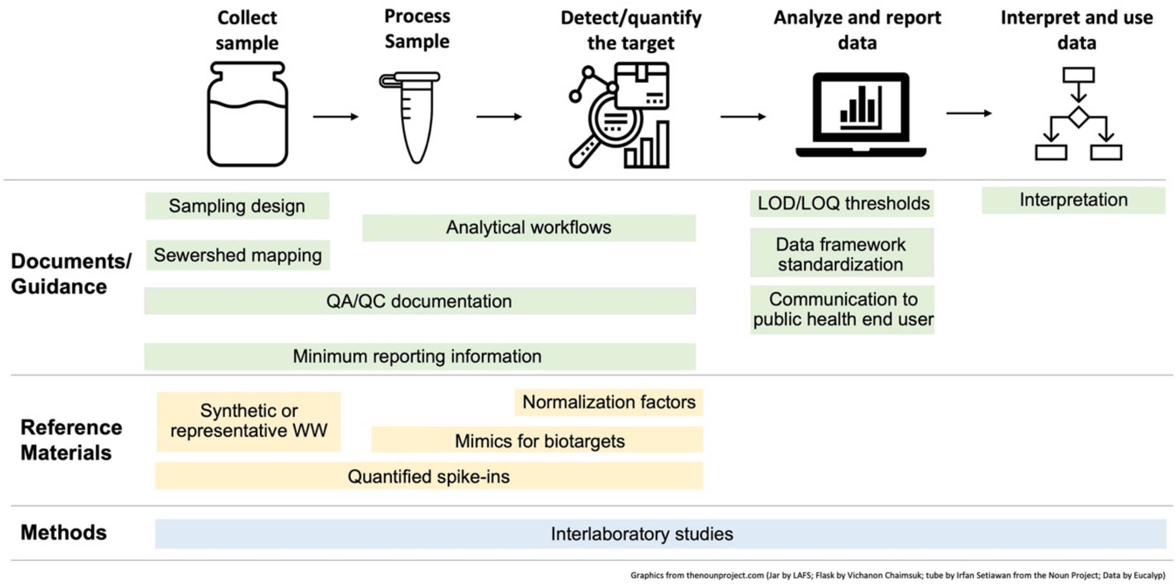

Fig. 16. Recommendations for Standards to Support WWS as an Enduring Capability.

(Wastewater, WW; Limit of detection, LOD; Limit of quantification, LOQ)

5.1. Concordance without Constraints

For all stages of the WWS workflow (from sampling to use of data) it was generally agreed that selecting a single standardized method was not the best option. First, multiple methods have been shown to be successful, and second WWS examines a complex network that will vary based on site-specific factors and limitations. Taken together, a one-size-fits-all approach is not feasible. The need expressed by the community was for standards that can enable lab to lab comparability. Different types or levels of standardization were identified based upon the stage in the process. For wastewater sampling, there are already well-established protocols for samples collected for regulatory purposes that are accessible and include QA/QC measures; therefore, the primary need identified was for guidance documents on how to identify the appropriate protocol. This selection guidance document should account for variables such as sewershed design, population served and site-specific limitations. To enable lab-to-lab comparability, the development of various calibrant materials (matrix spike-ins, extractions controls, and PCR controls) was proposed. While the exact form of these materials was not determined, the necessity of such materials for inter-lab studies to assess method comparability was highlighted by Dr. Kate Griffiths and Dr. Kimberli Miller. Standard samples allow for the comparison of different assays performed under different conditions and the results can then be used to develop guidelines to improve data reporting (e.g., setting thresholds). Finally, while a single national platform for data reporting would be the best option, it was agreed that independent dashboards don’t need to be standardized but the data should be interoperable. Data analysis and reporting would benefit from the development of a data framework.

5.2. Nationally-Consistent

The focus of this workshop was on the advancement of WWS towards an enduring capability. From the work that has been carried out during the COVID-19 pandemic and examples of other national surveillance initiatives, achieving national consistency was identified as an important goal for building the WWS capability. To this end, the need for guidance documents referenced above was reiterated. A major gap identified at the national level was in the area of data analysis and interpretation. The question of what units to report, relative versus absolute data reporting, and trend analysis are all areas for continued discussion and assessment for a path forward toward comparability and reproducibility. A dedicated effort should be given to providing guidance on understanding the data generated by WWS and how these data should be conveyed to the public health end-user. This should include not only the lab-generated data, but also integration of sewershed, population, and hospital data to understand demographics being impacted. To this end, improved sewershed mapping is required. Another critical area to be addressed at the national level was a coordinated program to provide resources including sustainable funding mechanisms and technical support. Ongoing efforts are already addressing some of these issues, but there is still much work to be done to enable and promote national consistency.

5.3. Building for the Next Pandemic

With an eye towards and enduring capability, developing standards that will support versatility and preparedness for the next pandemic was also highlighted. Many of the proposed standards specific to this theme can be categorized as reference materials. Reference materials come in a variety of forms and the development of both matrix RMs and calibrant RMs were proposed. Matrix RMs such as synthetic wastewater and wastewater that is representative of different steps in the process were both proposed. Calibrant RMs proposals included mimics of biotargets and normalization factors (human or fecal indicators versus other wastewater components). It was also observed that negative control standards were as important as the positive controls for evaluating processes. An additional proposal was for the development of a sample bank or archive.

5.4. Concluding Remarks

In conclusion, the workshop highlighted several gaps and provided the foundation for a path forward in building an enduring WWS capability. The initial focus should be on standards that support harmonization efforts including guidance documents for sampling and data interpretation and calibrant RMs for method comparability. Continued engagement with the stakeholder community will be crucial for success of this program. From the focus feedback pools, engagements through stakeholder working groups and cooperative efforts (agreements and partnerships) were identified as the best forum to continue WWS standards development.

5.5. Update following the workshop

The need for standards for WWS has received some additional recognition. In the time between the workshop and the publication of this report, the U.S. Government Accountability Office (GAO) published a Science and Tech Spotlight on wastewater surveillance [30]. In that publication, GAO recognized the immense potential of WWS for both biological and chemical targets and highlighted four major challenges. Echoing the findings from this workshop, lack of national coordination and standardization, specifically with respect to sample collection, data analysis, and data sharing was identified as one of the key challenges to widespread adoption of WWS. The GAO spotlight, published in April 2022 aligns with SWWS workshop findings and hopefully will help to pave a path toward standards development efforts that enable realization of the potential of WWS for improved public health.

References

[1] Water Research Australia (2021) *ColoSSoS - Collaboration on Sewage Surveillance of SARS-CoV-2*. Available at https://www.waterra.com.au/project-details/264.

[2] Vatovec C, Phillips P, Van Wagoner E, Scott TM, Furlong E (2016) Investigating dynamic sources of pharmaceuticals: Demographic and seasonal use are more important than down-the-drain disposal in wastewater effluent in a University City setting. *Sci Total Environ* 572:906-914. https://doi.org/10.1016/j.scitotenv.2016.07.199

[3] Univeristy of Louisville Christine Lee Brown Envirome Institute (2021) *The Co-Immunity Project*. Available at https://louisville.edu/envirome/thecoimmunityproject/covidstudy.

[4] Bivins A, Lott M, Shaffer M, Wu Z, North D, Lipp EK, Bibby K (2022) Building-level wastewater surveillance using tampon swabs and RT-LAMP for rapid SARS-CoV-2 RNA detection. *Environmental Science: Water Research & Technology* 8(1):173-183. https://doi.org/10.1039/D1EW00496D

[5] Pecson BM, Darby E, Haas CN, Amha YM, Bartolo M, Danielson R, Dearborn Y, Di Giovanni G, Ferguson C, Fevig S, Gaddis E, Gray D, Lukasik G, Mull B, Olivas L, Olivieri A, Qu Y, Consortium SA-C-I (2021) Reproducibility and sensitivity of 36 methods to quantify the SARS-CoV-2 genetic signal in raw wastewater: findings from an interlaboratory methods evaluation in the U.S. *Environmental Science: Water Research & Technology* 7(3):504-520. https://doi.org/10.1039/D0EW00946F

[6] The Water Research Foundation (2021) *COVID-19 Wastewater Surveillance Symposium – A Global Update*. Available at https://www.waterrf.org/resource/covid-19-wastewater-surveillance-symposium-global-update.

[7] Ohio Department of Health (2021) *COVID-19 Dashboard*. Available at https://coronavirus.ohio.gov/wps/portal/gov/covid-19/dashboards/other-resources/wastewater.

[8] Graham KE, Loeb SK, Wolfe MK, Catoe D, Sinnott-Armstrong N, Kim S, Yamahara KM, Sassoubre LM, Mendoza Grijalva LM, Roldan-Hernandez L, Langenfeld K, Wigginton KR, Boehm AB (2021) SARS-CoV-2 RNA in Wastewater Settled Solids Is Associated with COVID-19 Cases in a Large Urban Sewershed. *Environ Sci Technol* 55(1):488-498. https://doi.org/10.1021/acs.est.0c06191

[9] Wurtz N, Lacoste A, Jardot P, Delache A, Fontaine X, Verlande M, Annessi A, Giraud-Gatineau A, Chaudet H, Fournier P-E, Augier P, La Scola B (2021) Viral RNA in City Wastewater as a Key Indicator of COVID-19 Recrudescence and Containment Measures Effectiveness. *Frontiers in Microbiology* 12. https://doi.org/10.3389/fmicb.2021.664477

[10] Karthikeyan S, Ronquillo N, Belda-Ferre P, Alvarado D, Javidi T, Longhurst CA, Knight R, Cristea IM (2021) High-Throughput Wastewater SARS-CoV-2 Detection Enables

Forecasting of Community Infection Dynamics in San Diego County. *mSystems* 6(2):e00045-00021. https://doi.org/doi:10.1128/mSystems.00045-21
[11] Rasile B, Maas K (2021) SARS-CoV-2 Wastewater RNA Concentration and Extraction (Nanotrap® and NucleoMag® RNA Water). *Protocolsio*. https://doi.org/dx.doi.org/10.17504/protocols.io.bn58mg9w
[12] Mathematica (2021) *COVID-19 Curated Data, Modeling, and Policy Resources*. Available at https://www.mathematica.org/features/covid-19-curated-data-modeling-and-policy-resources.
[13] BiobotAnalytics (2021) *Nationwide Wastewater Monitoring Network*. Available at https://biobot.io/data/.
[14] COVID-19 WBE Collaborative (2021) *COVIDPoops19 Dashboard*. Available at https://www.covid19wbec.org/covidpoops19.
[15] DHS (2011) Framework for a Biothreat Field Response Mission Capability. Washington, D.C., Security USDoH.
[16] National Science and Technology Council (U.S.). Biological Response and Recovery Science and Technology Working Group (2013) *Biological response and recovery science and technology roadmap*. Washington, D.C.: Executive Office of the President, National Science and Technology Council.
[17] ASTM International (2017) *ASTM E2270-17– Standard Guide for Operational Guidelines for Initial Response to Suspected Biological Agents and Toxins*. https://doi.org/10.1520/E2770-17
[18] ASTM International (2017) *ASTM E2458-17 – Standard Practices for Bulk Sample Collection and Swab Sample Collection of Visible Powders Suspected of Being Biological Agents and Toxins from Nonporous Surfaces*. https://doi.org/10.1520/E2458-17
[19] Green HC, Dick LK, Gilpin B, Samadpour M, Field KG (2012) Genetic Markers for Rapid PCR-Based Identification of Gull, Canada Goose, Duck, and Chicken Fecal Contamination in Water. *Appl Environ Microb* 78(2):503-510. https://doi.org/10.1128/Aem.05734-11
[20] Green HC, Haugland RA, Varma M, Millen HT, Borchardt MA, Field KG, Walters WA, Knight R, Sivaganesan M, Kelty CA, Shanks OC (2014) Improved HF183 Quantitative Real-Time PCR Assay for Characterization of Human Fecal Pollution in Ambient Surface Water Samples. *Appl Environ Microb* 80(10):3086-3094. https://doi.org/10.1128/Aem.04137-13
[21] Shanks OC, Kelty CA, Sivaganesan M, Varma M, Haugland RA (2009) Quantitative PCR for Genetic Markers of Human Fecal Pollution. *Appl Environ Microb* 75(17):5507-5513. https://doi.org/10.1128/Aem.00305-09
[22] Stachler E, Kelty C, Sivaganesan M, Li X, Bibby K, Shanks OC (2017) Quantitative CrAssphage PCR Assays for Human Fecal Pollution Measurement. *Environmental Science & Technology* 51(16):9146-9154. https://doi.org/10.1021/acs.est.7b02703
[23] Mieszkin S, Yala JF, Joubrel R, Gourmelon M (2010) Phylogenetic analysis of Bacteroidales 16S rRNA gene sequences from human and animal effluents and assessment of ruminant faecal pollution by real-time PCR. *J Appl Microbiol* 108(3):974-984. https://doi.org/10.1111/j.1365-2672.2009.04499.x
[24] Mieszkin S, Furet JP, Corthier G, Gourmelon M (2009) Estimation of Pig Fecal Contamination in a River Catchment by Real-Time PCR Using Two Pig-Specific

Bacteroidales 16S rRNA Genetic Markers. *Appl Environ Microb* 75(10):3045-3054. https://doi.org/10.1128/Aem.02343-08

[25] Chern EC, Siefring S, Paar J, Doolittle M, Haugland RA (2011) Comparison of quantitative PCR assays for Escherichia coli targeting ribosomal RNA and single copy genes. *Lett Appl Microbiol* 52(3):298-306. https://doi.org/10.1111/j.1472-765X.2010.03001.x

[26] Siefring S, Varma M, Atikovic E, Wymer L, Haugland RA (2008) Improved real-time PCR assays for the detection of fecal indicator bacteria in surface waters with different instrument and reagent systems. *J Water Health* 6(2):225-237. https://doi.org/10.2166/wh.2008.022

[27] Kralj J, Servetas SL, Hunter M, Toman B, Jackson S (2021) *Certification of Standard Reference Material® 2917: Plasmid DNA for Fecal Indicator Detection and Identification*. Special Publication (NIST SP) - 260-221, National Institute of Standards and Technology, Gaithersburg, MD. https://doi.org/10.6028/NIST.SP.260-221

[28] Office of the Assistant Secretary for Preparedness and Response (2019) *2019 Biodefense Public Report Implementation of the National Biodefense Strategy*, Services USDoHaH.

[29] Symonds EM, Rosario K, Breitbart M (2019) Pepper mild mottle virus: Agricultural menace turned effective tool for microbial water quality monitoring and assessing (waste)water treatment technologies. *PLoS Pathog* 15(4):e1007639. https://doi.org/10.1371/journal.ppat.1007639

[30] USGAO (2022) *Science & Tech Spotlight: Wastewater Surveillance* (U.S. Government Accountability Office), U.S. Government Accountability Office.

List of Appendices

Appendix A
DHS/NIST Workshop: Standards to Support an Enduring Capability in Wastewater Surveillance for Public Health

AGENDA

Updated 6/15/2021

Day 1: Monday, June 14, 2021 (all times EDT)

10:00 – 10:50 AM	**WELCOME & INTRODUCTORY REMARKS**
	Welcome – Mr. Philip Mattson, DHS Standards Executive and Director, Office of Standards at US Department of Homeland Security
	Welcome Remarks – U.S. Senator Gary Peters (MI), Chair of the Homeland Security & Governmental Affairs Committee
	Welcome Remarks – Robert B. Newman, Jr., Office of Strategy and Policy at the Department of Homeland Security, Science and Technology Directorate
	Welcome Remarks – Dr. Eric K. Lin, Acting Associate Director for Laboratory Programs, NIST
	Workshop Logistics; Overview and Goals of the 2021 SWWS Workshop – Dr. Nancy Lin, Leader of the Biomaterials Group, Material Measurement Laboratory at the National Institute of Standards and Technology
	Welcome Remarks – Mr. Joseph Hamel, Director, ASPR Program Office for Innovation and Industrial Base Expansion (IBx) for the Department of Health and Human Services (HHS) Office of the Assistant Secretary for Preparedness and Response (ASPR)

10:50 AM – 12:35 PM	**STANDARDS FOR WASTEWATER SURVEILLANCE (SWWS) 101: INTRODUCTION TO WASTEWATER SURVEILLANCE (WWS)** **Chair:** Dr. Michael Focazio, Environmental Health Research Program Coordinator at the U.S. Geological Survey
10:50 – 10:55 AM	**Opening Remarks** – Dr. Michael Focazio, USGS
10:55 – 11:35 AM	**KEYNOTE PRESENTATION** – **National Wastewater Surveillance System: Implementation for COVID and Beyond,** Dr. Amy E. Kirby, National Wastewater Surveillance System Program Lead at the Centers for Disease Control and Prevention
11:30 – 11:55 AM	**Australia's ColoSSoS Project: Inter-laboratory Study for SARS-CoV-2 in Wastewater**, Dr. Kate R. Griffiths, Senior Molecular Biologist at the National Measurement Institute of Australia

11:55 AM – 12:15 PM	**Singapore's National Wastewater Based Surveillance Programme for COVID-19: Analytical Standards, Controls and Reference Materials,** Dr. Judith Wong, Director of the Microbiology and Molecular Epidemiology Division, Environment Health Institute, National Environment Agency, Singapore **Wastewater Based Epidemiology: Monitoring of SARS-CoV-2 and Related Markers in Singapore,** Dr. Shane Snyder, Professor of Civil & Environmental Engineering and Executive Director of the Nanyang Environment & Water Research Institute (NEWRI) at Nanyang Technological University (NTU), Singapore
12:15 – 12:35 PM	**Wastewater Infrastructure & Operations for an Enduring Public Health Partnership,** Dr. Beverley Stinson, Executive Vice President at AECOM

12:35 – 1:05 PM	**BREAK**

1:05 – 2:55 PM	**SWWS 201: SAMPLING** **Chair:** Ms. Renee Stevens, Program Manager, Department of Homeland Security
1:05 – 1:10 PM	**Opening Remarks,** Ms. Renee Stevens, DHS
1:10 – 1:25 PM	**Utilities Perspectives and Needs,** Mr. Claudio Ternieden, Senior Director of Government Affairs at Water Environment Federation
1:25 – 1:40 PM	**USGS Experience in Wastewater Sampling: 3 Issues to Guide Wastewater Surveillance for Public Health**, Mr. Patrick Phillips, Quality Assurance Specialist at US Geological Survey
1:40 – 1:55 PM	**Case Study** – **Wastewater monitoring for COVID-19 in Louisville**, Dr. Aruni Bhatnagar, Professor of Medicine at the University of Louisville
1:55 – 2:00 PM	**Rapid Fire** – **Wastewater sampling for WBE surveillance,** Mr. Kaushal Trivedi, Business Development Manager at Teledyne ISCO
2:00 – 2:05 PM	**Rapid Fire** – **Building-level Wastewater Monitoring for COVID-19 Using Tampon swabs and RT-LAMP for Rapid SARS-CoV-2 RNA Detection** – Dr. Aaron Bivins, Post Doctoral Research Fellow at University of Notre Dame
2:05 – 2:55 PM	**Panel Discussion: Need for Standards to Support Wastewater Sampling** **Moderator:** Ms. Sarah Wright, Environmental Laboratories Manager at the Association of Public Health Laboratories **Panelists:** ● Mr. John Birkner, Manager of Technical Services, Bergen County Utilities Authority ● Dr. Raul A. Gonzalez, Environmental Scientist at Hampton Roads Sanitation District ● Dr. Rochelle Holm, Associate Professor, Mzuzu University, Malawi ● Mr. Greg Kester, Director of Renewable Resource Programs at the California Association of Sanitation Agencies (CASA) ● Mr. Bruce Smith, Environmental Engineer at USEPA ● Mr. David Swain, Associate Vice President at AECOM

2:55 – 3:00 PM	**WRAP-UP: Closing Remarks for Day 1,** Mr. Philip Mattson, DHS; Dr. Nancy Lin, NIST

Day 2: Tuesday, June 15, 2021 (all times EDT)

10:00 – 10:05 AM	**WELCOME & LOGISTICS** – Mr. Philip Mattson, DHS; Dr. Nancy Lin, NIST

10:05 AM – 12:05 PM	**SWWS 202: TESTING METHODS** **Chair:** Ms. Sally C. Gutierrez, Senior Advisor for Water, United States Environmental Protection Agency
10:05 – 10:10 AM	**Opening Remarks,** Ms. Sally Gutierrez, USEPA
10:10 – 10:25 AM	**Global Update on Wastewater Surveillance for SARS-CoV-2,** Dr. Christobel M. Ferguson, Chief Innovation Officer at The Water Research Foundation
10:25 – 10:45 AM	**Case Study – Overview of the Ohio Wastewater Monitoring Network,** Dr. Nichole E. Brinkman, Biologist at USA Environmental Protection Agency and Ms. Rebecca Fugitt, Assistant Chief, Bureau of Environmental Health and Radiation Protection at Ohio Department of Health
10:45 – 10:55 AM	**Scalable, sensitive, representative, and comparable approach for high throughput SARS-CoV-2 RNA analysis in settled solids,** Dr. Alexandria Boehm, Professor of Civil and Environmental Engineering at Stanford University
10:55 – 11:00 AM	**Rapid Fire** – **Reducing Erosion: Using a rapid deployable testing solution to reduce time between sampling and action**, Dr. Jordan J. Schmidt, Director, Product Applications at LuminUltra Technologies
11:00 – 11:05 AM	**Rapid Fire** – **The Importance of Validation for Wastewater Surveillance**, Dr. Brian Swalla, Staff Scientist, IDEXX Laboratories, Inc.
11:05 – 11:10 AM	**Rapid Fire** – **SARS-CoV-2 Quantification in Wastewater by Droplet Digital PCR,** Ms. Carolyn Reifsnyder, Director, Global Product Marketing, Digital Biology Group at Bio-Rad Laboratories
11:10 – 11:15 AM	**Rapid Fire – Nanoplate Digital PCR for Wastewater Surveillance,** Dr. Michael Bussmann, Associate Director Global Product Management - dPCR, QIAGEN
11:15 – 11:20 AM	**Rapid Fire** – **SARS-CoV-2 Variant Profiling in Wastewater by Sequencing,** Dr. Ellen M. Beasley, CSO at Pangolin Health
11:20 – 11:25 AM	**Rapid Fire** – **BioFire Defense Overview,** Mr. Ryan Gregerson, Product Marketing Manager at BioFire Defense
11:25 – 11:30 AM	**Rapid Fire** – **Viral Concentration with Nanotrap Magnetic Virus Particles**, Dr. Roberto J. Barbero, Chief Business Officer at Ceres Nanosciences
11:30 – 12:05 PM	**Panel Discussion: Need for Standards to Support Testing Methods** **Moderator:** Dr. Jay Garland, Associate Director for Research at the US EPA Office of Research and Development

	Panelists: ● Dr. Kartik Chandran, Professor of Environmental Engineering, Columbia University ● Mr. Kahlil Lawless, Microbiology Segment Manager - Americas at Illumina ● Dr. Mia Mattioli, Environmental Engineer at the Centers for Disease Control and Prevention ● Dr. Gertjan Medema, Principal Microbiologist at KWR Water Research Institute ● Dr. Martin Shafer, Senior Scientist, University of Wisconsin-Madison, State Laboratory of Hygiene

12:05 – 12:30 PM	**BREAK**

12:30 – 2:15 PM	**SWWS 203: DATA REPORTING AND ANALYTICS** **Chair:** Mr. Paul Storella, Senior Vice President Water Business Line at AECOM
12:30 – 12:35 PM	**Opening Remarks,** Mr. Paul Storella, AECOM
12:35 – 12:50 PM	**Available Data Tools: National Wastewater Surveillance System,** Dr. Wiley Jennings, Health Scientist at the Centers for Disease Control and Prevention
12:50 – 1:10 PM	**Case Study – SARS-CoV-2 Wastewater Surveillance and Public Health Applications in Houston, TX,** Dr. Lauren Stadler, Assistant Professor, Environmental Engineering at Rice University AND Dr. Loren Hopkins, City of Houston Chief Environmental Science Officer, Chief of the Bureau of Community and Children's Environmental Health at the Houston Health Department, and a Professor in the Practice in the Department of Statistics at Rice University
1:10 – 1:25 PM	**Translating Wastewater Data for Policymaking,** Ms. Aparna Keshaviah, Senior Statistician at Mathematica
1:25 – 1:35 PM	**Biobot: Building early warning health analytics from data available in our sewers,** Dr. Mariana Matus, CEO and Cofounder at Biobot Analytics, Inc.
1:35 – 1:40 PM	**Rapid Fire – Global COVID-19 Wastewater Monitoring Efforts: Development of the COVIDPoops19 Dashboard,** Ms. Ana Grace Alvarado, Graduate Student, Department of Environmental Engineering, University of California Merced
1:40 – 2:15 PM	**Panel Discussion: Need for Standards to Support Data Reporting and Analytics** **Moderator:** Mr. Paul Storella, AECOM **Panelists:** ● Dr. Ellie Graeden, CEO at Talus Analytics ● Mr. Robert Greenberg, CEO, G&H International Services, Inc. ● Dr. Wiley Jennings, Health Scientist at the Centers for Disease Control and Prevention ● Mrs. Stacie Reckling, GIS Analyst, North Carolina Department of Health and Human Services, Division of Public Health

	• Dr. Rachel R. Spurbeck, Senior Genomics Research Scientist at Battelle Memorial Institute

2:15 – 2:55 PM	**SWWS 204: ROLE OF STANDARDS IN SUPPORTING THE USE OF WWS DATA,** **Chair:** Dr. Ted Smith, Associate Professor, University of Louisville School of Medicine
2:15 – 2:25 PM	**Communicating Sewage Surveillance Data for a Public Health Response,** Dr. Sandra McLellan, Professor at the University of Wisconsin School of Freshwater Sciences
2:25 – 2:55 PM	**Panel Discussion: Standards to Support Implementation of WWS Data for Public Health** **Moderator:** Dr. Ted Smith, University of Louisville School of Medicine **Panelists:** • Major Michael Dietrich, PhD, Bioenvironmental Engineering Flight Commander, USAF • Ms. Rosa Inchausti, Deputy City Manager, City of Tempe • Dr. Nathan LaCross, Wastewater Surveillance Program Manager at Utah Department of Health • Dr. Sandra McLellan, Professor at the University of Wisconsin School of Freshwater Sciences • Mrs. Halley Reeves, Vice President of Community Health Impact for Oklahoma University Medicine

2:55 – 3:00 PM	**WRAP-UP: Closing Remarks for Day 2,** Mr. Philip Mattson, DHS; Dr. Nancy Lin, NIST

3:00 – 4:00 PM	**VIRTUAL POSTER SESSION**

Day 3: Asynchronous (all materials available through at: https://www.nist.gov/news-events/events/dhsnist-workshop-standards-support-enduring-capability-wastewater-surveillance)

Recorded talk	**WELCOME & LOGISTICS,** Mr. Philip Mattson, DHS

SWWS 301: BUILDING AN ENDURING CAPABILITY

Examples from Related Fields	
Record Talk	**Example Surveillance Program: Making It Count - Keeping It Available: Wildlife Disease Situational Awareness,** Dr. Kimberli Miller, Wildlife Disease Specialist, US Geological Survey National Wildlife Health Center
Slides	**Example Tool: Documentary Standards for Biological Response,** Dr. Jayne B. Morrow, Assistant Vice President for Research and Economic Development at Montana State University
Slides	**Example Tool: Development and Performance of NIST SRM 2917 for Molecular Recreational Water Quality Testing**, Dr. Orin C. Shanks, Senior Scientist at the U.S. Environmental Protection Agency
Recorded Talk	**Example Tool: Accreditation: Draft Accreditation Checklist for Wastewater COVID PCR Testing,** Ms. Patsy Root, Regulatory Affairs Manager, IDEXX Water
Enduring Capability Perspectives	
Recorded Talk	**Enduring Capability: NWSS Perspective,** Dr. Amy E. Kirby, National Wastewater Surveillance System Program Lead at the Centers for Disease Control and Prevention
Recorded Talk	**Enduring Capability: One Health Perspective,** Dr. Tonya Nichols, Senior Science Advisor, US Environmental Protection Agency
Recorded Talk	**Enduring Capability: Public Health Decision Maker Perspective,** Mr. Jeffrey A. Wenzel, Chief of the Bureau of Environmental Epidemiology at the Missouri Department of Health and Senior Services
Recorded Talk	**Enduring Capability: A Local Health Department Perspective,** Dr. George A. Conway, Director at Deschutes County Health Services Department
Slides	**Enduring Capability: State Perspective,** Dr. Larry Madoff, Medical Director at the Bureau of Infectious Disease and Laboratory Sciences, Massachusetts Department of Public Health

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FOCUSED FEEDBACK POLLS	
Recorded talk	**Instructions for Focused Feedback Polls**, *Dr. Katrice Lippa, NIST*
slido.com #Methods	SWWS 302: Poll on Methods and Data Comparability
slido.com #Materials	SWWS 303: Poll on Reference Materials
slido.com #Documentary	SWWS 304: Poll on Documentary Standards-Guidance Documents

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NIST SP 1279
Appendix B: Workshop Program

DHS/NIST Workshop: Standards to Support an Enduring Capability in Wastewater Surveillance for Public Health *(SWWS Workshop)*

DAY 1: MONDAY, JUNE 14, 2021

Welcome & Introductory Remarks

Mr. Philip J. Mattson
Standards Executive
Department of Homeland Security (DHS), Science and Technology Directorate (S&T) | USA
Philip.mattson@hq.dhs.gov

Mr. Philip J. Mattson serves as the Department of Homeland Security (DHS) Standards Executive and Senior Standards Advisor in the DHS Science and Technology Directorate (S&T). He coordinates standards and conformity assessment activities across the Department and manages a broad portfolio of standards development activities including detection and personal protective equipment standards and response robot test method development.

Mr. Mattson is the DHS representative to the Interagency Committee on Standards Policy, and currently chairs the ASTM E54 Committee on Homeland Security Applications. He serves on the Board of Directors of the American National Standards Institute (ANSI) and also serves on several ASTM Technical Committees, the ANSI Unmanned Aircraft Systems Standardization Collaborative Steering Committee, and on standing committees in ANSI, the Society for Standards Professionals and in the Interagency Board for Emergency Preparedness and Response.

He holds a bachelor’s degree in Nuclear Engineering Technology from Oregon State University, and a master’s degree in Nuclear Physics from the Naval Postgraduate School. He has extensive training in nuclear weapons and radiological incident management and is a registered Professional Engineer. Mr. Mattson is a retired U.S. Army officer; serving over 20 years as a combat engineer and nuclear physicist.

U.S. Senator Gary Peters (MI)
Chair of the Homeland Security & Governmental Affairs Committee
U.S. Senate | USA

Mr. Robert B. Newman
Director of Engagement and Partnerships, Office of Strategy and Policy
Department of Homeland Security (DHS) | USA
robert.newman@hq.dhs.gov

Mr. Robert Newman has been with DHS S&T for over 2 years. He has served as a senior advisor to the under secretary and led the program office before assuming his new duties as Director of Engagement and Partnerships. In his new role, Mr. Newman leads efforts to identify technologies that can lead to new strategies for DHS for the security of our country and then to partner with organizations that can facilitate the adoption of those technologies. Mr. Newman is a retired Air Force general officer and has worked in the homeland security arena in state government for two Virginia governors.

Dr. Eric K. Lin

Acting Associate Director for Laboratory Programs
National Institute of Standards and Technology (NIST) | USA

Dr. Eric K. Lin is currently serving as the Acting Associate Director for Laboratory Programs at the National Institute of Standards and Technology (NIST). In this role, he provides direction and operational guidance for all of NIST's scientific and technical laboratory, among other duties. Before this role, Dr. Lin served as Director of the Material Measurement Laboratory (MML) at the National Institute of Standards and Technology (NIST). MML activities include fundamental research in the composition, structure and properties of industrial, biological, and environmental materials and processes, to the development and dissemination of certified reference materials, criticallyevaluated data and other measurement quality assurance programs. His contributions have been recognized with the Presidential Early Career Award for Scientists and Engineers (PECASE), the Department of Commerce Silver Medal, and the William P. Slichter Award.

Mr. Joseph Hamel
Director, ASPR Program Office for Innovation and Industrial Base Expansion (IBx)
Office of the Assistant Secretary for Preparedness and Response (ASPR)
Department of Health and Human Services (HHS) | USA
joseph.hamel@hhs.gov

Mr. Joseph Hamel is the Director, ASPR Program Office for Innovation and Industrial Base Expansion (IBx) for the Department of Health and Human Services (HHS) Office of the Assistant Secretary for Preparedness and Response (ASPR). He received his BA in molecular biology from Colgate University and his MS in biotechnology from John Hopkins University. Prior to joining the ASPR, Hamel worked as a biologist for the US Army, where he also served as a team leader and chief of planning and policy. Later, Mr. Hamel was a program director for the Department of Homeland Security, and then a program manager at Johns Hopkins University's applied physics laboratory. You can find him on Twitter (@JoeHamel9).

Dr. Nancy J. Lin

Biomaterials Group Leader, Material Measurement Laboratory
National Institute of Standards and Technology (NIST) | USA
nancy.lin@nist.gov

Talk Title: Overview and Goals for the 2021 SWWS Workshop

Dr. Nancy J. Lin is the Leader of the Biomaterials Group in the Biosystems and Biomaterials Division of the Material Measurement Laboratory at NIST. Her research focuses on developing measurements and standards to enable the detection and quantification of microbes and microbial communities, with an emphasis on total and viable cell count for microbial cell reference materials, biofilm-material interactions, antimicrobial efficacy, and biosurveillance. Dr. Lin holds a BS in Mechanical Engineering from Valparaiso University and a PhD in Biomedical Engineering from Case Western Reserve University.

SWWS 101: Introduction to Wastewater Surveillance (WWS)

***Session Chair*: Dr. Michael Focazio**
Environmental Health Research Program Coordinator
U.S. Geological Survey (USGS) | USA
mfocazio@usgs.gov

Dr. Michael Focazio earned his Ph.D. from the University of Connecticut. He has been a scientist and manager with U.S. Geological Survey for 30 years with research areas including drinking water, wastewater, environmental contaminants, ecosystems and human exposures. Dr. Focazio also serves on National Sewage Surveillance Interagency Leadership committee.

Speakers

Dr. Amy E. Kirby *(Keynote Presentation)*
National Wastewater Surveillance System Program Lead
Centers for Disease Control and Prevention (CDC) | USA
agk1@cdc.gov

Talk Title: National Wastewater Surveillance System: Implementation for COVID and Beyond
The National Wastewater Surveillance System (NWSS) launched in 2020 to coordinate wastewater surveillance programs implemented by state, tribal, local, and territorial health departments to support the COVID-19 pandemic response. Community-level infection trends for SARS-CoV-2 can be efficiently tracked through wastewater testing, providing an early indicator of changing infections trends in the community. Municipal wastewater surveillance is unique in that it can provide systematic information on both symptomatic and asymptomatic SARS-CoV-2 infections, and it is not influenced by healthcare access or clinical testing capacity. Robust and sustainable implementation of wastewater surveillance requires public health capacity for wastewater sampling, testing, analysis, and interpretation, as well as effective partnerships between wastewater utilities and public health departments. As of January 2021, the network comprises 33 jurisdictions. State and local public health agencies have used wastewater surveillance data to generate alerts to local jurisdictions, allocate mobile testing resources, evaluate potential testing artifacts in clinical surveillance, refine health messaging, and forecast hospital resource needs. NWSS provides a robust, highly

adaptable platform for community-level disease surveillance that can be expanded to collect data on multiple pathogens, such as antibiotic resistant bacteria and enteric pathogens, and leveraged for rapid assessment of emerging threats and preparedness for future pandemics.

Dr. Amy E. Kirby is an Environmental Microbiologist in the Waterborne Disease Prevention Branch and the Program Lead for the National Wastewater Surveillance System (NWSS) at the Centers for Disease Control and Prevention (CDC). She has a Bachelor of Science in Agriculture (BSA, major: Microbiology) from the University of Georgia, a PhD in Microbiology from the University of Buffalo, SUNY, and a Master's of Public Health in Epidemiology from Emory University. At CDC, Dr. Kirby is interested in leveraging environmental microbiology methods to measure pathogens, antibiotic resistance genes, and other health indicators in natural and man-made water systems. This data can be used to estimate health risks from environmental exposures, as well as measures of the health of the surrounding communities. Since February 2020, she has been working on the COVID-19 response as part of the Water, Sanitation, and Hygiene team. As part of that team, she led the development and implementation of NWSS.

Dr. Kate R. Griffiths

Senior Molecular Biologist
National Measurement Institute of Australia | Australia
kate.griffiths@measurement.gov.au

Talk Title: Australia's ColoSSoS Project: Inter-laboratory Study for SARS-CoV-2 in Wastewater

The WaterRA ColoSSoS project was initiated in the start of 2020 to co-ordinate the development of a national sampling and testing program for SARS-CoV-2 in wastewater within Australia. Once methods were developed across the country, the next phase was to compare the performance between the different testing approaches through participation in an inter-laboratory study. Twelve labs participated, representing a mix of water utilities, commercial labs, government labs and university research groups. Participants were provided with replicates of wastewater, inactivated SARS-CoV-2 virus for spiking and calibrant sets developed by Australia's National Measurement Institute. These calibrant sets consisted of a six-point dilution series of the inactivated virus quantified in "Copy Number Concentration of SARS-CoV-2 genome equivalents" using RT-dPCR. By providing the calibrant, all results were reported in the same units, allowing direct comparison of quantitative data from each lab. This study allowed protocols to be assessed, considering reproducibility, yield and RNA purity, identifying areas for improvement to increase testing consistency across the country.

Dr. Kate R. Griffiths is a senior molecular biologist working in the Bioanalysis Section of Australia's National Measurement Institute. She has many roles, including developing methods and managing the accredited DNA testing service, providing measurement uncertainty training to support biological measurements and designing bespoke DNA reference materials to achieve comparable quantitative data.

Dr. Judith Wong
Director
National Environment Agency | Singapore
Judith_Wong@nea.gov.sg

Talk Title: Singapore's National Wastewater Based Surveillance Programme for COVID-19: Analytical Standards, Controls and Reference Materials

Singapore's National Environment Agency, together with its partners, have developed a surveillance programme to monitor the presence of SARS-CoV-2 in wastewaters. Three use cases have been established. First, the surveillance at wastewater reclamation plants and at wide-area sentinel regional nodes provides situational awareness of COVID-19 spread in Singapore. Second, the monitoring of high-density living premises including workers' dormitories, nursing homes and student hostels provides early warning of emerging case(s), and thirdly, surveillance at clusters that have developed in the community provides an indicator if further case(s) are present. In the latter two use cases, data has translated into public health action, where targeted swab tests conducted have supported early case identification and isolation. As the programme relies on a network of testing laboratories, we will also describe analytical standards and proficiency testing criteria that have been implemented to ensure that tests conducted across laboratories are of high quality. Practical troubleshooting steps and corrective and preventive actions will also be discussed.

Dr. Judith Wong is the Director of the Microbiology and Molecular Epidemiology Division at the National Environmental Agency in Singapore. She oversees various COVID-19 related surveillance and research initiatives, including the development of Singapore's national wastewater-based epidemiology programme. The wastewater surveillance programme which she has set-up currently surveys wastewaters from more than a hundred sites across the country. Dr. Wong's other scientific interests include dengue diagnostics and surveillance, and the understanding anti-microbial resistance in the environment.

Dr. Shane Synder

Professor of Civil & Environmental Engineering and Executive Director of the Nanyang Environment & Water Research Institute (NEWRI)
Nanyang Technological University (NTU) | Singapore
ssnyder@ntu.edu.sg

Talk Title: Wastewater Based Epidemiology: Monitoring of SARS-CoV-2 and Related Markers in Singapore

The presentation focusses on optimisation to achieve a robust validated testing protocol for Covid-19 viral RNA.

Dr. Shane Snyder is a Professor of Civil & Environmental Engineering and is the Executive Director of the Nanyang Environment & Water Research Institute (NEWRI) at Nanyang Technological University (NTU), Singapore. For over 20 years, Dr. Snyder's research has focused on water quality, treatment, and sustainability, which resulted in over 300 published manuscripts with 28,000 citations. He serves as the Editor-in-Chief for the American Chemical Society journal, Environmental Science & Technology Water and is a Fellow of the International Water Association. Prof. Snyder was featured among the top 25 leading water researchers globally by Lux Research, and was awarded the Dr. Pankaj Parekh Research Innovation Award and the Agilent Thought Leader Award. Prof Snyder and his team were awarded the Nanyang Humanitarian Award for their philanthropic work in water and sanitation, which has benefited over two million people in underserved communities in Asia.

Dr. Beverley Margaret Stinson
Executive Vice President
AECOM | USA
Beverley.Stinson@aecom.com

Talk Title: Wastewater Infrastructure & Operations for an Enduring Public Health Partnership

Dr. Beverley Margaret Stinson is the Executive Vice President of AECOM Global Water with a technical background in water and wastewater treatment processes. Dr. Stinson has extensive experience nationally and internationally with wastewater epidemiology programs and a detailed understanding of wastewater infrastructure and operations. She will share her perspectives on the opportunity and challenges of establishing a sustainable and enduring wastewater surveillance program for the protection of Public Health.

SWWS 201: Sampling

***Session Chair*: Ms. Renee Stevens**
Program Manager
Department of Homeland Security (DHS) | USA
renee.stevens@hq.dhs.gov

Ms. Renee Stevens is currently on detail within DHS from Customs and Border Protection to the Science and Technology Directorate (S&T). In her role at S&T, Ms. Stevens coordinates voluntary consensus standards and related projects for DHS mission needs, including the integration of standards for unmanned aircraft systems (UAS).

Speakers

Mr. Claudio Ternieden
Senior Director of Government Affairs
The Waters Environment Federation

Talk Title: Utilities Perspectives and Needs

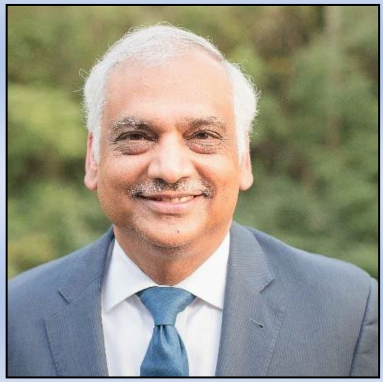
Mr. Claudio Ternieden is Sr. Director of Government Affairs & Strategic Partnerships for the Water Environment Federation (WEF). Ternieden is responsible for leading WEF's regulatory and legislative activities, including WEF's Global Programs. Ternieden is part of WEF's team working on wastewater-based epidemiology, coordinating with other organizations in the water sector such as municipalities, state agencies and universities. Ternieden served on WEF's Blue Ribbon Panel on wastewater operator safety due to COVID-19. Before coming to WEF, Claudio worked with Concurrent Technologies Corporation (CTC), a Department of Defense technologies innovator and integrator, and before that with the Water Environment Research Foundation (WERF) (now The Water Research Foundation) helping lead innovative research in infrastructure, disinfection, wet weather management, emerging contaminants, stormwater, climate adaptation, resilience, water reuse and decentralized systems. Ternieden has also worked with the American Association of Airport Executives (AAAE) where he worked in regulatory and legislative issues associated with environmental management at airports. Previously, he worked with the US EPA in Washington, DC and helped in the development of numerous federal regulations. Before the US EPA, Ternieden worked with the State of Indiana Department of Environmental Management (IDEM) supporting the implementation of the State's Great Lakes Water Quality Standards in NPDES permits, the pretreatment program, operators' certification program and the implementation of the drinking water capacity development program. Before working for the State of Indiana, Ternieden directed the City of Elkhart, IN pretreatment enforcement program. As part of his work with WEF over the last two years, Ternieden organized, presented and moderated sessions on technology innovation and transfer, water reuse and regulatory policy in Rio de Janeiro and Brasilia, Brazil, and in Cartagena de Indias, Colombia. As a volunteer, Ternieden has also organized and executed delegation visits to Addis Ababa, Ethiopia as part of development projects on water and sanitation with PRIDE (a non-profit organization doing development work in Ethiopia)

Mr. Patrick J. Phillips

Quality Assurance Specialist
US Geological Survey (USGS) | USA
pjphilli@usgs.gov

Talk Title: USGS Experience in Wastewater Sampling: 3 Issues to Guide Wastewater Surveillance for Public Health
The talk examines three crucial questions to guide wastewater sampling based on USGS experiences.

Mr. Patrick Phillips has been a USGS employee for over 35 years, focusing on data quality and the understanding of links between wastewater and public health.



Dr. Aruni Bhatnagar
Professor of Medicine
University of Louisville | USA
aruni@louisville.edu

Talk Title: Case Study-Wastewater Monitoring for COVID-19 in Louisville

Dr. Aruni Bhatnagar is Professor of Medicine and Distinguished University Scholar at the University of Louisville. He is the Director of the Christina Lee Brown Envirome Institute and Co-Director of the American Heart Association Tobacco Regulation Center. He is a leading expert on the mechanisms by which environmental exposures such as air pollution affect cardiovascular disease risk. Dr. Bhatnagar's initial work involved the purification and characterization of aldose reductase and its role in diabetic complications. To this end, he established the identity of this enzyme in several tissues and investigated its structural, kinetic, and inhibitory properties. His work has shown that increasing NO availability prevents aldose reductase activation and sorbitol accumulation in diabetic tissues. Additionally, his recent studies show that glucose activates multiple protein kinases and that the activation of these kinases is required for the inflammatory effects of glucose in vascular tissues. At UofL, Dr.

Bhatnagar's work also led to elucidation of the mechanisms by which free radicals and lipid peroxidation products affect the function of individual ion channels. He was the leader of a Program Project Grant from the NIEHS to study the cardiovascular toxicity of environmental aldehydes. In this program-project he directed a large multi-disciplinary team of investigators studying the molecular and cellular mechanisms of aldehyde toxicity. His studies at UofL have led to the development of the new field of Environmental Cardiology. He was the Deputy Editor of Circulation Research for 10 years. He has participated in over 50 NIH review panels and chaired several review panels. He was the recipient of the President's Award for Outstanding Scholarship, Research and Creative Activity, University of Louisville, and Partner in Healthcare Award – Contributing to Greater Louisville Healthcare Community, in 2007. In 2007, he also received the first Outstanding Faculty Mentor of Graduate Students, and the Outstanding Mentor Award from the Conference of Southern Graduate Schools. In 2017, he was designated Research Exemplar by Washington University. Dr. Bhatnagar has published 340 peer-reviewed manuscripts, 25 book chapters and reviews and over 200 abstracts. He has mentored 48 graduate students and post-doctoral fellows in his laboratory and has served on the dissertation committee of 18 Ph.D. students.

Mr. Kaushal Trivedi

Business Development Manager
Teledyne ISCO | USA
Kaushal.Trivedi@Teledyne.com

Talk Title: Wastewater Sampling for WBE Surveillance

Analysis of wastewater provides an early alert of WBE before symptoms surface out in individuals and clinical tests are performed. Proactive actions can be taken to prevent pandemic with alerts. In this presentation you will learn how to collect wastewater samples for WBE surveillance. Sampling types and methods will be presented. The location and purpose (WBE prevalence vs Trend) impact types and methods.

Mr. Kaushal Trivedi earned a Bachelor's in Electronics Engineering and a Bachelor's in Physics. Mr. Trivedi has been a water and wastewater professional for 32 years and spent with the last 21 years at Teledyne ISCO. He has managed large product portfolios of open channel flow meters and automatic samplers. He has also been involved in product development of field measurement technologies, interface protocols, m2m (machine to machine) remote communication, as well as sever and cloud-based software. Mr. Trivedi lives in Lincoln Nebraska, USA.

Dr. Aaron W. Bivins
Post-Doctoral Research Fellow
University of Notre Dame | USA
abivins@nd.edu

Talk Title: Building-level Wastewater Monitoring for COVID-19 using Tampon Swabs and RT-LAMP for Rapid SARS-CoV-2 RNA Detection

We piloted a wastewater surveillance workflow using tampons as passive swabs and reverse transcription loop-mediated isothermal amplification (RT-LAMP) to detect SARS-CoV-2 RNA in wastewater. Results for the developed workflow were available same day, with a time to result following tampon swab collection of approximately three hours. The workflow demonstrated a same-day positive predictive value (PPV) of 33% and negative predictive value (NPV) of 80% for incident COVID-19 cases. Even with lower analytical sensitivity the tampon swab and RT-LAMP workflow offers a cost-effective and rapid approach that could be leveraged for scalable same-day building-level wastewater monitoring for COVID-19.

Dr. Aaron W. Bivins is a public health engineer leading research at the intersection of microbiology, civil engineering systems, and human health. He seeks to characterize interactions between humans and pathogens mediated by water of all kinds – drinking, surface, ground, waste, and reuse. In response to the COVID-19 pandemic, he established the COVID-19 Wastewater-based Epidemiology (WBE) Collaborative, a global research collaboration. Dr. Bivins has led the development of robust and sensitive methods for detecting and quantifying SARS-CoV-2 RNA in wastewater and has contributed to WBE efforts throughout the state of Indiana. His research activity builds on his professional experience designing and permitting various hydraulic infrastructure including water distribution, wastewater collection, and stormwater systems.

***Panel Discussion:* Need for Standards to Support Wastewater Sampling**

***Moderator*: Ms. Sarah Wright**
Environmental Laboratories Manager
Association of Public Health Laboratories | USA
wright.sarah@gmail.com

Ms. Sarah Wright, MS, is an environmental laboratories manager at the Association of Public Health Laboratories in Silver Spring, MD. In this role, she works to strengthen state and local environmental laboratories. Current projects include working with the CDC National Wastewater Surveillance System to build public health laboratory wastewater surveillance testing capacity. Previously, she conducted watershed assessments in Wisconsin for the City of Racine and worked on national water policy at The Johnson Foundation at Wingspread. She has a master's degree in environmental monitoring from Ohio University and an environmental science and policy degree from Duke University.

Panelists:

Mr. John Birkner, Jr.
Manager Technical Services
Bergen County Utilities Authority | USA
jbirkner@bcua.org

Mr. John Birkner, Jr. attended Bergen Community College and Ramapo College as an undergraduate student. He has continued professional studies at Rutgers University/Cooke College and California State University with a focus on industrial wastewater treatment and wastewater microbiology.

Mr. Birkner is employed in the wastewater treatment industry where he is the Manager of Technical Services for the Bergen County Utilities Authority. Previously he was employed with Envirogen Technologies and Bigler Associates where served as the Industrial Pretreatment Program Manager at the Northwest Bergen county Utilities Authority and the Two Bridges Sewerage Authority. He has served 12 years as mayor of Westwood, NJ and currently sits on the Board of Trustees for Hackensack Riverkeeper, Inc. & Comprehensive Behavioral Health Care.

Dr. Raul A. Gonzalez
Environmental Scientist
Hampton Roads Sanitation District | USA
rgonzalez@hrsd.com

Dr. Raul A. Gonzalez is an Environmental Scientist at Hampton Roads Sanitation District (HRSD) where he applies molecular methods to manmade infrastructure and their adjacent waters. His current projects use DNA-based markers for a variety of applications, including identifying compromised sewer infrastructure and quantifying pathogen removal of various wastewater and water reuse treatment trains.

Dr. Rochelle Holm
Researcher
University of Louisville | USA
Rochelle.holm@louisville.edu

Dr. Rochelle Holm has broad interests in water and sanitation technologies to improve the well-being of the most vulnerable population groups, ranging from fecal sludge management to rural water supply. Her current research includes pathogen detection in pit latrines, SARS-COV-2 surveillance in wastewater, and the role of occupational mobility on the effects of household water, sanitation and hygiene access. Dr. Holm is currently with the University of Louisville, and also holds a faculty appointment at Mzuzu University, in Malawi, Africa.

Mr. Greg Kester

Director of Renewable Resources Programs
California Association of Sanitation Agencies (CASA) | USA
gkester@casaweb.org

Mr. Greg Kester is the Director of Renewable Resource Programs for the California Association of Sanitation Agencies (CASA). Mr. Kester serves as both the technical and programmatic contact for CASA members and conduit for emerging issues on the state and federal levels on all biosolids, renewable energy, and climate change mitigation issues. Recently this has included issues related to SARS-CoV-2 and wastewater-based epidemiology. He has worked with the wastewater sector in California, along with researchers and public health officials to advance the use of WBE since the pandemic began. He holds a BS in Civil and Environmental Engineering from the University of Wisconsin – Madison.

Mr. Bruce E. Smith
Environmental Engineer
Environmental Protection Agency (EPA) | USA
smith.bruce@epa.gov

Mr. Bruce E. Smith, now with EPA's Office of Research and Development, is formerly the Assistant Superintendent of the Compliance Services Division with the Metropolitan Sewer District of Greater Cincinnati, Ohio. In addition to regulatory compliance and divisional administrative duties he also led the innovation efforts, including applied research, and was actively involved in the district's efforts to identify, evaluate and adopt innovative technologies for the management and control of wet weather flows. He was a key team member developing MSDGC's next phase of its $3+ billion Wet Weather Improvement Program. Mr. Smith has served as a Senior Engineer with MSDGC managing large capital projects with Ohio EPA from 1990 – 2007 in its Surface Water Division.

Mr. Smith has a BS Chemical Engineering from the University of Cincinnati where he also completed Environmental Engineering Masters course curriculum. He has also served as an adjunct instructor at Cincinnati State Technical College from 2006 - 2016.

Mr. David Swain
Associate Vice President
AECOM | USA
David.swain@aecom.com

Mr. David Swain is a Department Manager with the EHS Practice of AECOM. Mr. Swain is currently supervising the field sampling effort on a wastewater sampling project for the Commonwealth of Kentucky. He has extensive experience with field environmental assessments and sampling of soil, soil vapor, ground water, wastewater, air and sediment.

Closing Remarks for Day 1

Mr. Philip J. Mattson

Department of Homeland Security (DHS), Science and Technology Directorate (S&T) | USA

Dr. Nancy J. Lin
National Institute of Standards and Technology (NIST) | USA

DAY 2: TUESDAY, JUNE 15, 2021

Welcome & Logistics

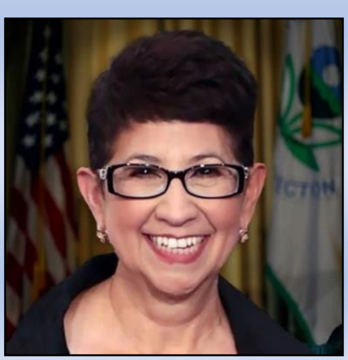
Mr. Philip J. Mattson

Department of Homeland Security (DHS), Science and Technology Directorate (S&T) | USA

Dr. Nancy J. Lin
National Institute of Standards and Technology (NIST) | USA

SWWS 202: Testing Methods

***Session Chair*: Ms. Sally C. Gutierrez**
Senior Advisor for Water
Environmental Protection Agency (EPA) | USA
Gutierrez.sally@epa.gov



Ms. Sally C. Gutierrez has recently returned to the Office of Research and Development as Senior Advisor to the Director of the Center for Environmental Solutions and Emergency Response. She is assisting in the development of wastewater-based epidemiology as an early warning of Covid-19 infections in communities, PFAS treatment for water matrices and advancing diversity, equity and inclusion.

From 2018 to mid-2000, she served as the Acting Director of Water Permits Division in the Office of Wastewater Management, Office of Water at USEPA in Washington, DC. She was responsible for leading the Agency's largest environmental permitting program, covering more than 800,000 wastewater facilities in the US through the National Pollutant Discharge Elimination Program. These include permits for stormwater, animal feeding operations, construction sites, and municipal and industrial wastewater. Before taking the detail to Washington DC, she was the Director of EPA's Environmental Technology Innovation Cluster Development and Support Program within the ORD. The program advanced environmental protection in tandem with economic development through the formation of public private partnerships. She was the lead on EPA's efforts to leverage its research and development capability in Cincinnati, Ohio with community-based assets to establish the region as a water technology innovation hub and leading an effort to network water innovation clusters across the globe. Prior to this new appointment, she was the Director of the National Risk Management Research Laboratory (NRMRL) in Cincinnati, Ohio for 8 years. NRMRL was one of three Federal research laboratories within the USEPA's ORD and consisted of 400 scientists and support staff. The Laboratory was responsible for conducting engineering and environmental technology research to support the Agency in policy and regulatory development and implementation. She was the Director of the Water Supply and Water Resources Division in NRMRL before becoming its Director and was responsible for leading a national technology demonstration program for control of arsenic in drinking water. Before coming to USEPA she was responsible for administering water programs for the State of Texas environmental agency in the areas of drinking water, water monitoring, wastewater permitting, dam safety, water rights and utility rates. She is a hydrologist by training and has completed over 21 years of service at EPA. When first appointed in 2000, she was the first Hispanic woman career Senior Executive Service member hired by USEPA.

Speakers

Dr. Christobel M. Ferguson
Chief Innovation Officer
The Water Research Foundation | USA
christobelf@gmail.com

Talk Title: Global Update on Wastewater Surveillance for SARS-CoV-2

In April 2020, The Water Research Foundation (WRF) convened global leaders in an international summit to identify best practices and high-priority research needs around wastewater-based epidemiology (WBE) in response to the COVID-19 pandemic. Because WBE approaches have continued to develop over the past 12 months, WRF convened a follow-up global symposium to share updates on WBE activities from around the world, with a focus on how this information is being used to support health agencies in their COVID-19 response.

Dr. Christobel M. Ferguson has a Biomedical Science degree and a Master of Science from the University of Technology Sydney and a Ph.D. from the University of New South Wales. Dr. Ferguson has led scientific, technical, research and consulting teams in the Australian water and environment sector for over 30 years and in early 2020 joined the Water Research Foundation as their Chief Innovation Officer. Her focus is supporting the implementation of innovation and new technologies to solve complex problems facing the water resource sector to enable utilities to mitigate risk and improve performance.

Dr. Nichole Brinkman
Biologist
US Environmental Protection Agency (EPA) | USA
brinkman.nichole@epa.gov

Talk Title: Overview of the Ohio Wastewater Monitoring Network

Dr. Nichole Brinkman is a Biologist with USEPA's Office of Research and Development. Her research focuses on the presence and dissemination of waterborne pathogens and antimicrobial resistance determinants in the environment. Nichole received her Ph.D. in Biological Sciences from the University of Cincinnati.

Ms. Rebecca Fugitt
Assistant Chief, Bureau of Environmental Health and Radiation Protection
Ohio Department of Health | USA
rebecca.fugitt@odh.ohio.gov

Talk Title: Overview of the Ohio Wastewater Monitoring Network

Ms. Rebecca Fugitt is the Assistant Chief of the Bureau of Environmental Health and Radiation Protection at the Ohio Department of Health where she oversees programs related to residential water and sewage, harmful algal blooms, fish consumption advisories and health assessment, Legionella, radioactive materials licensing, X-ray registration and inspection, and radiation health and safety. She holds a B.S. and M.S. degrees in Geological Sciences from Ohio University and is a registered sanitarian in the state of Ohio. She was the program manager for the Residential Water and Sewage program at ODH for 19 years, and program manager for the Water Resources Section at the Ohio Department of Natural Resources for 11 years. Prior to joining the state, Rebecca served as a research hydrogeologist for the National Ground Water Association.

Dr. Alexandria B. Boehm
Professor of Civil and Environmental Engineering
Stanford University | USA
aboehm@stanford.edu

Talk Title: Scalable, Sensitive, Representative, and Comparable Approach for High Throughput SARS-CoV-2 RNA Analysis in Settled Solids
We have been quantifying "pan-SARS-CoV-2" RNA targets (N, S, ORF1a) and variant mutations (HV69-70, E484K/N501Y) in settled solids from eight wastewater treatment plants in Northern California on a daily basis since November 2020. A focus on SARS-CoV-2 RNA in solids

enabled us to scale-up our measurements with a commercial lab partner. Samples were collected daily and results were posted to a website within 24-hours. SARS-CoV-2 RNA in daily samples correlated to incidence COVID-19 cases in the sewersheds; a 1 log10 increase in SARS-CoV-2 RNA in settled solids corresponds to a 0.58 log10 (4X) increase in sewershed incidence rate. SARS-CoV-2 RNA signals measured with the commercial laboratory partner were comparable across plants and to measurements conducted in a university laboratory when normalized by pepper mild mottle virus PMMoV RNA. Results suggest that SARS-CoV-2 RNA should be detectable in settled solids for COVID-19 incidence rates at least as low as 1/100,000. Variant mutations are detectable in settled solids matching clinical data on variant circulation. These sensitive, representative, scalable, and comparable methods will be valuable for future efforts to scale-up wastewater-based epidemiology.

Dr. Alexandria B. Boehm is a Professor with the Department of Civil and Environmental Engineering, at Stanford University. Dr. Boehm is also a Senior Fellow of the Woods Institute for the Environment and a Faculty Fellow at the Center for Innovation in Global Health. She received her Ph.D. in Environmental Engineering from the University of California, Irvine, and holds an M.S. in Environmental Engineering from the same university and a B.S. in Engineering and Applied Science from California Institute of Technology. (See full bio at www.stanford.edu/~aboehm).

Dr. Jordan J. Schmidt

Director, Product Applications
LuminUltra Technologies | Canada
Jordan.schmidt@luminultra.com

Talk Title: Reducing Erosion: Using a Rapid Deployable Testing Solution to Reduce Time Between Sampling and Action

Wastewater surveillance has become a useful tool for monitoring the trend of COVID-19 cases at a community level. Wastewater testing for SARS-CoV-2, the virus that causes COVID-19, is a non-invasive method that delivers accurate results that have been shown to give advanced warning of clinical case trends. Conventional methods typically require specialized equipment and skilled operators to pre-concentrate large volumes of wastewater prior to analysis for SARS-CoV-2. LuminUltra® has developed the GeneCount® SARS-CoV-2 Wastewater Test Kit for extracting SARS-CoV-2 RNA directly from a 1 mL sample of raw wastewater using magnetic binding bead technology thereby removing the need for pre-concentrating samples. Furthermore, it allows for simultaneous testing of both the liquid and solids fraction of the wastewater increasing the accuracy of the result. By simplifying the protocol testing can be done near-sample reducing the time between sampling and action.

Dr. Jordan J. Schmidt is the Director of Product Applications at LuminUltra Technologies. Dr. Schmidt holds a PhD in Civil Engineering specializing in wastewater treatment. During his academic career he spent time assessing treatment systems in one of the most remote areas of the world - the Canadian Arctic. This experience reinforced his need for rapid, accurate tools to be used in a variety of environments and lab infrastructure. At LuminUltra, Dr. Schmidt spends his days developing and supporting customers with our accurate, rapid, field-ready tests kits for the measurement of microorganisms.

Dr. Brian Swalla
Staff Scientist
IDEXX Laboratories, Inc. | USA
brian-swalla@idexx.com

Talk Title: The Importance of Validation for Wastewater Surveillance

Wastewater surveillance has delivered actionable public health data over the course of the COVID-19 pandemic. Because of the urgent nature of the pandemic, the extent of validation for currently used methods varies widely. Many methods were deployed before significant data was gathered on performance, and may not have been widely tested with a diversity of wastewater samples. As wastewater surveillance moves into the mainstream, it is critical that methods be thoroughly validated using real-world wastewater samples, that the validation captures as much natural variation as possible (such as ensuring the validation is performed with samples from many different geographies), and that validation data be made available to laboratories interested in using the method. Such data are critical to evaluate method performance capabilities and limitations, facilitate successful adoption in new laboratories, and ensure consistent and reliable results can be achieved over time.

Dr. Brian Swalla received a Ph.D. in Microbiology from the University of Illinois and has 17+ years of experience developing new technologies and products in a wide array of microbiological and molecular applications, including microbial and protein biocatalysts, cellulosic biofuels, and water quality. Since 2011, he has worked for IDEXX Laboratories Inc. on new products for water microbiology, where he is currently a Staff Scientist and most recently led the technical development and validation of a RT-qPCR test for SARS-CoV-2 in wastewater.

Ms. Carolyn Reifsnyder

Director, Global Product Marketing, Digital Biology Group
Bio-Rad Laboratories | USA
carolyn_reifsnyder@bio-rad.com

Talk Title: SARS-CoV-2 Quantification in Wastewater by Droplet Digital PCR

Ms. Carolyn Reifsnyder leads a high-performing team of product marketers and application scientists in the development and execution of DBG's strategic plans and product roadmaps to conceive, incubate and launch differentiated products to customers in the translational research, biopharma and molecular diagnostic markets. (See full bio at: https://www.linkedin.com/in/carolyn-reifsnyder-a269102/.)

Dr. Michael Bussmann
Associate Director, Global Product Management - dPCR
QIAGEN | Germany
michael.bussmann@qiagen.com

Talk Title: Nanoplate Digital PCR for Wastewater Surveillance

Dr. Michael Bussmann has spent 6 years at QIAGEN Product Management for Assay Technologies. He has 10 years industry experience in total and earned his Ph.D. in Biochemistry.

Dr. Ellen M. Beasley
CSO
Pangolin Health | USA

ellen.beasley@panoglinhealth.com

Talk Title: SARS-CoV-2 Variant Profiling in Wastewater by Sequencing

Novel viral strains continue to emerge and to spread, complicating management and containment of the COVID-19 pandemic. A smaller number of "Variants of Concern" and "Variants of Interest" have been defined by the CDC and WHO. Of these, at present, 13 variants, all have multiple mutations documented in the spike protein gene, as well as further mutations across their entire genome. Next generation sequencing (NGS) of the S gene allows unambiguous quantitative identification of these strains in wastewater samples. The advantages of this approach are that it is less likely to require frequent versioning of the assay as new strains emerge, and the results generally have multiple points of support for viral strain identification or elimination. Disadvantages are the complexity of developing a robust amplicon set for profiling S gene mutations, which covers all variants and avoids positioning primers over variants; and the data management and analysis pipeline required for analysis and interpretation of the results. We have a version 1 assay that provides excellent profiling of the current CDC Variant Strains. We have been able to apply this assay to longitudinal characterization of a specific outbreak, by testing dorms on a campus to ascertain and describe variant succession.

Dr. Ellen M. Beasley is an experienced biotechnology executive with a history of working at the intersection between diagnostics and genomics. She has had success moving technology into products, DNS sequencing technologies, intellectual property, and diagnostic test development (LDT & IVD). Dr. Beasley has a passion for team development, organizational design, communication, and collaboration. (full bio at https://www.linkedin.com/in/ellen-beasley-b58577/)

Mr. Ryan Gregerson
Product Marketing Manager
BioFire Defense | USA
Ryan.Gregerson@BioFireDefense.com

Talk Title: BioFire Defense Overview
An introduction to the BioFire FilmArray and available PCR test panels.

Mr. Ryan Gregerson is the Product Marketing Manager for BioFire Defense. He started is career in molecular biology labs and has moved into customer facing roles. Ryan previously worked in technical support and helped BioFire customers on-board and troubleshoot their products. In his current role he acts as an evangelist for the BioFire FilmArray products.

Dr. Roberto J. Barbero
Chief Business Officer
Ceres Nanosciences | USA
rbarbero@ceresnano.com

Talk Title: Viral Concentration with Nanotrap Magnetic Virus Particles
Nanotrap Magnetic Virus Particles, from Ceres Nanosciences, rapidly capture and concentrate SARS-CoV-2 from raw sewage, requiring no filtration or centrifugation. Automated and manual methods available. The automated method enables 96 raw sewage samples to be processed in 4.5 hours (from concentration to RT-qPCR result) and has higher recovery efficiencies than conventionally used methods for viral wastewater concentration. At University of California San Diego campus, the automated method has been used to process over 9,500 samples from 121 autosamplers covering 350 buildings. The sensitivity of the high-throughput protocol was shown to detect 1 asymptomatic individual in a building of 415 residents. To date, nearly 85% of the individual cases on the UCSD campus have been preceded by positive wastewater samples. Extracted RNA samples from this high-throughput method can be used for genome sequencing.

Dr. Roberto J. Barbero has over 15 years of experience operating across a range of responsibilities in the biotechnology sector, including in public policy, product development, process engineering, R&D, quality control, manufacturing, and customer-facing roles in startups, at MIT, and in the White House. Dr. Barbero has extensive experience building and maintaining strategic partnerships with leading foundations, universities, private research institutes, non-profit organizations, federal agencies, and companies across a variety of scientific and technical topics.

In the White House, Dr. Barbero spent more than four years developing and implementing policy on global and national life science issues, including the BRAIN Initiative, the Precision Medicine Initiative, biotechnology regulatory policy, emerging biotechnologies, genome editing, reducing the organ transplant waiting list, cancer diagnostics for the developing world, the federal government's response to the Zika virus, student innovation and entrepreneurship, and federal R&D agency budgets.

Dr. Barbero received his Ph.D in biological engineering from MIT, where he led or co-led projects on antimicrobial peptides, carbon capture, and industrial biocatalysts. He was named an MIT Presidential Fellow and a Siebel Scholar and his research resulted in four publications and two granted patents. Before graduate school, he spent five years working for three biotechnology startups – GlycoFi (acquired by Merck), Quantum Dot Corporation (acquired by Invitrogen), and Nanostream. He also holds A.B. and B.E. degrees in engineering sciences from Dartmouth College. (full bio at https://www.linkedin.com/in/robbie-barbero-6b18a31/)

***Panel Discussion:* Need for Standards to Support Testing Methods**

***Moderator*: Dr. Jay L. Garland**
Associate Director for Research, Office of Research and Development
Environmental Protection Agency (EPA) | USA
garland.jay@epa.gov

Dr. Jay L. Garland joined the EPA's Office of Research and Development in 2011. Dr. Garland received a Ph.D. in Environment Science from the University of Virginia and spent over 20 years working on NASA's efforts to develop closed, bioregenerative life support systems for extended human spaceflight. NASA recognized him for innovative technical achievements four separate times. He has worked on a range of topics, including methods for microbial community analysis, factors affecting survival of human associated pathogens, and various biological approaches for recycling wastes. Dr. Garland has completed visiting fellowships and professorships at the Institute for Environment Sciences in Japan, the University of Innsbruck in Austria, and the University of Buenos Aires in Argentina. His current efforts focus on advancing innovative approaches to water infrastructure, including decentralized water reuse, and mitigating risks associated with antimicrobial resistance in the water cycle.

Panelists:

Dr. Kartik Chandran
Professor of Environmental Engineering
Columbia University | USA
kc2288@columbia.edu

Dr. Kartik Chandran is an environmental engineer at Columbia University, where he is a Professor in the Department of Earth and Environmental Engineering. He primarily works on the interface between environmental molecular and microbiology, environmental biotechnology and environmental engineering. Applications of his work have ranged from energy and resource efficient treatment of nitrogen containing wastewater streams, development and implementation of sustainable approaches to sanitation to novel models for resource recovery. Under his stewardship, the directions of biological wastewater treatment and biological nutrient removal were established for the first time ever in the history of Columbia University. In 2015, he received the MacArthur Fellowship for his innovative work on "integrating microbial ecology, molecular biology, and engineering to transform wastewater from a troublesome pollutant to a valuable resource". (See full bio at: www.columbia.edu/~kc2288)

Mr. Kahlil Lawless

Microbiology Segment Manager – Americas
Illumina | Canada
klawless@illumina.com

Mr. Kahlil Lawless is currently the Agrigenomics and Microbiology Segment Manager for the Americas at Illumina, based in Toronto, Canada. After studying genetics at Victoria University of Wellington in New Zealand he worked in Australia as a researcher and molecular biologist in the Department of Primary Industries and the Environment before joining Illumina.

Dr. Mia Mattioli
Environmental Engineer
Centers for Disease Control and Prevention (CDC) | USA
kuk9@cdc.gov

Dr. Mia Mattioli is the Principle Investigator for the CDC's Waterborne Disease Prevention Branch's Domestic WASH Lab within Environmental Microbiology and Engineering Laboratory Team. Her research focuses on the intersection between the environment and human health with a specific interest in the relationship between, and fate and transport of, fecal indicators and enteric pathogens. Her lab is engaged in a wide range of environmental research areas including drinking water, irrigation water, wastewater, recreational water, soil, food, water distribution systems, and the development of advanced molecular detection technologies. Dr. Mattioli also leads CDC's environmental investigations of waterborne outbreak responses and currently serves as the Science Lead for the CDC National Wastewater Surveillance System. She has a Bachelor of Science in Biological Engineering from the University of Georgia and a Master and Ph.D. in Environmental Engineering from Stanford University.

Dr. Gertjan Medema
Principal Microbiologist
KWR Water Research Institute
gertjan.medema@kwrwater.nl | Netherlands

Dr. Gertjan Medema is principal microbiologist at KWR Water Research Institute in the Netherlands. He is part-time professor of Water & Health at Delft University of Technology and Visiting Hannah Professor at Michigan State University. His main area of expertise is detection methods, transmission, risk assessment and epidemiology of waterborne pathogens. He has been the scientific coordinator of the joint research program of the Netherlands water utilities. He is advisor of the WHO and the European Commission on microbial safety of water and initiated research on sewage surveillance of COVID-19.

Dr. Martin Shafer
Senior Scientist
University of Wisconsin-Madison, State Laboratory of Hygiene | USA
mmshafer@wisc.edu

Dr. Matt Shafer is a Senior Scientist with the University of Wisconsin-Madison State Laboratory of Hygiene (WSLH). He is an environmental biogeochemist, working at the interface of chemistry, biology and toxicology. He serves as research lead at the Environmental Health Division of the WSLH, directs the WSLH Trace Element Research Group, and oversees Quality Assurance for the National Atmospheric Deposition Program (NADP). His research program addresses the cycling of metals and emerging contaminants in aquatic, biologic, and atmospheric systems with a focus on the interface of environmental chemistry and human health. Dr. Shafer's current studies focus on interrelationships between trace element speciation and biological effect and element source attribution using elemental and isotopic fingerprinting. He is an expert in the analytical chemistry (particularly plasma mass spectrometry and electrochemistry) of metals in clinical and environmental matrices and helped develop many of the clean sampling and analytical approaches for trace elements now used by the scientific and regulatory communities. His research group developed new tools for quantification of the oxidative activity of atmospheric aerosols and has applied this toolbox to address chemical drivers of aerosol-associated toxicity in a large body of publications. Dr. Shafer has authored and co-authored more than 185 peer reviewed manuscripts.

SWWS 203: Data Reporting and Analytics

***Session Chair*: Mr. Paul Storella**
Senior Vice President Water Business Line
AECOM | USA
paul.storella@aecom.com

Mr. Paul Storella leads the New York Metro Water Business Line and AECOM's national SARS CoV-2 wastewater surveillance program. The Water business line provides planning, design, and construction/program management services for large-scale water and wastewater infrastructure projects throughout the globe. Under Paul's leadership, the Water has expanded AECOM's portfolio to include a number of wastewater surveillance projects including the Bergen County Utility Authority and the Commonwealth of Kentucky Department of Corrections programs. In his more than 30-year career, Paul has worked on many significant water and wastewater projects, including the Deer Island Sewage Treatment Plant, the centerpiece of the Massachusetts Water Resource Authority's program to protect Boston Harbor; the F. Wayne Hill Water Resources Center in Atlanta, a tertiary treatment facility; and the US Agency for International Development's expansion of the wastewater collection system in Alexandria, Egypt.

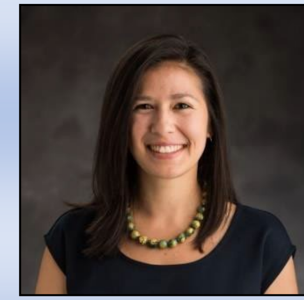
Speakers

Dr. Wiley Jennings
Health Scientist
Centers for Disease Control and Prevention (CDC) | USA
oht6@cdc.gov

Talk Title: Available Data Tools: National Wastewater Surveillance System
This talk will provide an overview of CDC's National Wastewater Surveillance System DCIPHER data platform, data submission process, and analytics. It will touch on the utility of standard materials for data comparability and the CDC-NCBI collaboration to develop a BioSample attribute package for submission of wastewater surveillance sequencing data to NCBI.

Dr. Wiley Jennings serves as the Data Lead for CDC's National Wastewater Surveillance System. He holds a Ph.D. in Environmental Engineering and Science from Stanford University.

Dr. Lauren Stadler
Assistant Professor
Rice University | USA
lauren.stadler@rice.edu



Talk Title: SARS-CoV-2 Wastewater Surveillance and Public Health Applications in Houston, TX
In this talk we describe the SARS-CoV-2 wastewater monitoring program implemented in Houston, TX. In this collaborative effort, we have been collecting, processing, and analyzing weekly wastewater samples over the past 13 months from 39 wastewater treatment plants in Houston that serve 2.3M+ people, 53 schools, and 22 congregate living facilities. We describe the types of data that are generated, and how the data are used to inform public health action, both at the wastewater treatment plant level, and at upstream (manhole) sampling locations.

Dr. Lauren Stadler is an assistant professor in the Department of Civil and Environmental Engineering at Rice University. Her research is focused on advancing sustainable and safe wastewater management systems that can be used to generate valuable resources and monitored to inform public health.

Dr. Loren Hopkins

City of Houston Chief Environmental Science Officer, Chief of the Bureau of Community and Children's Environmental Health at the Houston Health Department, and a Professor in the Practice in the Department of Statistics at Rice University
Houston Health Department and Rice University | USA
loren.hopkins@houstontx.gov

Talk Title: SARS-CoV-2 Wastewater Surveillance and Public Health Applications in Houston, TX
In this talk we describe the SARS-CoV-2 wastewater monitoring program implemented in Houston, TX. In this collaborative effort, we have been collecting, processing, and analyzing weekly wastewater samples over the past 13 months from 39 wastewater treatment plants in Houston that serve 2.3M+ people, 53 schools, and 22 congregate living facilities. We describe the types of data that are generated, and how the data are used to inform public health action, both at the wastewater treatment plant level, and at upstream (manhole) sampling locations.

Dr. Loren Hopkins is the City of Houston Chief Environmental Science Officer, Chief of the Bureau of Community and Children's Environmental Health at the Houston Health Department, and a Professor in the Practice in the Department of Statistics at Rice University. In this dual capacity, she conducts applied research and uses the results to inform policies at the City of Houston to improve the health of the community. She leads the city's COVID-19 data science team.

Ms. Aparna Keshaviah
Senior Statistician
Mathematica | USA
akeshaviah@mathematica-mpr.com

Talk Title: Translating Wastewater Data for Policymaking

With hundreds of communities across the country now implementing wastewater surveillance for the SARS-CoV-2 virus, standards for analysis and reporting are needed to ensure that the resulting data are reliable and comparable. More work is needed to identify robust metrics for reporting wastewater viral concentrations and criteria for triggering alerts and action. To communicate findings for policymakers charged with pandemic management and response decisions, lab results can be presented alongside administrative data sources that officials are already monitoring. Such data contextualization should move beyond confirmed cases to also include local and regional indicators of risk and population vulnerability. Aligning and synthesizing wastewater data with community data can provide a more holistic picture of the threat, facilitate data triangulation, and yield novel policy insights. For the opioid epidemic, such approaches have been used to predict the need for overdose response, assess the impacts of law enforcement, and measure black-market activity.

Ms. Aparna Keshaviah is a senior statistician at Mathematica who brings advanced analytics and innovative data to clarify urgent questions across multiple public health arenas. Her translational approach to wastewater surveillance uses data integration and dynamic visualization to help officials manage infectious diseases and drug epidemics. Her work aims to communicate scientific findings to technical audiences, academic research communities, and the general public alike. Prior to joining Mathematica, she conducted head-to-head comparisons of the safety and efficacy of breast cancer treatments to help clinicians tailor patient management decisions. She also analyzed and validated psychiatric symptom profiles to inform the diagnosis and treatment of debilitating mental health conditions. Her research has been widely published in leading journals such as the New England Journal of Medicine, JAMA Psychiatry, and Environmental Health Perspectives. Ms. Keshaviah is a 2006-2007 Fulbright fellow and holds a Master’s degree in biostatistics from the Harvard School of Public Health.

Dr. Mariana Matus
CEO and Cofounder
Biobot Analytics, Inc. | USA
mariana@biobot.io

Talk Title: Biobot: Building early warning health analytics from data available in our sewers

Over the past year, Biobot has tested close to ten thousand wastewater samples from across the U.S. for SARS-CoV-2. The communities that have worked with Biobot include large metro areas, cities, rural areas, Indian territories, as well as building-level communities such as nursing homes, prison systems, places of work, and places of study. More recently, Biobot got an award from HHS to collect a nationwide dataset covering 320 communities and 100 million people. Outside some early method development, the vast majority of Biobot's data has been produced with a single standardized method that relies on commercially-available kits. The depth of data collected plus close collaborations with public health officials and academics have highlighted three successful applications of wastewater epidemiology data: i) Independent confirmation of clinical data trends; ii) Outbreak detection in a background of little disease activity; iii) Nationwide ranking based on all other recent samples. Before Covid-19, Biobot had an opioid use monitoring product on the market to inform harm-reduction programs in cities.

Dr. Mariana Matus is CEO and Cofounder at Biobot Analytics where she leads the development of a wastewater epidemiology data platform to make public health more proactive and equitable. With a background in microbiology and computational biology, Dr. Matus specialized in wastewater epidemiology in Professor Eric Alm's laboratory at the Biological Engineering Department at MIT. Through Biobot, aims to bring wastewater epidemiology platform across the globe to serve all of the world's cities and stop outbreaks before they become pandemics. She values wastewater epidemiology for its accurate and privacy-protected data as an invaluable tool for public health and public safety officials.

Ms. Ana Grace Alvarado
Graduate Student
University of California Merced | USA
aalvarado55@ucmerced.edu

Talk Title: Rapid Fire – Global COVID-19 Wastewater Monitoring Efforts: Development of the COVIDPoops19 Dashboard

A year since the declaration of the global coronavirus disease 2019 (COVID-19) pandemic there have been over 110 million cases and 2.5 million deaths. Using methods to track community spread of other viruses such as poliovirus, environmental virologists and those in the wastewater-based epidemiology (WBE) field quickly adapted their existing methods to detect SARS-CoV-2 RNA in wastewater. Unlike COVID-19 case and mortality data, there was not a global dashboard to track wastewater monitoring of SARS-CoV-2 RNA worldwide. Here, we'll discuss the development of the

COVIDPoops19 dashboard to disseminate information regarding sites, universities, research institutions and private laboratories in countries that are involved in WBE for SARS-CoV-2. Methods to assemble the dashboard combined standard literature review, direct submissions, and daily, social media keyword searches. Over 200 universities, 1,000 sites, and 50 countries with 59 dashboards monitor wastewater for SARS-CoV-2 RNA. However, data is not widely shared publicly or accessible to researchers to inform public health actions, meta-analysis, better coordinate, and determine equitable distribution of monitoring sites. For WBE to be used to its full potential during COVID-19 and beyond, show us the data.

Ms. Ana Grace F. Alvarado is a graduate student in Environmental Systems at the University of California Merced. She received a BA in Chemistry and Hispanic Studies at the College of Saint Benedict in Minnesota and her research interests include Life Cycle Assessment, the effects of COVID-19 on the food supply chain, and how wastewater based epidemiology can be used to inform actions to improve public health.

***Panel Discussion:* Need for Standards to Support Data Reporting and Analytics**

***Moderator*: Mr. Paul Storella** (see above for photo and bio)
Senior Vice President Water Business Line
AECOM | USA
paul.storella@aecom.com

Panelists:

Dr. Ellie Graeden
Chief Executive Officer
Talus Analytics | USA
egraeden@talusanalytics.com

Dr. Ellie Graeden is the founder and CEO of Talus Analytics and an associate adjunct professor with the Georgetown University Center for Global Health Science and Security (GHSS). She leads an interdisciplinary research and development team that applies data analysis, modeling, and visualization to solve challenging problems at the intersection of policy, science, and strategy. Dr. Graeden has applied her expertise to developing quantitative approaches for global-scale decision making. With an emphasis on applying the best available data to decision making during emergencies, she has led projects in support of FEMA and the White House National Security Council to coordinate data-driven decision making for public health emergencies and other hazards. Most recently, Dr. Graeden and her team have worked with CDC National Center of Immunization and Respiratory Diseases to develop platforms for health care visibility, vaccination coverage, and response efforts for influenza and COVID-19. In collaboration with the Georgetown University GHHS, her team helped lead development of a comprehensive inventory of policies implemented to mitigate COVID-19 and model the impact of those policies.

Dr. Graeden earned her undergraduate degree in microbiology from Oregon State University and her doctorate in biology from the Massachusetts Institute of Technology (MIT), where she held a National Science Foundation Graduate Research Fellowship. She was named a 2013 Emerging Leader in Biosecurity Fellow with the Johns Hopkins Bloomberg School of Public Health Center for Health Security.

Mr. Robert Greenberg
CEO
G&H International Services, Inc. | USA
rgreenberg@ghinternational.com

Talk Title: Ensuring Trusted Data to Ensure Trustworthy Analysis

Mr. Robert Greenberg is founder and Chief Executive Officer of G&H International Services Inc., a Washington D.C. based and national consulting firm that provides services to enhance the safety and security of communities across the nation. G&H is a national leader in developing processes and the cost-effective technical capabilities to enable organizations to identify, acquire, store, manage, share, analyze and report on the data needed to make timely and informed decisions. During the COVID 19 pandemic G&H developed logistics management systems for various State emergency management agencies and partnered with AECOM to develop the Data Integrity and Reporting System as a chain of custody system for quality control over the data being analyzed from wastewater sampling.

Mrs. Stacie Reckling
GIS Analyst
NC Department of Health and Human Services, Division of Public Health | USA
Stacie.reckling@dhhs.nc.gov

Mrs. Stacie Reckling is a GIS analyst at the NC Department of Health and Human Services. She also works as a Research Associate at the Center for Geospatial Analytics at NC State University. By wearing 'two hats' Mrs. Reckling can use her geospatial research to enhance public health programs. Her creative thinking has led to the development of new GIS methods for wastewater surveillance including systematic sewershed delineation, and population weighted estimates of COVID-19 cases in the sewershed. In her free time, she enjoys hiking with her family, fiber arts and brewing new flavors of kombucha.

Dr. Rachel R. Spurbeck

Senior Genomics Research Scientist
Battelle Memorial Institute | USA
spurbeck@battelle.org

Dr. Rachel R. Spurbeck is a senior genomics research scientist at Battelle Memorial Institute and serves as a subject matter expert and principal investigator for wastewater-based epidemiology, metagenomics, forensic genomics, and emerging genomic technology studies. She has over 15 years of professional laboratory experience and is trained in genetics, bacterial pathogenesis, and biotechnology research and development. Dr. Spurbeck previously worked in the biotechnology industry, developing novel Next Generation Sequencing (NGS) library preparation kits and applications for whole genome sequencing, targeted sequencing methods including both amplicon and hybridization sequencing, transcriptomics, metagenomics, and epigenomics. She has published 19 peer-reviewed publications and book chapters with two manuscripts currently in press (full bibliography is at https://www.ncbi.nlm.nih.gov/sites/myncbi/1bgwvVrdtVTQQr/bibliography/57543137/public/?sort=date&direction=ascending).

Dr. Spurbeck will bring experience with wastewater sequencing the whole genome of SARS-CoV-2 to detect variants of importance to diagnostics and vaccine efficacy to the proposed study. She is currently the principal investigator on National Science Foundation grant number 2033137 where her team has successfully sequenced and distinguished variants in SARS-CoV-2 across time and space within the city of Toledo, Ohio. Her genomics laboratory will contribute biweekly sequencing for sites within the Ohio Coronavirus Wastewater Monitoring Network.

SWWS 204: Role of Standards in Supporting the Use of WWS Data

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***Session Chair*: Dr. Ted Smith**
Associate Professor
University of Louisville School of Medicine | USA
ted.smith@louisville.edu

Dr. Ted Smith has a breadth of experience and record of accomplishment in the academic, civic, and private sectors that will contribute to the success of the proposed research. In his career, he has specifically focused on delivering new models and methods for clinical research. Many of Dr. Smith's projects have included robust engagement between academic institutions and local government, and they often involve applications of new technologies with significant community participation. His interest in novel technological approaches dates back to his graduate and post- doctoral studies when he was awarded a NASA graduate fellowship to develop first-generation virtual environment technology studies to demonstrate a promising theory of sensorimotor integration which has since informed a generation of virtual reality space medical applications. Dr. Smith possess a decade of experience in developing new applications of digital technology for respiratory disease, directly associated with environmental factors such as air pollution. He also has extensive experience working in local government, having served as the city of Louisville, Kentucky's first Chief Innovation Officer. In that role, a major focus of his work was translating research to provide public health benefits. Dr. Smith's work was funded, in part, by the Robert Wood Johnson Foundation and was foundational to the city government prioritizing place-based clinical research. In his recent role as the Research Translation Core leader for the Louisville Superfund Research Center he has developed place-based environmental monitoring methods. Most recently, these methods have included wastewater monitoring of SARS-CoV-2 in Louisville Metropolitan area. Today Dr. Smith leads the adaptation and build-out of the university's wastewater-based epidemiology capability.

Speakers

Dr. Sandra McLellan
Professor
University of Wisconsin School of Freshwater Sciences | USA
mclellan@uwm.edu

Talk Title: Communicating Sewage Surveillance Data for a Public Health Response

Dr. Sandra McLellan's training in clinical laboratory sciences, medical microbiology, and environmental toxicology has given her the unique skill set to pursue questions related to microorganisms that flux between the primary habitat of human hosts and environmental reservoirs. For the past 12+ years Dr. McLellan has been funded by NIH to identify new indicators of waterborne disease by sequencing sewage and other fecal pollution sources and characterizing the microbial population structure. In her lab she has used novel computational approaches to detect ecologically relevant patterns and thus identified a number of human cut microbiome members that are specific to humans, highly abundant, and appear to be "steady state" in human population. She has also developed a sequenced based source identification platform FORENSIC that she has made available to the larger scientific community as an open source, web-based tool. A primary goal of her lab is to translate their discoveries into tools that can be used by public health. (See full bio at https://www.ncbi.nlm.nih.gov/myncbi/sandra.mclellan.1/cv/497151/)

Panel Discussion: **Need for Standards to Support Data Reporting and Analytics**

***Moderator*: Dr. Ted Smith** (see above for photo and bio)
Associate Professor
University of Louisville School of Medicine | USA
ted.smith@louisville.edu

Panelists:

Major Michael Dietrich, Ph.D.
Bioenvironmental Engineer
U.S. Air Force | USA
michael.t.dietrich4.mil@mail.mil

Dr. Michael Dietrich is a Bioenvironmental Engineer stationed at Tyndall Air Force Base in Panama City, Florida, where he is responsible for occupational health, environmental health, and radiation safety across the installation. Prior to his current assignment, he was an Air Force environmental health consultant. He has recently led a wastewater surveillance pilot study and participated in USAF and US Government working groups focused on the topic of wastewater surveillance, which has provided a glimpse of the promise and challenge associated with wastewater surveillance.

Ms. Rosa Inchausti
Deputy City Manager
City of Tempe | USA
Rosa_inchausti@tempe.gov

Ms. Rosa Inchausti's 28 years with the City have included many firsts. She began her career as the first bilingual Marriage and Family Therapist then forged the City's diversity efforts as the first Diversity Director. Her most recent promotion was to the role of the first Strategic Management and Diversity Director. In this role she is responsible for transforming the organization into a data-driven municipality and aligning the City Council's priorities into an actionable Strategic Plan. As co-chair of Tempe's Technology and Innovation Steering Committee, Ms. Inchausti identifies and recommends the adoption of new technologies and innovations that advance the quality of life for residents and businesses. Two notable projects that stemmed from this role was the creation of a roadmap for the integration of Autonomous Vehicles into a municipality and the launch of wastewater epidemiology via municipal infrastructure. Ms. Inchausti's areas of responsibility are Special Projects: Public Safety Advisory Task Force and Covid19 Recovery efforts. Most importantly, Ms. Inchausti's true passion and dedication is in prioritizing equity and inclusion in all city operations.

Ms. Inchausti was raised in Southern California and received a Bachelor's degree in Psychology from Loyola Marymount University. She then moved to Arizona and received a Master's in Counseling from Northern Arizona University. She has been a proud resident of Tempe for over 25 years where she raised her daughters Alexia and Andrianna.

Dr. Nathan LaCross

Wastewater Surveillance Program Manager
Utah Department of Health | USA
nlacross@utah.gov

Dr. Nathan LaCross is an epidemiologist with the COVID Active Response Team at the Utah Department of Health (UDOH). He received a Master of Public Health in 2006 and a doctorate in epidemiology in 2011, both from the University of Michigan. He first joined the Utah Department of Health in 2013 working with the Environmental Epidemiology Program on environmental health issues in Utah. Since March of 2020, he has been working on COVID-19 surveillance and assisting the state's pandemic response. In November 2020, Dr. LaCross moved to a new position within UDOH to manage Utah's wastewater surveillance program.

Dr. Sandra McLellan (see above for photo and bio)
Professor
University of Wisconsin School of Freshwater Sciences | USA
mclellan@uwm.edu

Mrs. Halley Reeves

Vice President of Community Health Impact
Oklahoma University Medicine | USA
Halley.Reeves@oumedicine.com

As Vice President of Community Health Impact for OU Medicine, Halley Reeves brings her public health practice and economic development background to bear in her work, offering health data analysis, planning and assessment, and various approaches to thinking about community health improvement efforts for the state's only comprehensive academic medical system. through her oversight of the health system's community benefit and as a result of the multi-disciplinary nature of her work, she focuses on cross-sectorial collaboration building with the aim of collectively impacting health and defining new ways to measure that impact.

Closing Remarks for Day 2

Mr. Philip J. Mattson

Department of Homeland Security (DHS), Science and Technology Directorate (S&T) | USA

Dr. Nancy J. Lin
National Institute of Standards and Technology (NIST) | USA

DAY 3: FRIDAY, JUNE 18, 2021

Welcome & Logistics

Mr. Philip J. Mattson

Department of Homeland Security (DHS), Science and Technology Directorate (S&T) | USA

Dr. Nancy J. Lin
National Institute of Standards and Technology (NIST) | USA

SWWS 301: Building an Enduring Capability

***Session Chair*: Mr. Philip J. Mattson**
Standards Executive
Department of Homeland Security (DHS), Science and Technology Directorate (S&T) | USA
Philip.mattson@hq.dhs.gov

Mr. Philip J. Mattson serves as the Department of Homeland Security (DHS) Standards Executive and Senior Standards Advisor in the DHS Science and Technology Directorate (S&T). He coordinates standards and conformity assessment activities across the Department and manages a broad portfolio of standards development activities including detection and personal protective equipment standards and response robot test method development.

Mr. Mattson is the DHS representative to the Interagency Committee on Standards Policy, and currently chairs the ASTM E54 Committee on Homeland Security Applications. He serves on the Board of Directors of the American National Standards Institute (ANSI) and also serves on several ASTM Technical Committees, the ANSI Unmanned Aircraft Systems Standardization Collaborative Steering Committee, and on standing committees in ANSI, the Society for Standards Professionals and in the Interagency Board for Emergency Preparedness and Response.

He holds a bachelor's degree in Nuclear Engineering Technology from Oregon State University, and a master's degree in Nuclear Physics from the Naval Postgraduate School. He has extensive training in nuclear weapons and radiological incident management and is a registered Professional Engineer. Mr. Mattson is a retired U.S. Army officer; serving over 20 years as a combat engineer and nuclear physicist.

Speakers

Dr. Kimberli Miller
Wildlife Disease Specialist
U.S. Geological Survey (USGS) National Wildlife Health Center | USA
kjmiller@usgs.gov

Talk Title: Making It Count-Keeping It Available: Wildlife Disease Situational Awareness

Healthy and resilient wildlife, domestic animal, plant, human, and water resources all contribute to a healthy thriving ecosystem. Compounding stressors affect resiliency and can lead to disease outbreaks. Worldwide, over 40% of emerging diseases are estimated to have a wildlife connection1. Knowing where and when wildlife diseases are occurring and what species are involved is important for not only for understanding immediate disease risks but also the factors contributing to wildlife resiliency. The USGS National Wildlife Health Center (NWHC) is the only federal Biosafety level 3 diagnostic and research facility in the US focused on wildlife health and disease. Working with State, Federal, and Tribal partners, NWHC has amassed an extensive 45+ year collection of wildlife diagnostic and mortality event information including passive and active disease surveillance. In addition, state department of natural resource agencies generate data through their own wildlife health programs. These disparate data sources are important for real-time situational awareness and biosurveillance efforts but difficult to access. To address this data sharing need, NWHC created the Wildlife Health Information Sharing Partnership-event reporting system (WHISPers) through partnership and funding by the Department of Homeland Security – Countering Weapons of Mass Destruction - National Biosurveillance Integration Center. This on-going project has required careful attention and creative solutions to curating disease data for public and partner use. (Jones and others. 2008. Global trends in emerging infectious diseases. Nature.)

Dr. Kimberli Miller has been a Wildlife Disease Specialist at the USGS National Wildlife Health Center since 1992. She has a Doctor of Veterinary Medicine degree and a BS degree in Animal Science from the University of Missouri-Columbia. Since joining the NWHC, Kim has worked on disease issues and questions across the country. This work has allowed Kim to practice non-traditional veterinary medicine and be involved in wildlife conservation on a large scale. One long term project involved representing NWHC as a founding partner in reintroducing whooping cranes to the Eastern US. Presently her efforts have been focused on data management and making Center wildlife mortality information more available for use by internal and external users. She has a DVM and a BS in Animal Science from University of Missouri-Columbia.

Dr. Jayne B. Morrow
Assistant Vice President of Research and Economic Development
Montana State University | USA
jayne.morrow@montana.edu

Talk Title: Documentary Standards for Biological Response

Dr. Jayne B. Morrow has led a broad portfolio of research program and science policy development relevant to a range of priorities in the United States and abroad including dynamic technical and policy challenges presented by the biosurveillance and biological threat response communities. Dr. Morrow has demonstrated a career working across stakeholders to foster engagement, create strategic vision and build consensus including development of standards on a range of technical program and public policy areas including national security, environmental health, public health and safety and law enforcement for response to Anthrax, Ebola, SARS-CoV-2 and applied these same principles to the analytical characterization of the opioid epidemic. Dr. Morrow formerly led national science and technology (S&T) strategic policy development as the Executive Director of the National Science and Technology Council in the Executive Office of the President during the Obama Administration. Prior to that position she led biothreat response and metrology for biological science programs at the National Institute of Standards and Technology. Her research efforts have resulted in 45 peer-reviewed articles, reports and standards; over 200 technical presentations and operational exercises. Recently, to enhance the response to COVID-19, Dr. Morrow partnered with motivated volunteers to form a non-for-profit entity, CLEAN2020 Summit, to bring together leaders from business, policy, standards development, science and engineering to better understand current knowledge and identify opportunities to work together to control viral transmission in the built environment. These efforts continue to help translate the research, standards and guidance into practice. She currently serves as the Assistant Vice President of Research and Economic Development at Montana State University where she is working to develop stronger connections between science, technology and research among academia, industry and government agencies. She has a B.Sc. degree in Civil Engineering from Montana State University as well as a M.S. and Ph.D. in Environmental Engineering with a specialty in molecular and microbiology from the University of Connecticut.

Dr. Orin C. Shanks
Senior Scientist
Environmental Protection Agency (EPA) | USA
shanks.orin@epq.gov

Talk Title: Development and Performance of NIST SRM 2917 for Molecular Recreational Water Quality Testing

Dr. Orin Shanks is a Senior Scientist for the United States Environmental Protection Agency in the Office of Research and Development Center for Environmental Measurement and Modeling. Dr. Shanks has been with the EPA for over 15 years. His chief research interests include molecular method development and implementation, fecal source identification, nucleic acid fate and transport, and wastewater surveillance. Dr. Shanks received his undergraduate and master's degrees from the University of Wyoming and his Ph.D. from Oregon State University.

Ms. Patsy Root

Regulatory Affairs Manager
IDEXX Water | USA
patsy-root@idexx.com

Talk Title: Draft Accreditation Checklist for Wastewater COVID PCR Testing
This talk will review the need for, and development of, a checklist for laboratories to follow when performing and requesting accreditation for methods that test for SARS CoV-2 virus in wastewater.

Ms. Patsy Root received her M.S. in Biochemistry from University of Maine, Orono. Ms. Root has over 14 years' experience in water microbiology, water-related regulations and environmental laboratory accreditation. She has worked with a variety of regulatory agencies worldwide in attaining regulatory approval for IDEXX methods and on Validation/verification of methods. She is a long-time participant in various standards development organizations including with TNI, Standard Methods for the Examination of Water and Wastewater and ASHRAE. Ms. Root is an active member of the following organizations:

- American Public Health Laboratories; Environmental Laboratory Science Committee, member ASHRAE, Standard 188:2015, Guideline 12:2000, ASHRAE 514 on Legionellosis prevention
- American Water Works Association (AWWA), member
- AOAC, Water and Wastewater subcommittee, Chair
- American Council of Independent Laboratories (ACIL),
- Environmental Science Section (ESS), member
- Wastewater/COVID Accreditation Task Group, Chair
- Canadian Water Works Association (CWWA), Drinking Water Committee, member
- Environmental Laboratory Advisory Board (ELAB), a US EPA Federal Advisory Committee Act (FACA) Board, reporting the US EPA Administrator; past Chair
- National Environmental Monitoring Conference (NEMC), Session Coordinator
- Standard Method for the Examination of Water and Wastewater, Part 9000, reviewer and contributor; Part 9213 Joint Task Group
- TNI, The National Environmental Laboratory Accreditation Conference
- Executive Board of Directors, Secretary of the Board
- Policy Committee, Chair

- Microbiology Expert Committee, Past Co-Chair
- Microbiology Proficiency Testing Committee, member

Dr. Amy E. Kirby

National Wastewater Surveillance System Program Lead
Centers for Disease Control and Prevention (CDC) | USA
agk1@cdc.gov

Talk Title: Enduring Capability: NWSS Perspective

Dr. Amy E. Kirby is an Environmental Microbiologist in the Waterborne Disease Prevention Branch and the Program Lead for the National Wastewater Surveillance System (NWSS) at the Centers for Disease Control and Prevention (CDC). She has a Bachelor of Science in Agriculture (BSA, major: Microbiology) from the University of Georgia, a PhD in Microbiology from the University of Buffalo, SUNY, and a Master's of Public Health in Epidemiology from Emory University. At CDC, Dr. Kirby is interested in leveraging environmental microbiology methods to measure pathogens, antibiotic resistance genes, and other health indicators in natural and man-made water systems. This data can be used to estimate health risks from environmental exposures, as well as measures of the health of the surrounding communities. Since February 2020, she has been working on the COVID-19 response as part of the Water, Sanitation, and Hygiene team. As part of that team, she led the development and implementation of NWSS.

Dr. Tonya Lynn Nichols
Senior Science Advisor
Environmental Protection Agency (EPA) | USA
Nichols.tonya@epa.gov

Talk Title: Enduring Capability: One Health Perspective

Mr. Jeffrey A. Wenzel
Chief, Bureau of Environmental Epidemiology
Missouri Department of Health and Senior Services | USA
Jeff.Wenzel@health.mo.gov

Talk Title: Enduring Capability: Public Health Decision Maker Perspective
Presentation will discuss what would be needed for the nation to build an enduring capability in wastewater surveillance overall as it relates to standards (e.g., documentary standards, reference, materials, methods and data comparability).

Mr. Jeffrey A. Wenzel has worked for the Missouri Department of Health and Senior Services for 19 years. During that time, he has served multiple roles including laboratory scientist, environmental specialist, epidemiologist, and has served in his current role of Bureau Chief of the Bureau of Environmental Epidemiology for the last 3 years.

Dr. George A. Conway
Director
Deschutes County Health Services Department | USA
george.conway@deschutes.org

Talk Title: Enduring Capability: A Local Health Department Perspective
We have piloted and now routinely use wastewater sampling for supplemental SARS-CoV2 (COVID-19) genomic surveillance. This method is promising for early detection of arrival or re-emergence of pathogens, and also adds to our local screening for variants of interest (more pathogenic or more readily transmissible strains). To become a generally effective, reliable surveillance tool for early warning for this and other pathogens, the methods used for concentrating and testing samples will need to be better standardized, and the overall detection level made more sensitive, reliable, and reproducible in different laboratories.

George A. Conway, MD, MPH is a physician and epidemiologist known for his work in outbreak investigation, epidemic response, environmental health, and human adaptation to extreme environments. Director of the Health Services Department for Deschutes County, Oregon since 2016, Dr. Conway previously served in multiple senior level positions with the US Centers for Disease Control and Prevention (CDC), including 2012 through 2015 as CDC Senior Advisor to the Chinese Centers for Disease Control and as Public Health Attaché for the U.S. Embassy in Beijing, concentrating on the epidemiology and mitigation of air pollution health effects and epidemic response. He also served as Senior Medical Officer and Epidemiologist for the United Nations Mission for Ebola Emergency Response in Liberia during the West Africa epidemic, November 2014 to June 2015.

Dr. Larry Madoff

Medical Director, Bureau of Infectious Disease and Lab Science
Massachusetts Department of Public Health | USA
larry.madoff@mass.gov

Talk Title: Wastewater Surveillance for COVID in Massachusetts

Dr. Larry Madoff is an infectious disease physician specializing in the epidemiology of emerging pathogens, bacterial pathogenesis, and international health. He is Professor of Medicine at the University of Massachusetts Medical School and Lecturer on Medicine at Harvard Medical School. Dr. Madoff serves as Medical Director of the Bureau of Infectious Disease and Laboratory Sciences for the Massachusetts Department of Public Health.

Dr. Madoff directed the International Society for Infectious Diseases' Program for Monitoring Emerging Diseases (ProMED), from 2002 to 2021. He is a member of the American Society for Microbiology, Massachusetts Medical Society, past President of the U.S. Lancefield Streptococcal Research Society, a Fellow of the Infectious Diseases Society of America and a Fellow of the American College of Physicians. A graduate of Yale College and Tufts Medical School, he performed his Internal Medicine Residency at New York Hospital-Cornell Medical Center and his Infectious Disease Fellowship at the Harvard Medical School-Longwood program.

***Panel Discussion:* Enduring Capability and Role of Standards**

***Moderator*: Mr. Philip J. Mattson** (see above for photo and bio)
Standards Executive
Department of Homeland Security (DHS), Science and Technology Directorate (S&T) | USA
Philip.mattson@hq.dhs.gov

Panelists:

Dr. George A. Conway (see above for photo and bio)
Director

Deschutes County Health Services Department | USA
george.conway@deschutes.org

Dr. Amy E. Kirby (see above for photo and bio)
National Wastewater Surveillance System Program Lead
Centers for Disease Control and Prevention (CDC) | USA
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Dr. Larry Madoff (see above for photo and bio)
Medical Director, Bureau of Infectious Disease and Lab Science
Massachusetts Department of Public Health | USA
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Dr. Tonya Lynn Nichols (see above for photo and bio)
Senior Science Advisor
US Environmental Protection Agency | USA
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Mr. Jeffrey A.Wenzel (see above for photo and bio)
Chief, Bureau of Environmental Epidemiology
Missouri Department of Health and Senior Services | USA
Jeff.Wenzel@health.mo.gov

CONCURRENT BREAKOUT SESSIONS

SWWS 302: Methods and Data Comparability Breakout

Session Co-Leads

Dr. Scott A. Jackson

Leader of Microbial Metrology Group, Material Measurement Laboratory
National Institute of Standards and Technology (NIST) | USA
scott.jackson@nist.gov

Dr. Scott A. Jackson leads efforts internationally to improve microbiome and pathogen detection measurements through the development of standards. (See full bio at: https://www.linkedin.com/in/thescottjackson/)

Dr. Mia Mattioli
Environmental Engineer
Centers for Disease Control and Prevention (CDC) | USA
kuk9@cdc.gov

Dr. Mia Mattioli is the Principle Investigator for the CDC's Waterborne Disease Prevention Branch's Domestic WASH Lab within Environmental Microbiology and Engineering Laboratory Team. Her research focuses on the intersection between the environment and human health with a specific interest in the relationship between, and fate and transport of, fecal indicators and enteric pathogens. Her lab is engaged in a wide range of environmental research areas including drinking water, irrigation water, wastewater, recreational water, soil, food, water distribution systems, and the development of advanced molecular detection technologies. Dr. Mattioli also leads CDC's environmental investigations of waterborne outbreak responses and currently serves as the Science Lead for the CDC National Wastewater Surveillance System. She has a Bachelor of Science in Biological Engineering from the University of Georgia and a Master and Ph.D. in Environmental Engineering from Stanford University.

Ms. Sarah Wright
Environmental Laboratories Manager
Association of Public Health Laboratories | USA
wright.sarah@gmail.com

Ms. Sarah Wright, MS, is an environmental laboratories manager at the Association of Public Health Laboratories in Silver Spring, MD. In this role, she works to strengthen state and local environmental laboratories. Current projects include working with the CDC National Wastewater Surveillance System to build public health laboratory wastewater surveillance testing capacity. Previously, she conducted watershed assessments in Wisconsin for the City of Racine and worked on national water policy at The Johnson Foundation at Wingspread. She has a master's degree in environmental monitoring from Ohio University and an environmental science and policy degree from Duke University.

Session Scribe: **Dr. Paulina K. Piotrowski**
Research Chemist
National Institute of Standards and Technology (NIST) | USA
paulina.piotrowski@nist.gov

Dr. Paulina Piotrowski is currently a Research Chemist within the Organic Chemical Metrology Group at the National Institute of Standards and Technology (NIST) in Gaithersburg, MD. Dr. Piotrowski is an expert in untargeted mass spectrometry-based analyses of complex matrix samples. At NIST, she develops omics-based measurement techniques and standards for the microbiome and complex microbial systems.

SWWS 303: Reference Materials Breakout

Session Co-Leads

Dr. Katrice A. Lippa

Leader, Organic Chemical Metrology Group
National Institute of Standards and Technology (NIST) | USA
katrice.lippa@nist.gov

Dr. Katrice Lippa obtained her Ph.D. in environmental chemistry from Johns Hopkins University in 2002 and then joined NIST as a research chemist after completing an NRC postdoctoral fellowship in 2005. Dr. Lippa is currently Leader of the Organic Chemical Metrology Group within the Material Measurement Laboratory at NIST, a position she has held since 2016. She manages numerous laboratory QA/QC products and programs for clinical diagnostics and metabolomics, food nutrition and safety, natural and plant-based products, chemical manufacturing, forensics and drug standard sectors.

Dr. Orin C. Shanks
Senior Scientist
Environmental Protection Agency (EPA) | USA
shanks.orin@epq.gov

Dr. Orin Shanks is a Senior Scientist for the United States Environmental Protection Agency in the Office of Research and Development Center for Environmental Measurement and Modeling. Dr. Shanks has been with the EPA for over 15 years. His chief research interests include molecular method development and implementation, fecal source identification, nucleic acid fate and transport, and wastewater surveillance. Dr. Shanks received his undergraduate and master's degrees from the University of Wyoming and his Ph.D. from Oregon State University.

Ms. Briana Benton
Technical Manager
ATCC | USA
bbenton@atcc.org

Ms. Briana Benton is the Technical Manager for ATCC's Sequencing and Bioinformatics Center (SBC). She is a key member leading the development of the ATCC Genome Portal and is currently responsible for the next-generation sequencing (NGS) of the various collections within

ATCC. During her time with ATCC, she has developed numerous microbiome standards, as well as molecular assays for the authentication of infectious viral and bacterial pathogens.

Session Scribe: **Dr. Stephanie L. Servetas**
Microbiologist
National Institute of Standards and Technology (NIST) | USA
stephanie.servetas@nist.gov

Dr. Stephanie L. Servetas is a microbiologist in the Complex Microbial Systems Group at the National Institute of Standards and Technology (NIST). Her research is focused on whole cell and multi-omic measurements of microbial communities, both natural and contrived. While at NIST, Dr. Servetas has worked on the development of reference materials for pathogen detection and development of the Whole Stool Gut Microbiome reference material.

SWWS 304: Documentary Standards – Guidance Documents Breakout

Session Co-Leads

Dr. Nancy J. Lin

Biomaterials Group Leader, Material Measurement Laboratory
National Institute of Standards and Technology (NIST) | USA
nancy.lin@nist.gov

Dr. Nancy J. Lin is the Leader of the Biomaterials Group in the Biosystems and Biomaterials Division of the Material Measurement Laboratory at NIST. Her research focuses on developing measurements and standards to enable the detection and quantification of microbes and microbial communities, with an emphasis on total and viable cell count for microbial cell reference materials, biofilm-material interactions, antimicrobial efficacy, and biosurveillance. Dr. Lin holds a BS in Mechanical Engineering from Valparaiso University and a PhD in Biomedical Engineering from Case Western Reserve University.

Ms. Patsy Root

Regulatory Affairs Manager
IDEXX Water | USA
patsy-root@idexx.com

Ms. Patsy Root received her M.S. in Biochemistry from University of Maine, Orono. Ms. Root has over 14 years' experience in water microbiology, water-related regulations and environmental laboratory accreditation. She has worked with a variety of regulatory agencies worldwide in attaining regulatory approval for IDEXX methods and on Validation/verification of methods. She is a long-time participant in various standards development organizations including with TNI, Standard Methods for the Examination of Water and Wastewater and ASHRAE.

Session Scribe: **Dr. Sandra Da Silva**
Research Chemist
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***Session Scribe:* Dr. Joy P. Dunkers**
Physical Scientist
National Institute of Standards and Technology (NIST) | USA
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SWWS 401: Summary of Breakout Sessions

***Session Chair*: Mr. Yonas Nebiyeloul-Kifle**
Program Manager
Department of Homeland Security (DHS), Science and Technology Directorate (S&T) | USA
yonas.nebiyeloul-kifle@hq.dhs.gov

Mr. Yonas Nebiyeloul-Kifle coordinates voluntary consensus standards and related measurement science R&D projects for DHS mission needs to include grants, procurement, systems engineering, S&T policy and tech-transfer projects.

Final Remarks & Next Steps

Mr. Philip J. Mattson

Department of Homeland Security (DHS), Science and Technology Directorate (S&T) | USA

Dr. Nancy J. Lin
National Institute of Standards and Technology (NIST) | USA

ACKNOWLEDGEMENTS

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Mr. Philip J. Mattson
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Dr. Ted Smith
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Mr. Paul Storella
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Ms. Sarah Wright (also Session Chair or Panel Moderator, Breakout Co-Lead)
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Workshop Logistics Team

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Ms. Lori Clark
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Ms. Jennifer Dootz
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Dr. Joy Dunkers
National Institute of Standards and Technology (NIST) | USA

Ms. Alexis Ecarma
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Ms. Monique Hunter
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Dr. Christina Jones
National Institute of Standards and Technology (NIST) | USA

Mr. Shaswat Koirala
National Institute of Standards and Technology (NIST) | USA

Dr. Jason Kralj
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Dr. Alshae' Logan
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Dr. Kirsten Parratt
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Dr. Paulina Piotrowski
National Institute of Standards and Technology (NIST) | USA

Dr. Stephanie Servetas
National Institute of Standards and Technology (NIST) | USA

Appendix C

Workshop Overview Slides

DHS/NIST Virtual Workshop: Standards to Support an Enduring Capability in Wastewater Surveillance for Public Health

GOAL: To identify and prioritize standards needs and technology/measurement gaps and propose a path forward to develop standards that enable a sustained capability in wastewater surveillance that provides high confidence, representative, comparable results to inform public health and safety decisions across the nation.

EXPECTED OUTCOME: Stakeholder input will inform standards development activities, including consensus-based documentary standards, reference methods and protocols, and reference materials, and ensure that efforts are fit for purpose and aligned with the needs of the community.

INTENDED PARTICIPANTS: Those involved in all aspects of wastewater surveillance from sampling to results/reporting, including representatives from government, public health, testing and manufacturing, academia, and non-profit organizations.

Technical Program

Day 1: Monday, June 14, 10 AM to 3 PM (ET)

Day 2: Tuesday, June 15, 10 AM to 3 PM (ET)

- National and International Case Studies in COVID-19 Surveillance: Lessons Learned and Challenges Remaining

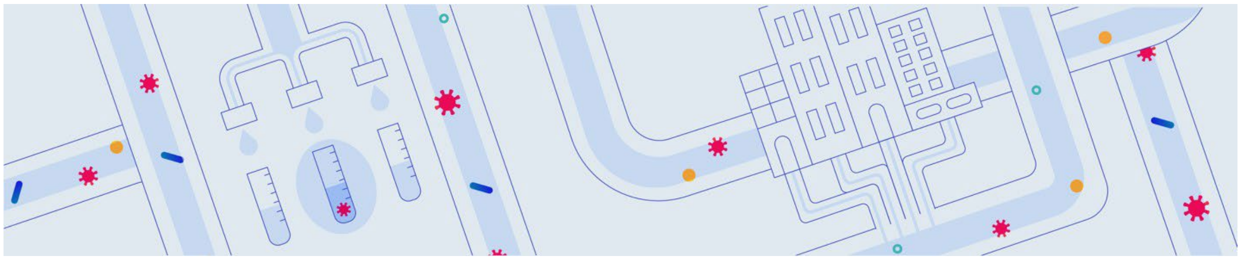
Day 3: Friday, June 18, 10 AM to 3 PM (ET)

- Next Steps Toward Standards to Help Build and Sustain an Enduring Wastewater Surveillance Capability

Organizing Committee Members

- AECOM
- Army Public Health Center
- Association of Public Health Laboratories
- CDC
- DHS S&T
- EPA
- NIST
- University of Louisville
- USGS

Appendix D: Poster Abstracts



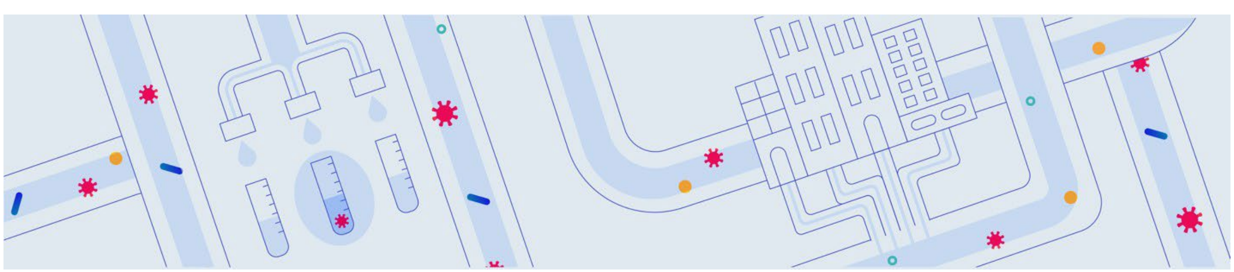
DHS/NIST Workshop: Standards toSupport an Enduring Capability in Wastewater Surveillance for PublicHealth

Poster Abstracts

June 14-18th 2021

Virtual Poster Session

https://swwsworkshop.virtualpostersession.org/



CEDAR-MC: Clinical and Environmental Dynamics of Antibiotic Resistance withinMicrobial Communities

George Hanna[1,2], Bashir Hamidi[1], Scott Curry[1], Cheryl Carmack[2], Alexander V.

Alekseyenko[1][1]Medical University of South Carolina; [2]Charleston Waterkeeper

Introduction: Escape into the environment and the persistence of antibiotic resistance is an imminent threat to the healthcare advances attained in the 20th century. Microbial communities that co-exist with resistant bacteria may help uncover novel strategies for global antimicrobial control and curb emergence and maintenance of resistance. However, availability of clinically relevant specimens with complementary samples from the built andnatural environment is a major obstacle to effective studies of the dynamics of resistance in the affected human populations and in their surroundings.

Methods: We bring together environmental and clinical measurements of the microbial communities with evidence for emerging resistance by linking existing local clinical and environmental surveillance programs. The clinical specimens are sourced from the Medical University of South Carolina (MUSC) infection surveillance culture program that routinely samples the MUSC patient population for clinically relevant pathogens. The environmental specimens are the result of partnership with a local non-profit, Charleston Waterkeeper, that performs water quality monitoring at popular recreational spots in the area.

Results: The surplus infection surveillance specimens are made available for research via a Living µBiome Bank system that enables nuanced electronic phenotyping of patient populations for just-in-time capture of clinical microbiology specimens. We show the feasibility of sequencing these clinical specimens for microbiota composition, which in combination with resistance status is invaluable in characterizing the broader structure of resistant microbial communities. The environmental specimens collected from May 2018 through October 2020 have been banked to be assessed for evidence of antibiotic resistance of their constituent microbiota.

Discussion: We continue to expand the resources and the biobanks associated with the clinical and environmental surveillance programs with an overarching goal to control infections by understanding the dynamics of emergence and maintenance of resistance. This project demonstrates the value of collaboration between academic healthcare and community-funded environmental monitoring programs.

Key words: antibiotic resistance, living biobank, water quality

Standards and control materials used: *Yes*

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A Step-By-Step Approach to Evaluating SARS-CoV-2 Methods: Adaptability for Future Use with Other Microorganisms

Brienna L. Anderson-Coughlin[1] and Kalmia E. Kniel[1]

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There is a breadth of research regarding enteric viruses in agricultural waters and using viruses such as pepper mild mottle virus (PMMoV) as fecal contamination indicators. Traditionally used in food safety research, PMMoV is now being employed for wastewater surveillance efforts during the COVID-19 pandemic. The purpose of this study was to determine how existing wastewater surveillance methods for SARS-CoV-2 could be adapted for other microorganisms, using PMMoV as a model. Wastewater was collected across New Castle County, Delaware, incubated, filtered, and concentrated. Viral concentrates were extracted, and detection performed via RT-qPCR for PMMoV and the N1 and N2 SARS-CoV-2 targets. Variability in recovery along with impacts of incubation (none or 60°C for 60 min) and refrigerated storage (0-hour or 24-hour) were evaluated. Delta cycle threshold (dCT), the number of cycles before completion (CT=40) at which the target amplified across the threshold (e.g., CT38=dCT2).

PMMoV was detected in all (n=48) replicates for incubated and non-incubated samples with 0- and 24-hour holds. SARS-CoV-2 N1and N2 were detected in 95.8% (n=46) of incubated samples in each treatment of 0 and 24 hour holds, in 79.2% (n=19) of non-incubated with 0-hour hold, and 50% (n=12) of non-incubated with 24-hour hold. PMMoV detection in samples (n=6) was significantly ($p<0.05$) greater than N1 and N2. PMMoV increased by 3.16 ± 1.18 dCT in processed (9.25 ± 1.15 dCT) samples compared to unprocessed (6.09 ± 0.98 dCT). N1 and N2 increased by 0.77 ± 1.38 and 0.63 ± 1.32 dCT in processed (0.95 ± 1.37 and 1.04 ± 1.29 dCT) compared to unprocessed (0.18 ± 0.61 and 0.41 ± 0.90 dCT), respectively.

The detection and recovery efficacy of the method explored here confirmed the potential for its adaptation for use with additional organisms. These data provide evidence that with appropriate modifications, the methods and infrastructure created during the COVID-19 pandemic and currently used for SARS-CoV-2 surveillance, can be successfully utilized for viral, bacterial, and parasitic organisms of interest.

Key words: Wastewater surveillance, Method Adaptation, Viruses

Standards and control materials used: Positive and negative controls for this study consisted of plasmid gene sequences (IDTDNA: 10006625) and nuclease-free water (Qiagen: 129114), respectively.

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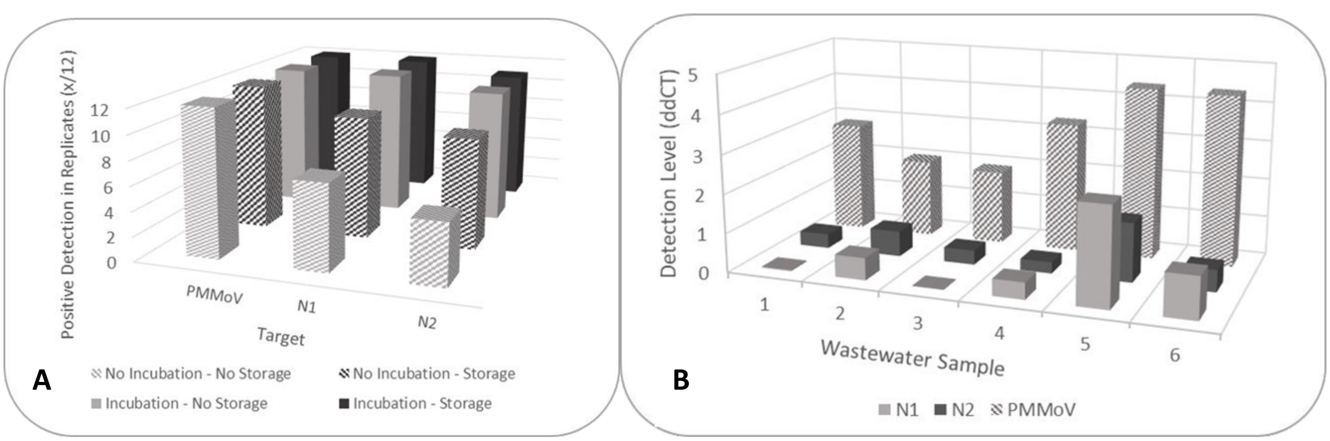

Figure 1. The detection of PMMoV and SARS-CoV-2 (N1 and N2 genes) from wastewater samples. A. The number of replicates (n=12) for each target in which positive detection occurred, treatments evaluated included the incubation (60C for 60 minutes) or no incubation and storage (4C for 24 hours) or no storage prior to completing wastewater filtration, concentration, nucleic acid extraction and detection. B. The detection level, represented by ddCT values (the difference in CT between unprocessed and processed samples), of targets for each of the six samples processed in triplicate. Large variation in recovery was observed across wastewaters and between PMMoV and SARS-CoV-2.

The impact of data on source control

Anne-li Steutel-Maron
Kando

This abstract will elaborate on how we should rethink wastewater for water reuse, and data's role in securing consistently high-quality wastewater. Raw wastewater is a valuable raw material. Having a consistent, stable, and high quality raw material is essential for maximizing its value. For wastewater, controlling the quality of inputs to the collection network is vital to realizing its value as a resource. Optimized 'source control' cannot rely on random sampling, as water-reuse requires high quality water that needs to rely on precise, reliable, and continuous flow of data, giving reuse plants and service providers a total understanding of their water quality.

Key words: waterreuse Key word 1; source control Key word 2; data

Standards and control materials used: **ISO 5667-10:2020**

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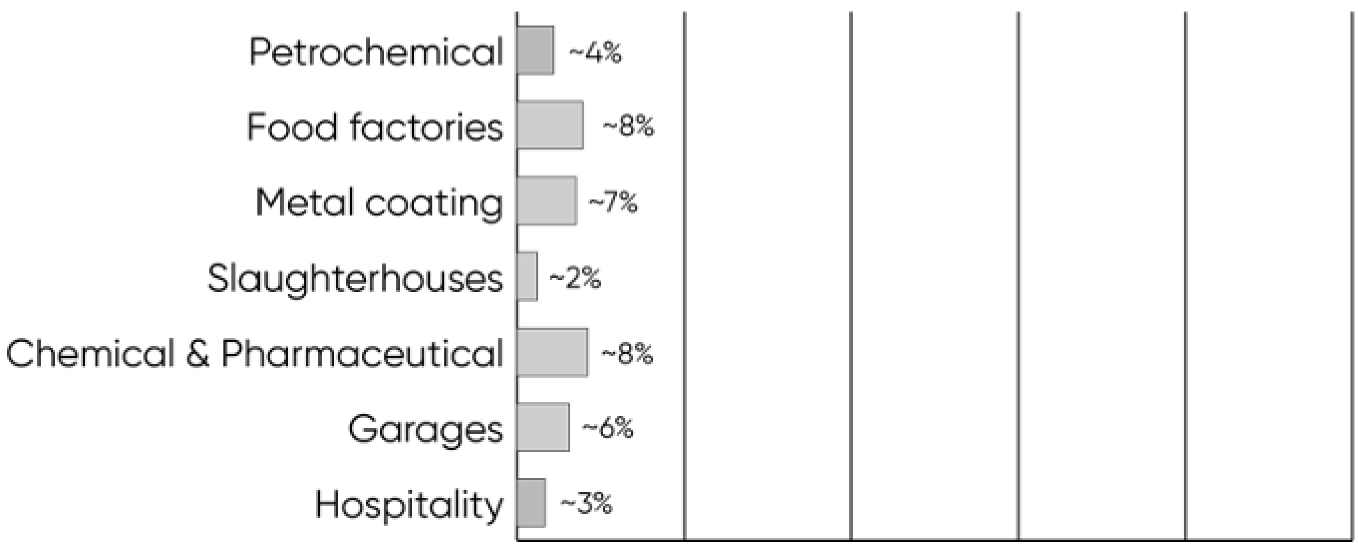

Figure 1. Pollution found with grab sampling

Figure 2. Pollution found with smart monitoring & sampling

Optimizing Sampling Strategies for Large Rural Regions

Anuj Tiwari, Aaron Packman, Charles Williams, Wilnise Jasmin, Rachel Poretsky, Wayne Duffus, SarahPatrick, Leslie Wise, Charlie Catlett[1]

Corresponding author: Charlie Catlett, ccatlett@uillinois.edu

Abstract

Wastewater Based Epidemiology (WBE) typically focuses on wastewater treatment plants (WWTPs) and the corresponding community. However, many public health questions require insight across many communities such as at the county or state level. What is the optimal sampling strategy in regions such as rural counties with dozens of treatment plants and private septic systems, or in entire states with many such counties?

The State of Illinois is a primarily rural region comprising 58k square miles with a population of roughly 16M, over half of whom live within 50 miles of Chicago. Outside of the Chicago area, Illinois has roughly 140 cities with populations greater than 10k and nearly 1,000 with populationsunder 10k. To understand and track infectious disease such as COVID-19 across these communities, a comprehensive WBE sampling plan would involve over 1,000 WWTPs and countless private septic systems.

In Sep 2020, we introduced a machine learning based COVID-19 Vulnerability Index (C19VI) using CDC's six themes: (a) socioeconomic status, (b) household composition & disability, (c) minority status & language, (d) housing type & transportation, (e) epidemiological factors, and (e)healthcare system factors[2]. This model uses an ensemble learning approach with recursive partitioning to optimally compute non-linear relationships between input themes. We refined the model in early 2021 with additional demographic and sequencing information to evaluate WBE sampling strategies across Illinois counties to support an expansion of WBE from currently severaldozen to over 150 WWTPs. The vulnerability index supported the evaluation of various samplingstrategies, such as selecting the largest population centers within each of the eleven COVID-19 "Restore Illinois" regions[3], and eventually the current strategy of sampling from the largest population center in each of Illinois' 102 counties. In this poster we outline the models and methods used, including various sampling strategies explored, the current strategy, and experiments planned to evaluate the sampling strategy as we expand to 150 WWTPs in 2021.

Keywords: COVID-19; Wastewater Based Epidemiology (WBE); Wastewater Sampling Strategy; COVID-19 Vulnerability Modeling; Machine Learning (ML)

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[1] Tiwari and Catlett are from the University of Illinois Discovery Partners Institute; Packman is from Northwestern University; Poretsky is from the University of Illinois-Chicago; Jasmin is from the Chicago Department of Public Health; and Williams, Duffus, Patrick, and Wise are from the Illinois Department of Public Health.

[2] Tiwari, Anuj, Arya V. Dadhania, Vijay Avin Balaji Ragunathrao, and Edson RA Oliveira. "Using machine learning to develop a novel COVID-19 Vulnerability Index (C19VI)." *Science of The Total Environment* 773(2021): 145650.

[3] Restore Illinois. Available at https://www.dph.illinois.gov/restore

Parameters influencing the methods of detection for SARS-CoV-2 RNA wastewater surveillance using RT-PCR quantification

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[a]The University of Ontario Institute of Technology

Abstract

The COVID-19 pandemic presented many challenges to public health units attempting to track infected individuals by performing individual clinical testing of large populations. Due to the lack of trained personnel and shortage of testing materials, many undiagnosed but infected individuals caused substantial viral spread within communities[1]. Thus, it is crucial that alternative surveillance methods are explored, which can provide public health organizations with information on the number of infected individuals. Such methods allow for real-time allocation of resources, staff, and restrictive measures to reduce further spread. Wastewater surveillance of viral RNA has emerged as a surprising approach to track and monitor the presence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in communities. Considering the novelty of the methods used, the aim of this study was to determine which parameters are suitable in order to store, pasteurize, and concentrate the viral particles found in raw wastewater influent. Six wastewater treatment plants (WWTPs) provided 500mL samples three times a week from different municipalities in the Durham Region, Ontario, Canada. Storage conditions were investigated by storing 30mL raw influent at 4◦C, -20◦C, -80◦C for 7, and 14 days and analyzed using a Three-Way ANOVA ($p<0.005$). Pre-treatment of the samples included pasteurization for 60 mins at 60◦C analyzed using t-tests ($p<0.005$), and concentrated

using polyethylene glycol (PEG) for either 0 or 12 hours and analyzed using a t-test ($p<0.005$). Combined, this study presents recommendations for developing reliable, accurate, sensitive and reproducible estimation of the evolution of the SARS-CoV-2 virus in wastewater.

Keywords: Covid-19, SARS-CoV-2, Wastewater, RT-qPCR, Pasteurization, Viral Concentration

Standards and control materials used: Sars-CoV-2 Standard (EDX), pMMoV gBlock (IDT)

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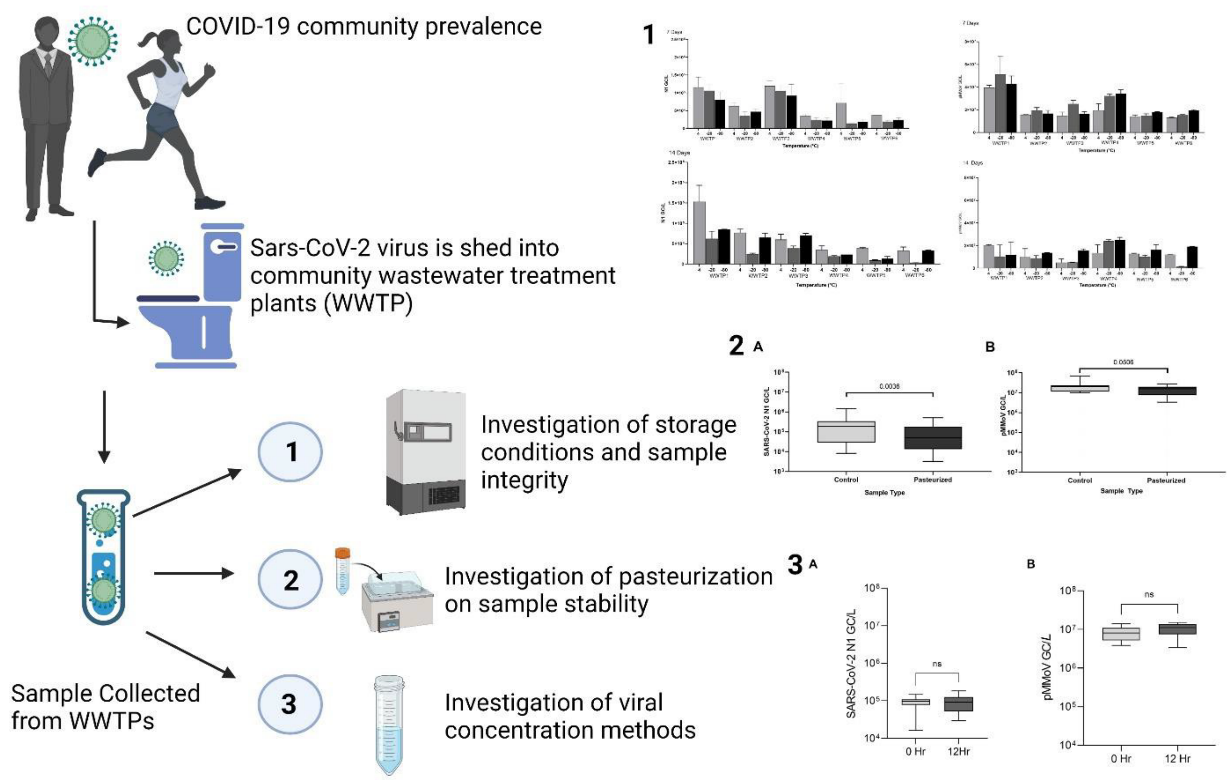

References

1. Wang,W., et al., Detection of Sars-CoV-2 in Different Types of Clinical Specimens. JAMA, 2020 323(18): p.1843-1844.

Metagenomics for Monitoring Antibiotic Resistance in Water and Wastewater: Key Considerationsand a Path Towards Standard Protocols

Benjamin C. Davis[1], Jeannette Calarco[2], Krista Liguori[1], Erin Milligan[1], Valerie J. Harwood[2], Amy Pruden,[1] Ishi Keenum[1*]

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Environmental dimensions of antibiotic resistance are increasingly being recognized and are essential to a One-Health framework for combatting antibiotic resistance [1]. Strategies are emerging for comprehensive surveillance of resistomes (i.e., all antibiotic resistance determinants carried across a microbial community) in surface water, wastewater, and recycled water matrices to both establish a baseline and to monitor for changes in resistance patterns that occur over time [2]. Metagenomics, i.e., the study of an entire microbial community's genomic information via shotgun next-generation sequencing (NGS), is emerging as a powerful tool for characterizing these aquatic resistomes. Metagenomics is an attractive approach to monitoring because it theoretically allows for the simultaneous detection of all antibiotic resistance genes (ARGs), mobile genetic elements (MGEs), as well as the microbial composition in an environmental matrix without *a priori* knowledge of targets, circumventing the narrow target scopes of both quantitative polymerase chain reaction and culturing. With short- and long-read sequencing technologies, the contextualization of ARGs (e.g., their positions on MGEs and taxonomic affiliation) are also further being realized [3]. However, NGS approaches to water quality monitoring have only been applied in the last decade and guidance is still needed with respect to sampling, DNA extraction, library preparation, sequencing platforms, sequencing depths required for unique matrices and targets, and metrics that are ultimately mined and derived from the raw sequencing data.

Here we conducted a comprehensive, systematic literature review of articles that use NGS to investigate the resistomes of surface water, wastewater, and recycled water. We identified 98 peer-reviewed papers meeting our search criteria and systematically compiled and compared sample processing workflows, sequencing approaches, and bioinformatic analyses to find commonalities in data generation and data reporting. We also performed a robust meta-analysis of raw sequencing data retrieved from each study's accompanied Sequence Read Archive (SRA) Bioproject to extract summary statistics as well as determine the intrinsic sequence diversities using Nonpareil [4]. The findings help inform a framework for standardized metagenomic monitoring of antibiotic resistance in aquatic environments.

Key words: metagenomics, water quality monitoring, antibiotic resistance, standardization

Standards and control materials used: Mock Community [5]

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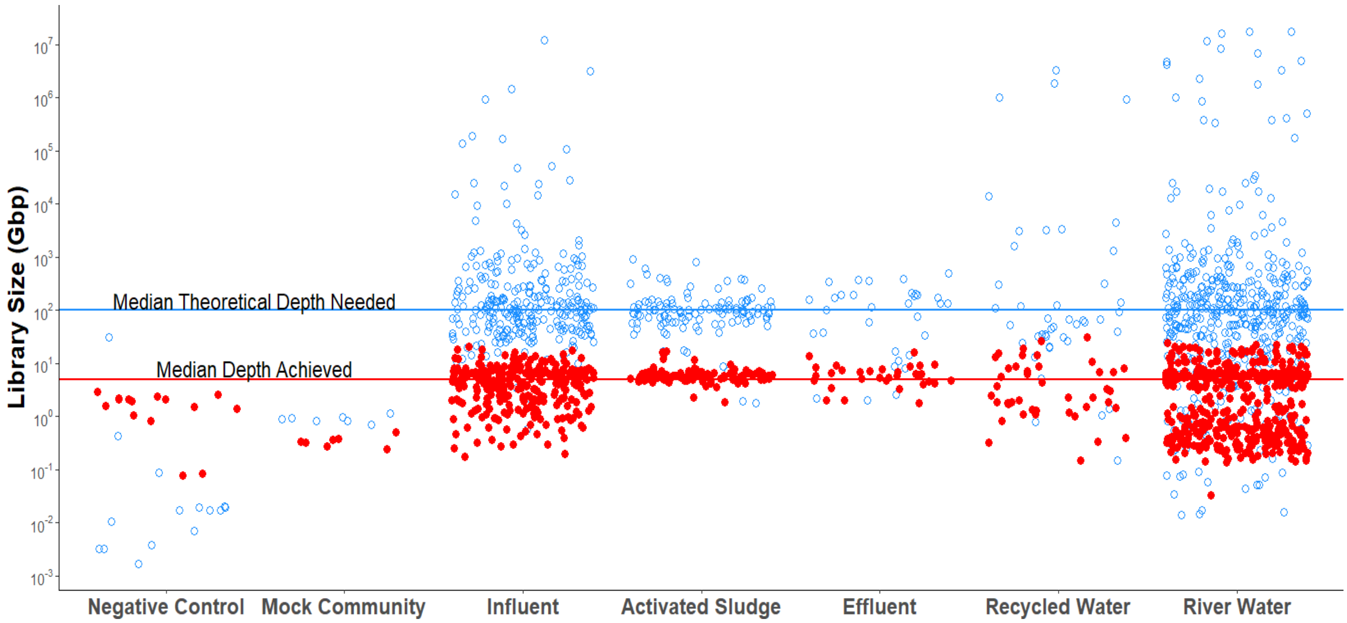

Figure 1. Results of Nonpareil (Rodriguez et al. 2018), an estimator of sequence diversity and depth needed to achieve 100% coverage of metagenomes. Metagenomes (n=946) of diverse water and wastewater matrices were downloaded from the Sequence Read Archive (SRA) from all papers identified in a comprehensive literature review (98 articles). Trends show that an order of magnitude deeper sequencing (measured in giga base pairs (Gbp)) is needed to achieve 100% coverage of the complex microbial communities in many aquatic matrices. Negative Controls = miliq water; Influent = raw sewage entering a wastewater treatment plant; Effluent = treated wastewater.

References:

1. European Commission: *A European One Health Action Plan against Antimicrobial Resistance (AMR)*. 2017.
2. JPIAMR: *Strategic Research and Innovation Agenda on Antimicrobial Resistance*. 2019.
3. Che Y, Xia Y, Liu L, Li AD, Yang Y, Zhang T: **Mobile antibiotic resistome in wastewater treatment plants revealed byNanopore metagenomic sequencing**. *Microbiome* 2019, **7**:1–13.
4. Rodriguez-R LM, Gunturu S, Tiedje JM, Cole JR, Konstantinidis KT: **Nonpareil 3: Fast Estimation of MetagenomicCoverage and Sequence Diversity**. *mSystems* 2018, **3**:1–9.
5. Peabody MA, Van Rossum T, Lo R, Brinkman FSL: **Evaluation of shotgun metagenomics sequence classificationmethods using in silico and in vitro simulated communities**. *BMC Bioinformatics* 2015, **16**.

Importance of Validation for Wastewater Surveillance

Brian M. Swalla
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Wastewater surveillance has delivered actionable public health data over the course of the COVID-19 pandemic. Because of the urgent nature of the pandemic, the extent of validation for currently used methods varies widely. Many methods were deployed before significant data was gathered on performance, and may not have been widely tested with a diversity of wastewater samples. As wastewater surveillance moves into the mainstream, it is critical that methods be thoroughly validated using real-world wastewater samples, that the validation captures as much natural variation as possible (such as ensuring the validation is performed with samples from many different geographies), and that validation data be made available to laboratories interested in using the method. Such data are critical to evaluate method performance capabilities and limitations, facilitate successful adoption in new laboratories, and ensure consistent and reliable results can be achieved over time.

Key words: method validation, process controls, matrix controls, reference materials, quantification, wastewater, RT-qPCR

Standards and control materials used: PCR positive and negative controls (IDEXX), Extraction and PCR Internal Control (IDEXX), SARS-CoV-2 reference RNA (ATCC), BRSV matrix recovery control, PMMoV and crAssphage human fecal controls

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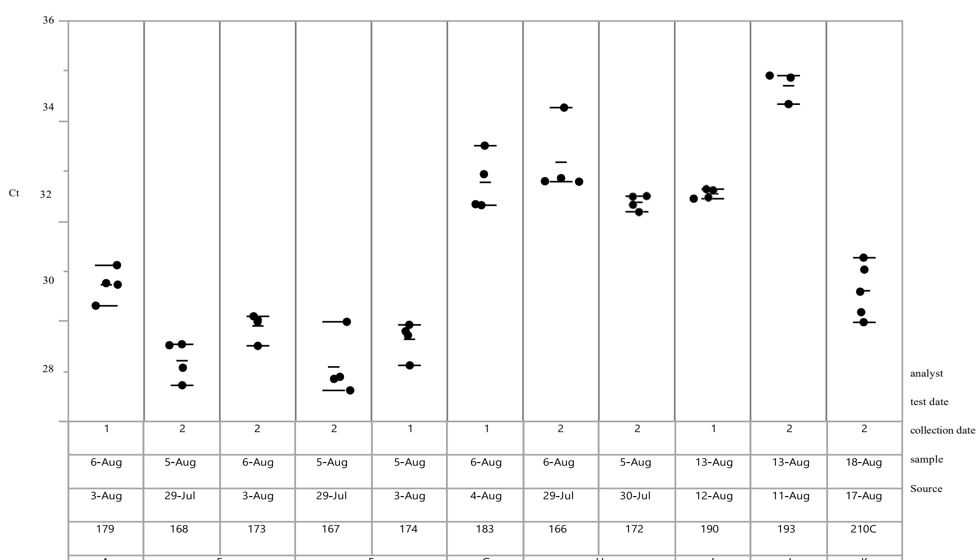

Example validation data demonstrating high Repeatability for detection of SARS-CoV-2 N1 and N2 from a geographically diverse collection of raw wastewaters. Samples were processed using PEG-based concentration, RNA purification with the IDEXX Water DNA/RNA Magnetic Bead Kit, and viral quantification with the IDEXX Water SARS-CoV-2 RT-PCR Test kit following validated protocols for each step.

Early Warnings of COVID-19 Second Wave in Detroit MI

Miyani Brijen[1], Zhao Liang[2], Spooner Maddie[3], Gentry Zachary[4], Mehrotra Anna[5], Norton John[6], Xagoraraki Irene[7*]

[1,2,]PhD candidate, Michigan State University; [3,4]Michigan State University; [5]Environmental Engineering, CDM Smith, Inc; [6]Director of Energy, Research and Innovation, Great Lakes Water Authority; [7]Professor of Environmental Engineering, Michigan State University; [*]corresponding author

Abstract:
This study focuses on using wastewater-based-epidemiology to provide early warnings of the second COVID-19 wave in Detroit metropolitan area in MI, USA. SARS-CoV-2 RNA from untreated wastewater samples was compared to reported public health records. Untreated wastewater samples were collected from the Great Lakes Water Authority (GLWA) Water Resource Recovery Facility (WRRF), located in southeast Michigan, between Sept 6, 2020 and Dec 14, 2020. The WRRF receives wastewater from its service area via three main interceptors: Detroit River Interceptor (DRI), North Interceptor-East Arm (NIEA), and Oakwood-Northwest-Wayne County Interceptor (ONWI). A total of 144 untreated wastewater samples were collected (45, 48, and 51 for ONWI, NIEA and DRI respectively) at the point of intake into the WRRF. Virus-selective sampling was conducted, and viruses were isolated from wastewater using electropositive NanoCeram column filters. For each sample, an average of 33 L of wastewater was passed through NanoCeram electropositive cartridge filters at an average rate of 11 L/m. Viruses were eluted and concentrated and SARS-CoV-2 RNA concentrations were quantified with RT-qPCR. SARS-CoV-2 RNA was detected in 98% of samples and measured concentrations were in the range of 4.45E+04 to 5.30E+06 genomic copies/L. Early warnings of COVID-19 peaks were observed approximately four weeks prior to reported publicly available clinical data. This was confirmed by statistical analysis as well.

Key words: SARS-CoV-2, coronavirus, COVID-19, wastewater, wastewater-based-epidemiology (WBE), Detroit

Standards and control materials used: *SARS-CoV-2 synthetic control as positive control in RT-qPCR.*

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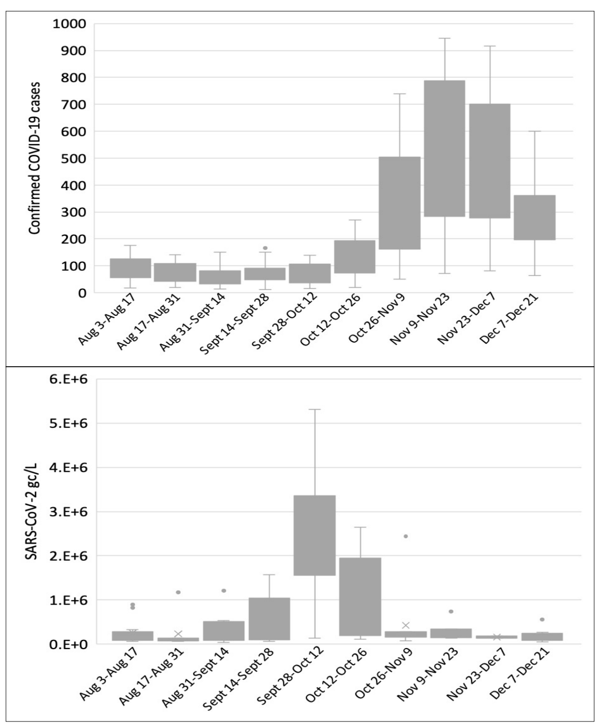

Figure: Biweekly confirmed COVID-19 cases and SARS-CoV-2 RNA concentrations

Wastewater SARS Public Health Environmental Response (W-SPHERE) GlobalData Center

Krystin Kadonsky[1], Colleen C. Naughton[1], Gertjan Medema[2], Panagis Katsivelis[3], Vajra Allan[4], Joan B. Rose[5]

[1,2]University of California Merced; [2]KWR Water Research Institute, [3]Venthic Technologies, [4]PATH, [5]Michigan State University

Over a year since the declaration of the global coronavirus disease 2019 (COVID-19) pandemic there have been over 173 million cases and 3.7 million deaths. Using methods to track community spread of other viruses such as poliovirus, environmental virologists and those in the wastewater based epidemiology (WBE) field quickly adapted their existing methods to detect SARS-CoV-2 RNA in wastewater. Unlike COVID-19 case and mortality data, there was not a global dashboard to track wastewater monitoring of SARS-CoV-2 RNA worldwide. We first created COVIDPoops19, a global dashboard for wastewater monitoring of SARS-CoV-2, that has grown into a global data center.

Methods for the COVIDPoops19 ArcGIS online dashboard included google form submission of direct sampling of wastewater for SARS-CoV-2 in several countries and stakeholder engagement, literature review, social media and news key-word searches, and attendance at online wastewater surveillance webinars worldwide. After a year of data tracking, wastewater surveillance for SARS-CoV-2 is conducted in over 55 countries, 2,276 sites, and 263 universities/institutions. A small subset (86) of those monitoring for SARS-CoV-2 in wastewater provide their data publicly and less than 20 provide downloadable data. Of the 55 that are conducting wastewater monitoring: 36 (65%) are in high-income countries, 11 (20%) are upper middle income, 8 (15%) are lower middle income, and 0% are low income countries.

COVIDPoops19 is informing a global data center W-SPHERE global data center (Wastewater SARS Public Health Environmental REsponse) using open data from individual country/city wastewater dashboards and soliciting data submissions and agreements from the research community. The mission of W-SPHERE is to advance environmental surveillance of sewage to inform local and global efforts for monitoring and supporting public health measures to combat COVID-19.

Wastewater surveillance has been a powerful tool to build resilience to the COVID-19 pandemic. However, there is a lack of data standards, limited use in low-income countries, limited data sharing publicly and challenges in analysis of the data to communicate to public health officials for decision making. We will provide a global data center and standards to build resilience beyond COVID-19 in the face of climate change and increased pathogens in the environment.

Key words: Dashboard, COVID-19, Geographic Information System (GIS), Wastewater Based Epidemiology (WBE)

Standards and control materials used: *Various standards and control methods used since data from many sources are aggregated for the data center.*

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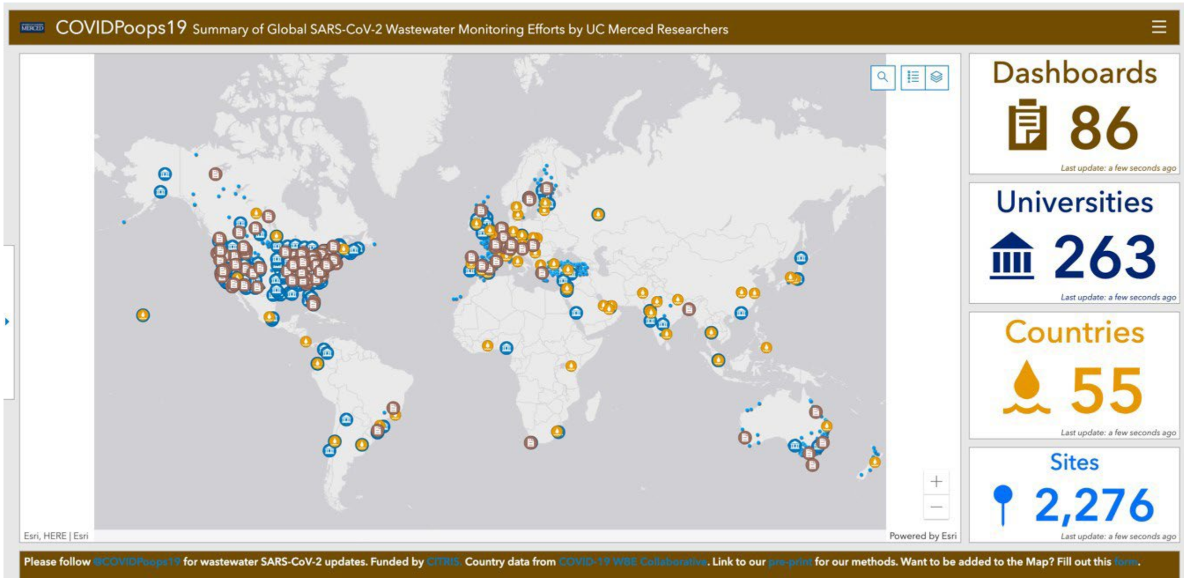

Figure 1. COVIDPoops19 Global Dashboard of Wastewater Monitoring for SARS-CoV-2

Rapid Sample Concentration for Streamlined Workflow in the Wastewater Laboratory

Alburty, David[1], David Goad, PhD[1], and James Brayer[2]

[1]InnovaPrep LLC; [2] Oxford Nanopore

Wastewater-based epidemiology (WBE) is a valuable tool for assessing population dynamics, infection rates, and most recently population-based assessment of vaccine efficacy and detection of variant strains. Collected samples are commonly concentrated for identification of viruses in liquid WW samples via PEG precipitation, ultrafiltration, or electronegative filters. RNA from concentrated samples is extracted and purified by COTS rapid methods such as RT-qPCR or ddRT-PCR and via genetic sequencing. To speed up sample processing through automation while eliminating the potential for sample carryover, an automated bioconcentrator can be used. In the concentrator, samples are aspirated into a single-use, high surface-area hollow fiber filter tip. Particles larger than the filter pore size are retained while the permeate fluid passes through. When the sample has been filtered the device stops and alerts the user to elute the sample. With a button press, a viscous, expanded wet foam is pushed through the retentate tangential to the filter surface recovering the particles in a user-selected volume of clean buffer (wet foam elution) which collapses immediately into a final volume of approximately 200 microliters, providing key benefits of decreased processing time, repeatability, and scalable concentration factor based on the input volume, output volume, and high efficiency to improve the limit of detection. Data are presented from a 3rd party investigation that showed better results for SARS-CoV-2 concentration vs PEG method when analyzed using ddPCR. Published data showed faster processing times and better performance than electronegative filtration[1] and equivalent concentration using centrifugal ultrafiltration.[2, 3] In these studies, bovine coronavirus or MS-2 were used as process controls. Details are presented including summarized materials and methods and comparative data for the complete processes from sample to analysis.

[1] *Juel, A.I. et.al. 2021;* [2] *Rusinol, M. et. al 2021* [3] Forés et. al. 2021.

Key words: wastewater based epidemiology, SARS-CoV-2, sample preparation, viruses

Standards and control materials used: *Bovine Coronavirus as a process control and internal standard for quantification of SARS-CoV-2.*

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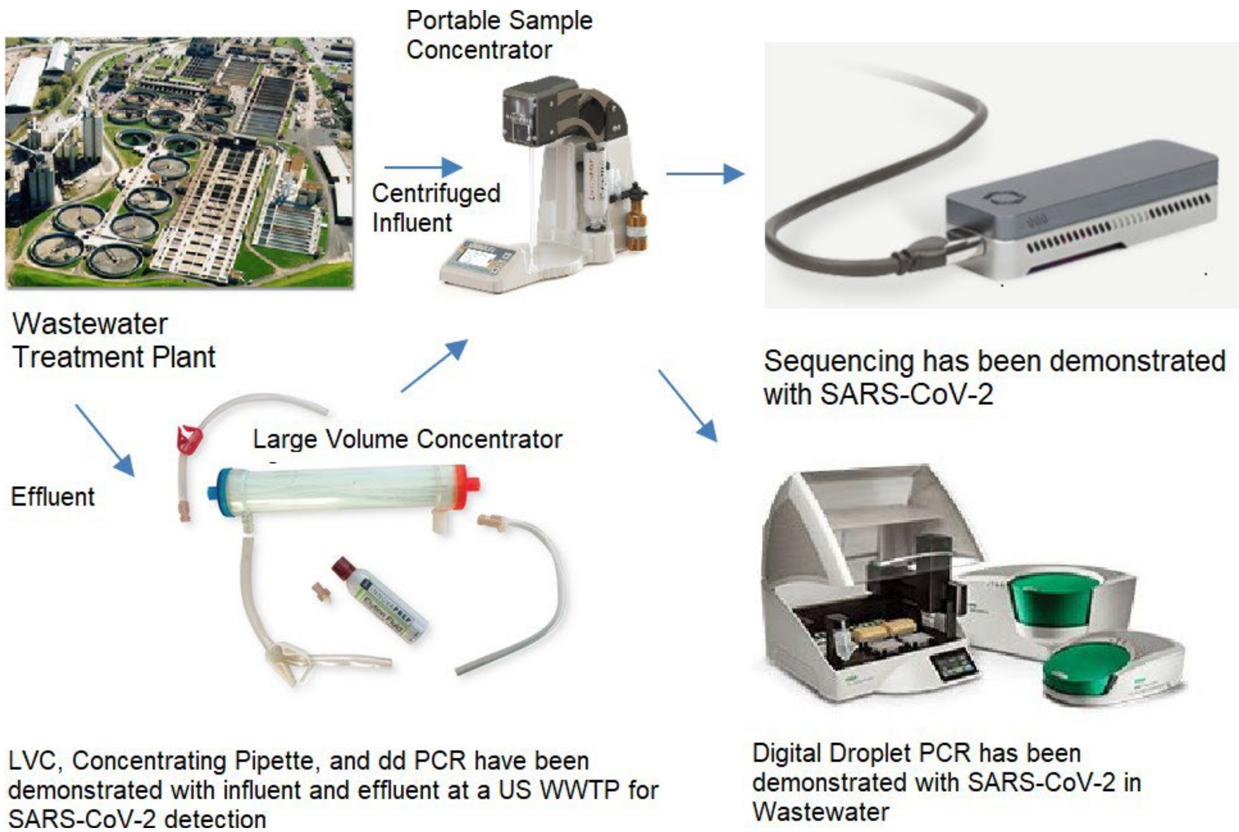

Figure 1. Concentrating Pipette Select for Faster Wastewater Processing.

The Development of a Sensitive and Reliable qRT-PCR-Based SARS-CoV-2 Wastewater Analysis Protocol

Agustin Pierri[1] and Douglas Sieglaff[2]
[1]Weck Laboratories, [2]Agilent Technologies

Wastewater testing can offer valuable insight on community-level occurrence of infectious disease- causing microorganisms, including enveloped ssRNA+ viruses such as SARS-CoV-2. Quantitative reverse transcriptase PCR (qRT-PCR) is well suited for wastewater microbial surveillance due to its sensitivity, specificity, scalability, rapid implementation and cost-effectiveness. Weck Laboratories developed a qRT-PCR-based SARS- CoV-2 wastewater testing procedure that includes sample concentration by ultrafiltration, and nucleic-acid extraction andanalysis using Agilent Technologies reagents with the AriaDx Real-Time PCR System. The CDC EUA N1 and N2 primer probes were used to analyze wastewater samples. Each qRT-PCR run batch included wastewater samples analyzed in duplicate, a 5-log dilution standard line, a matrix spike-in process control, a negative template control, no template control, and PCR- inhibition assessment of each wastewater sample tested. The validated procedure reliably interpreted SARS-CoV-2 viral RNA levels within a variety of raw wastewater samples, along withdelivering high sensitivity (as low as 8,000 viral genome copies per liter of wastewater). The procedure developed and employed by Weck laboratories is an easily implemented, robust methodology for community-level SARS-CoV-2 surveillance.

Figure

Overview of Weck Laboratories' workflow for extracting and detecting SARS-CoV-2 RNA inwastewater

Review of culture methods for monitoring antibiotic resistant *Acinetobacter*, *Aeromonas*, and *Pseudomonas* in wastewater, recycled and receiving water

Erin Milligan[1,2], Jeanette Calarco[3], Ben Davis[1], Ishi Keenum[1], Krista Liguori[1], Amy Pruden[1,2], Valerie J. Harwood[3*]

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The continued and growing health threat posed by antibiotic-resistant bacterial pathogens has led to increased interest in antibiotic resistance outside of hospital settings, e.g., in wastewater and wastewater-influenced aquatic environments. Environmental dimensions to the antibiotic resistance problem are increasingly being recognized and there has correspondingly been an increase in calls for comparable means to assess antibiotic resistance in environmental samples (Larsson et al., 2018; Smallaet al., 2018; JPIAMR, 2019). Recently, substantial progress has been made in the standardization of methodologies for monitoring antibiotic resistant fecal indicator bacteria in the environment, e.g., extended-spectrum beta lactamase (ESBL)-producing *Escherichia coli* (World Health Organization, 2021) and ESBL-producing Enterobacteriaceae (Marano et al., 2020). However, non-fecal bacteria with environmental niches, especially those that are adept at persisting and growing in aquatic environments, may be a more meaningful target for assessing potential for evolution and dissemination of antibiotic resistance in environmental matrices. For example, the presence of such bacteria in wastewater treatment plants and their versatile ability to survive and grow in receiving environments presents an opportunity to interact with bacteria in multiple niches, where they could potentially acquire and transfer antibiotic resistance genes along the way. Further, such organisms have greater potential than fecal bacteria to regrow in recycled water distribution systems. Human opportunistic pathogens that proliferate in aquatic environments are particularly key targets to consider for monitoring purposes, especially those that have developed a reputation for multi-drug resistance in clinical infections.

Studying these organisms in culture provides the advantage of being able to confirm their viability while also being able to further characterize multi-drug resistance, both phenotypically and genotypically. We conducted a systematic literature review and extracted data from studies that quantified antibiotic-resistant *Acinetobacter, Aeromonas,* and *Pseudomonas* by culture methods. The search criteria yielded 50 peer-reviewed articles across 25 countries over the past 20 years. Based on a systematic comparisonof the isolation, confirmation, and antibiotic resistance assaying methods reported in these articles, we suggest a path forward for standardizing methodologies for monitoring antibiotic resistant strains of these bacteria in the water environment.

Keywords: antibiotic resistance, wastewater, recycled water, surface water, culture, opportunistic pathogens

Standards and control materials used: *not applicable*

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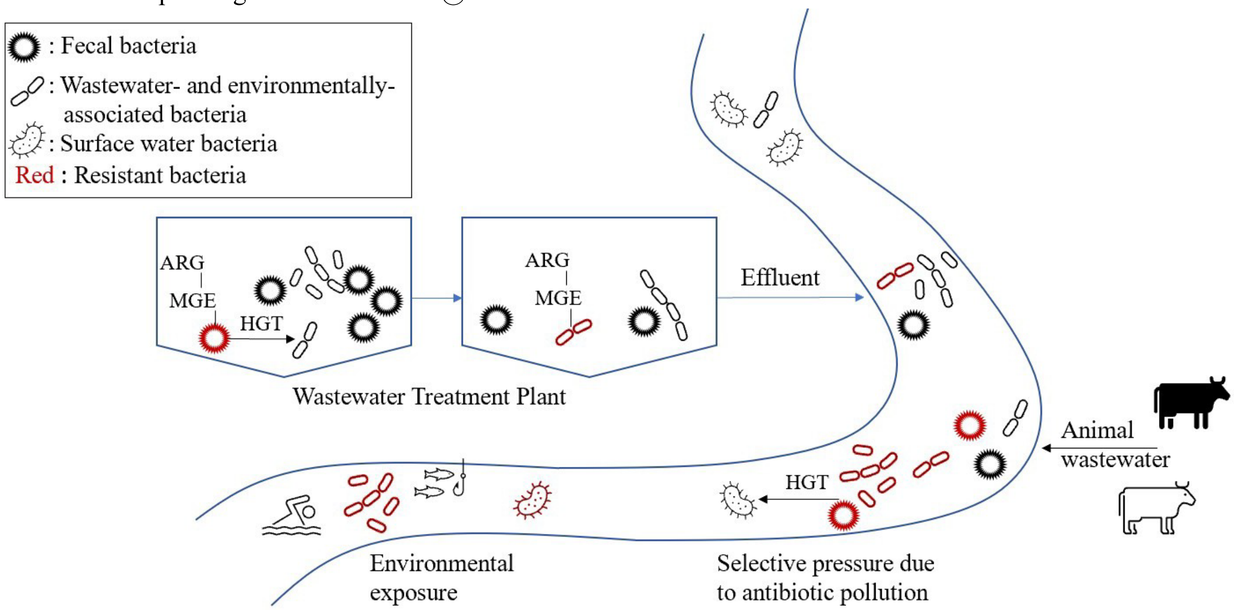

Figure 1. Potential for acquisition of antibiotic resistance genes (ARGs) by environmentally-associated bacteria during wastewater treatment and in affected surface waters.

References:

JPIAMR. (2019). Strategic Research and Innovation Agenda on Antimicrobial Resistance. In Strategic Research andInnovation Agenda on Antimicrobial Resistance (pp. 1–54).

Larsson, D. G. J., Andremont, A., Bengtsson-Palme, J., Brandt, K. K., de Roda Husman, A. M., Fagerstedt, P., Fick, J.,Flach, C. F., Gaze, W. H., Kuroda, M., Kvint, K., Laxminarayan, R., Manaia, C. M., Nielsen, K. M., Plant, L., Ploy, M. C., Segovia, C., Simonet, P., Smalla, K., … Wernersson, A. S. (2018). Critical knowledge gaps and research needs related to the 196 environmental dimensions of antibiotic resistance. Environment International, 117(January), 132–138. https://doi.org/10.1016/j.envint.2018.04.041

Marano, R. B. M., Fernandes, T., Manaia, C. M., Nunes, O., Morrison, D., Berendonk, T. U., Kreuzinger, N., Telson, T.,Corno, G., Fatta-Kassinos, D., Merlin, C., Topp, E., Jurkevitch, E., Henn, L., Scott, A., Heß, S., Slipko, K., Laht, M., Kisand, V., … Cytryn, E. (2020). A global multinational survey of cefotaxime-resistant coliforms in urbanwastewater treatment plants. Environment International, 144(August), 106035. https://doi.org/10.1016/j.envint.2020.106035

Smalla, K., Cook, K., Djordjevic, S. P., Klümper, U., & Gillings, M. (2018). Environmental dimensions of antibioticresistance: assessment of basic science gaps. FEMS Microbiology Ecology, 94(12), 1–6. https://doi.org/10.1093/femsec/fiy195

World Health Organization. (2021). Global Tricycle Surveillance.

Minimizing uncertainties of COVID-19 prevalence by establishing standards forwastewater surveillance

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Wastewater-based epidemiology (WBE) is a promising approach for monitoring population-wide COVID-19 prevalence through detection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) RNA in wastewater. However, various methodological challenges associated with WBE affect the accuracy of prevalence estimation. Our previous study investigated the overall uncertainty of WBE and the impact of each step on the prevalence estimation. The uncertainties associated with the different steps in the WBE approach (i.e., virus shedding; in-sewer transportation; sampling and storage; analysis of SARS-CoV-2 RNA concentration in wastewater; back calculation) were quantified through systematic review. The uncertainties of virus shedding and in-sewer transportation are largely uncontrollable, but fortunately not a major contributor to the overall uncertainty in estimated COVID-19 prevalence. The uncertainty for the shedding of SARS-CoV-2 RNA becomes limited when there are more than 10 infected persons in the catchment area. Also, the relative stability of SARS- CoV-2 in wastewater and moderate hydraulic residence time in the sewer system (normally within 12 hr) indicates a mild uncertainty due to in-sewer transportation.

Based on the analysis of different uncertainties, the overall WBE uncertainty is mainly due to sampling and storage; analysis of SARS-CoV-2 RNA concentration in wastewater; back calculation. It is critical to minimize uncertainties of estimated COVID-19 prevalence through establishing and adopting standards for wastewater surveillance. The uncertainty can be reduced mostly by using a high-frequency flow-proportional or time-proportional sampling and estimating the prevalence through actual water usage data. And under such a scenario, the overall uncertainty can be further reduced by improving SARS-CoV-2 RNA detection in wastewater. It is critical to determine the virus recovery efficiency for the various concentration methods being used in different labs. A best-practice RT-qPCR protocol, including choice of primer-probe sets, should be encouraged.

Key words: COVID-19; SARS-CoV-2; Wastewater-based epidemiology; Uncertainty; Prevalence estimation;

StandardsStandards and control materials used: *No*

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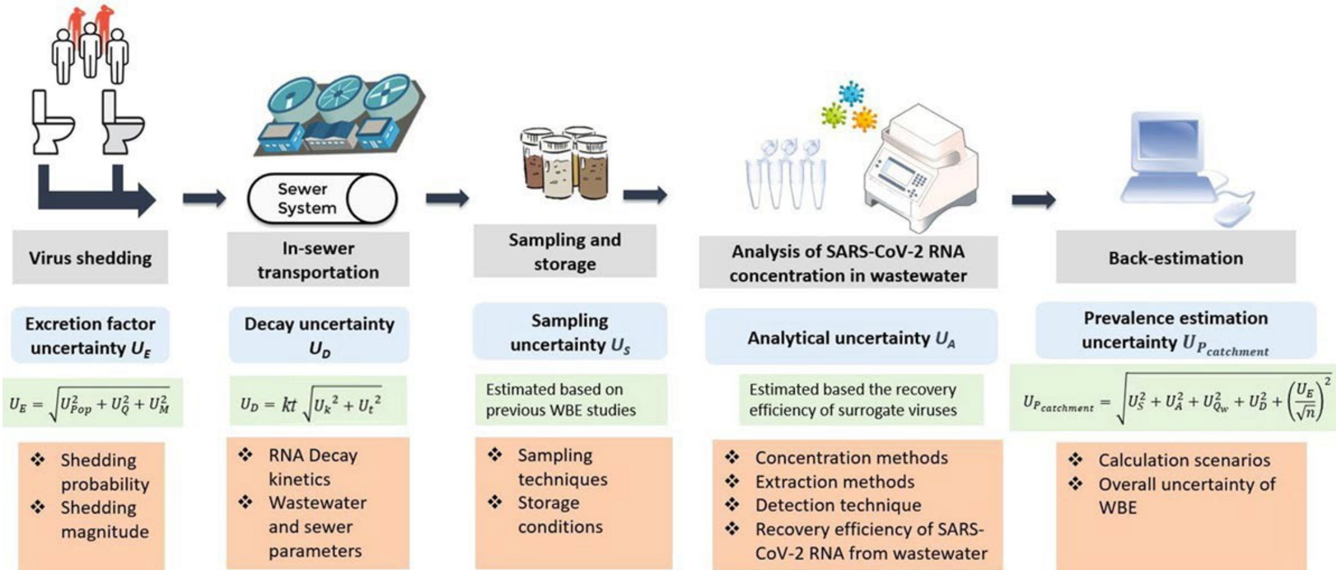

Figure 1. Uncertainty analysis for monitoring COVID-19 community prevalence through wastewater surveillance.

Using a high-resolution sampling in Davis, CA to understand how to best analyze and act on WBE data

H. Safford[1], R. Zuniga-Montanez[1], M. Kim[2], L. Rueda[2], R. Pechacek[1], R. Guerrero[1], M. Clauzel[1], X. Liu[3], L. Wei[3], M. Daza,[3] M. Nuno,[3] J. Sharpnack[3], K. Shapiro[2], H. Bischel[1]

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Wastewater-based epidemiology (WBE) is a useful complement to clinical testing for pandemic response. The public-health benefits of WBE depend on the scale at which WBE is carried out. **Samples from building or neighborhood outflows** can support direct and targeted interventions. **Samples isolating different sub-regions of a community sewershed** can help officials decide how to allocate resources (e.g., testing and public messaging) within the community. **Samples from wastewater treatment plant (WWTP) influent** provide information that can reinforce confidence in clinical trends or suggest when clinical testing may be missing key population segments. WBE data can also be incorporated into education and outreach campaigns.

The Healthy Davis Together (HDT) program in Davis, CA applies WBE at all three of the above scales. Since Fall 2020, HDT has been collecting and analyzing levels of SARS-CoV-2 in wastewater collected from the following sites:

- **Building/neighborhood outflow [collected 1–3x/week]:**
  - 22 UC Davis residential buildings or complexes
  - 6 Davis neighborhoods
  - 1 apartment complex
  - 1 elementary school
- **Sewershed sub-regions [3x/week]:**
  - 15 sub-regions of the Davis sewershed
- **WWTP influent [7x/week]:**
  - City of Davis WWTP
  - UC Davis WWTP

We are leveraging the high temporal and spatial resolution of HDT wastewater analysis to understand how to best analyze and act on WBE data. We are pleased to present and discuss early insights related to the following key topics and questions:

- **Treatment of non-detects.** Relatively low concentrations of SARS-CoV-2 in environmental matrices like wastewater mean that qPCR technical replicates of the same sample frequently yield a mix of positive (“detect”) and negative (“non-detect”) results. Researchers commonly substitute a single constant value (e.g., zero or half the detection limit) for non-detects during data analysis. We are (i) illustrating how this crude approach biases results, and (ii) exploring multiple-imputation methods for more sophisticated handling of non-detects.
- **Sub-regional comparison of wastewater and clinical data.** Many groups have demonstrated strong correlation between trends in wastewater SARS-CoV-2 concentrations and clinically confirmed COVID-19 cases at the city or college level. We are exploring whether similar relationships exist at the sub-regional level.

Key words: Wastewater-based epidemiology, public health, SARS-CoV-2, COVID-19, data imputation, data correlation

Standards and control materials used: Phi6 bacteriophage (process control), PMMoV (fecal strength indicator), N1/N2 gene regions (target for SARS-CoV-2 quantification), Oregon RNA and N1/N2 plasmids (positive control)

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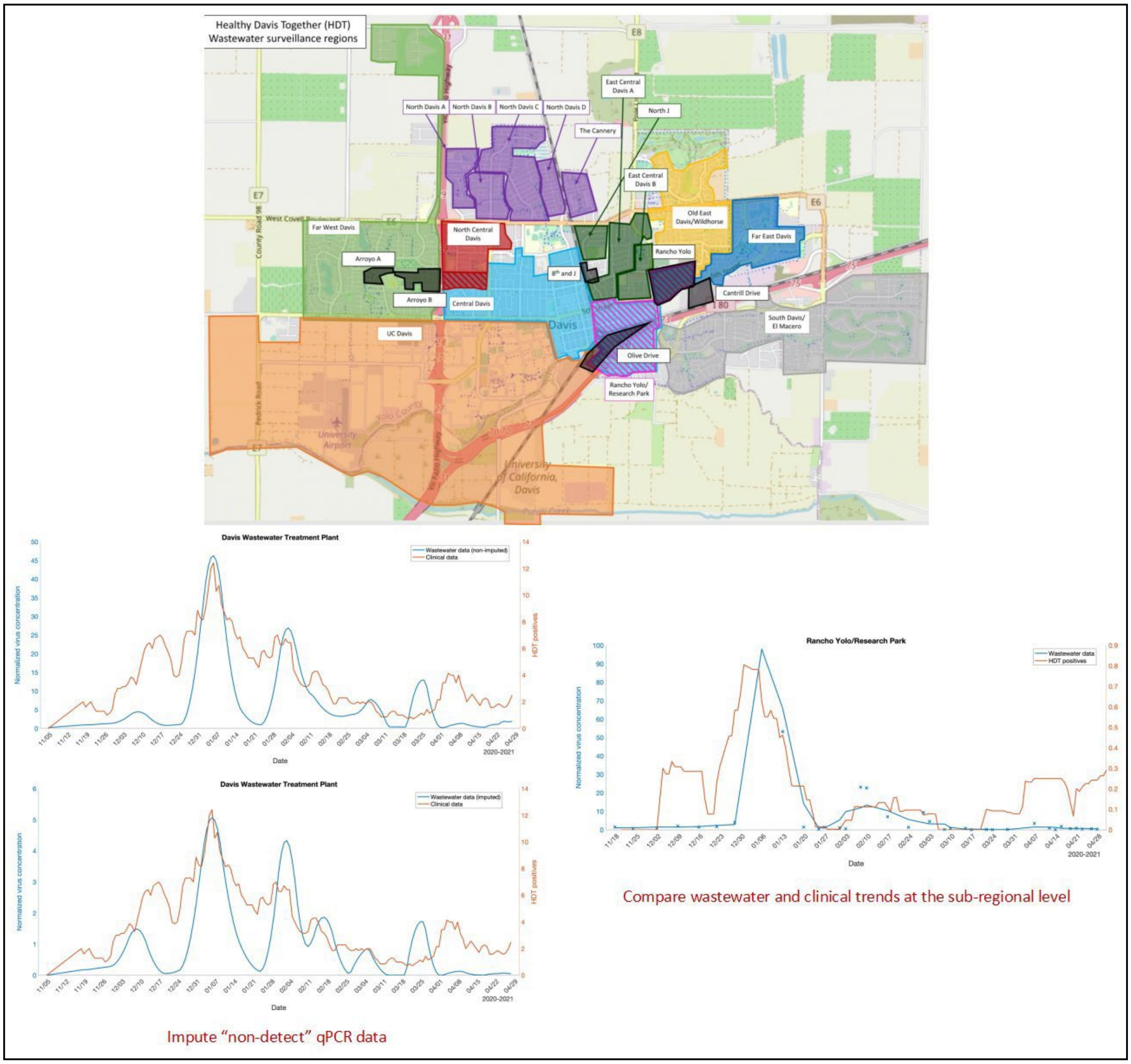

Figure 1. The rich dataset provided by the granular wastewater-sampling campaign and large-scale asymptomatic testing conducted through Healthy Davis Together enables novel insights into key questions such as "How do we handle non-detects in qPCR data collected on wastewater samples?" and "How well do WBE data correlate with clinical test results at the sub-city level?

EzCOVID19: A Cloud-based Bioinformatics Platform for Rapid Detection, Characterization and Epidemiological Sub-typing of SARS-CoV-2

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Background: The COVID-19 pandemic has caused enormous social and economic disruption worldwide. Tracing the evolutionary development and spread of SARS-CoV-2 across the globe requires continuous and exhaustive genomic sequencing. With the development of next generation sequencing (NGS) technology, genomic information is accessible to many researchers. However, the data generated by scientists, globally, comes in varying formats and from different sources. Additionally, despite the need for consensus genome assemblies, there is no single consensus protocol for sequencing SARS-CoV-2. Such information requires standardizing and compiling before it can be accurately analyzed. EzCOVID19, is a cloud-based bioinformatics platform that enables researchers the ability to rapidly detect, standardize, and characterize SARS-CoV-2 genomes from any NGS data suspected of containing SARS-CoV-2.

Method: EzCOVID19 generates consensus genome assembles, locates genetic variations from the SARS-CoV-2 Wuhan-Hu-1 reference (NC_045512.2), utilizes a novel SNV-based classification system for accurate identification, and produces parsimony trees based on public GISAID genomes.

Results: This user-friendly tool provides a platform for scientists to submit samples for rapid detection of SARS-CoV-2, download assembled genomes and profile tables, identify genetic variations, and compare with related genomes in public databases. EzCOVID19 also provides in-depth analyses and visualizations of the data with parsimony and maximum likelihood trees based on novel SNV sites. Its cloud-based system and online support provide immediate results from regularly updated public databases, including monitoring of variants of concern. To analyze your samples, access EzCOVID19 here https://www.ezbiocloud.net/tools/sc2.

Conclusion: Many scientists and public health workers from various research backgrounds have been required to adapt viral genomics into their research as a means of understanding SARS-CoV-2 without prior knowledge of virology or genomics. EzCOVID19 is a cloud-based, user-friendly, robust platform to assist scientists from different disciplines to research SARS-CoV-2. Regardless ofmethod or sequencing instrument used, EzCOVID19 is able to integrate raw metagenomic or isolate sequence data for standardised bioinformatic analyses. Processed data are presented with detailed visuals and relevant information related to concurrently circulated publicly available SARS-CoV-2 genomes.

Key words: COVID19 Surveillance, Bioinformatics, rapid detection, epidemiological sub-typing,mutational analysis.
Standards and control materials used: N/A
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Targeted communications using wastewater monitoring at the sub-sewershed scale in Davis, CA

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Healthy Davis Together (HDT), a joint project between UC Davis and the City of Davis, collects wastewater samples from nodes within the city's sewer system and monitors the City of Davis and the UC Davis Wastewater Treatment Plants. Samples are collected three times per week at 24 sewer nodes in the cityand seven days per week from wastewater influent at the City of Davis and UC Davis WWTPs. Sample processing takes about 24 hours, and SARS-CoV-2 concentrations are normalized to pepper mild mottle virus (PMMoV) for presentation and analysis.

HDT formed a Wastewater Action Committee (WAC) to coordinate communications of all wastewater results between HDT scientists and City leadership on a weekly basis and to develop and implement responses to wastewater results. A press release invited community members to opt into the pre- existing Yolo Alert messaging system to receive notifications when elevations are detected.

Virus levels in wastewater that exceed pre-defined action thresholds are reviewed by the WAC with three primary response strategies, together intended to encourage participation in HDT's widely available, free asymptomatic COVID-19 testing program. First, HDT posts results on its website, broken down by sampling region to keep the public informed each week. The slope of a two-week moving average is evaluated to denote on the HDT website if levels in each sampling region are increasing, staying about the same, or decreasing. Second, the highest absolute normalized values of detection areused by HDT and the WAC to identify priority regions within the city and to cross-check participation in asymptomatic individual testing programs within different regions. Third and finally, geo-targeted text, email, and social media messages can be sent to a sub-sewershed region following a sustained elevationof normalized virus concentrations above the limit of detection. Sub-sewershed samples represent smaller populations (hundreds to low thousands of people) and are expected to frequently yield nondetects; an increase in levels above the detection limit represents a significant and easily identifiablechange. The city used its opt-in text message alert system as well as Nextdoor to notify residents withinthe associated sub-sewershed region based on sewer system data collected in March 2021.

Key words: Sub-sewershed wastewater; actionable response strategies; COVID-19

Standards and control materials used: *(short answer)*

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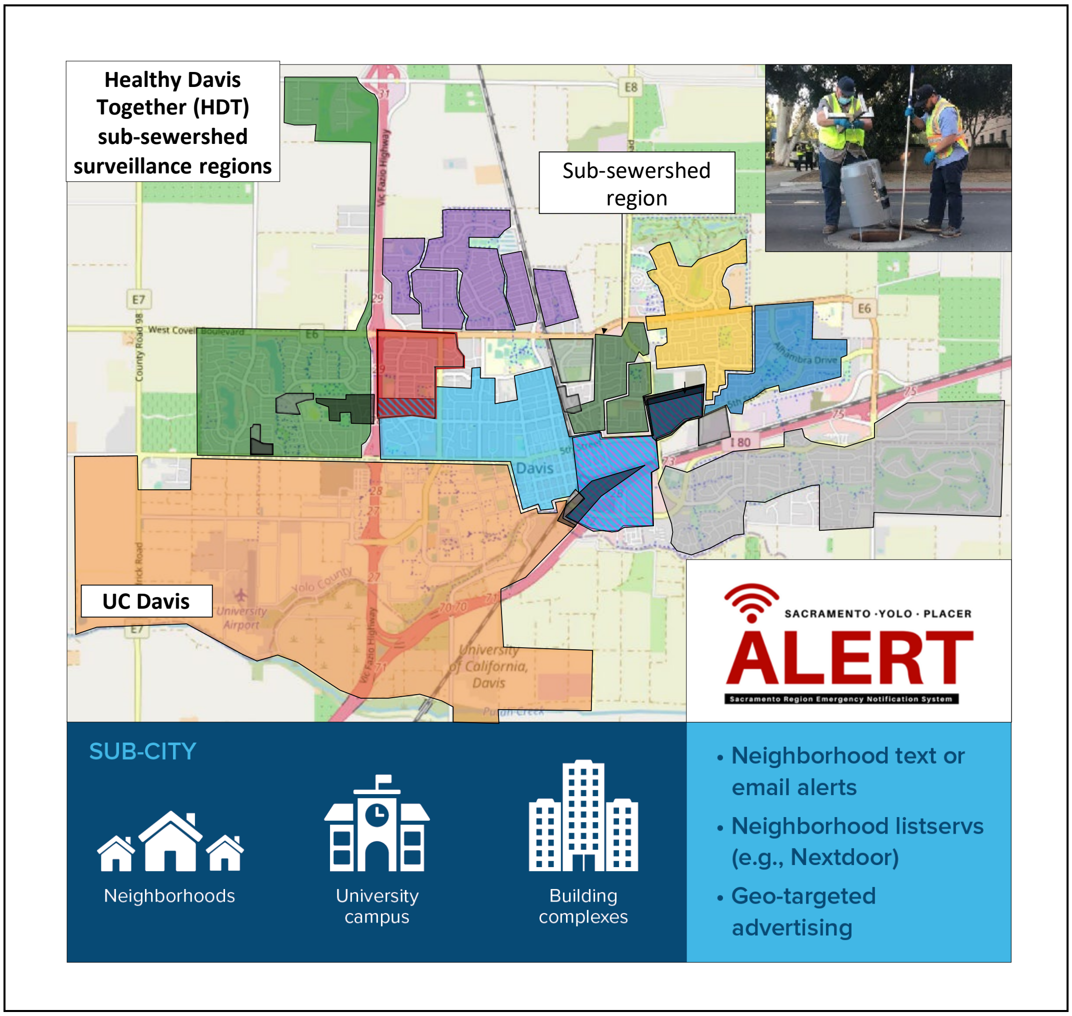

Figure 1. Sub-sewershed regions monitored in the City of Davis and

Assessment of qPCR- targets and protocols for quantifying anthropogenic impacts of antibiotic resistance to the water environment

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There is growing recognition of the need for standardizing monitoring of antibiotic resistance in the aquatic environment. Quantitative polymerase chain reaction (qPCR) is attractive as a quantitative and sensitive means of enumerating antibiotic resistance genes (ARGs) that has been applied broadly over the past two decades to various water matrices. qPCR circumvents challenges and biases associated with culture-based methods, providing a reproducible, quantitative, and highly sensitive measure of specific ARGs carried across a bacterial community. qPCR-based measurements can serve to address key goals for monitoring antibiotic resistance in water environments (Berendonk et al., 2015; Huijbers et al., 2019). Measuring the incidence of specific ARGs can help to predict the potential for the accelerated evolution of antibiotic resistance in pathogens through pollution with selective agents (e.g., antibiotics, heavy metals, personal care products) and bacteria of human or animal origin (Karkman et al., 2018; Kohanski et al., 2010). Additionally, qPCR can be used to estimate the risk of ARB infection in humans by directly measuring the carriage of specific ARGs with clinical implications in a given microbial community.

However, there are thousands of known ARGs that could be targeted, each varying in their relevance to human health and their overall contribution to dissemination of antibiotic resistance. Further, there are various methodological aspects, such as sample concentration, extraction, and PCR inhibition that need to be evaluated to ensure that measurements are representative and comparable across studies. Here we conducted a critical review to identify ARGs and assays that are most commonly measured by qPCR in wastewater, recycled water, and surface water, specifically: *sul1*, *intI1, vanA*, *blaCTX-M*, and *tetA.* We identified 117 peer-reviewed studies meeting the search criteria and systematically assessed the corresponding workflows reported, including sample collection and concentration, DNA extraction, amplification conditions, amplicon length, and level of validation. Resulting concentrations reported for various water matrices were compared across the studies. Based on this evaluation, recommend assays, a standardized workflow, and reporting guidelines for the five genes of interest. Implications for emerging qPCR approaches, such as droplet digital qPCR and high-throughput qPCR, are also discussed and a path forward for standardization is proposed.

Key words: antibiotic resistance, quantitative polymerase chain reaction, standardization, wastewater, surface water, environment
Standards and control materials used: *not applicable.*
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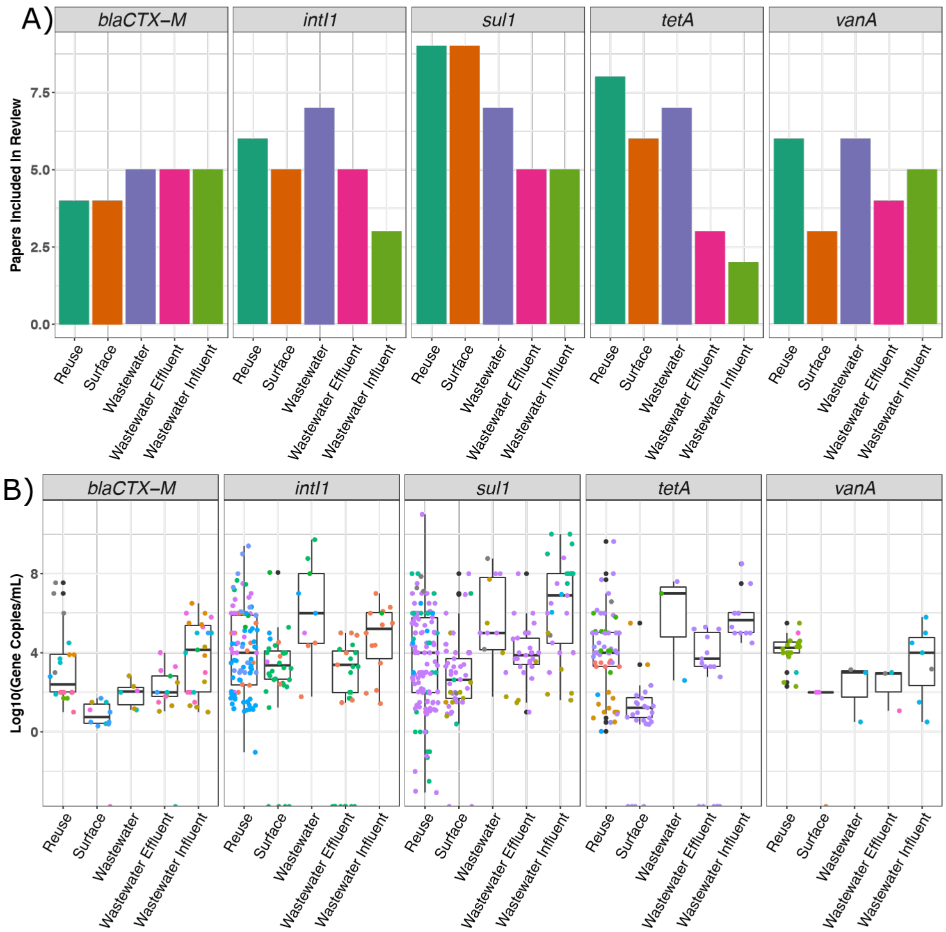

Figure 1**(A)** Number of articles that met the search criteria for each gene by water matrix (recycled/reuse, surface, and wastewater). Articles that examined more than one water matrix are double counted. **(B)** Target gene concentrations measured in each water matrix . Dots are colored by the studythe reported data was obtained from**.** Box plots indicate the median, first, and third quartiles and whiskers extend no more than 1.5 times the interquartile range.

Berendonk, T.U., Manaia, C.M., Merlin, C., Fatta-Kassinos, D., Cytryn, E., Walsh, F., Bürgmann, H., Sørum, H., Norström, M., Pons, M.-N., 2015. Tackling antibiotic resistance: the environmental framework. Nat. Rev. Microbiol. 13, 310–317.

Huijbers, P.M.C., Flach, C.-F., Larsson, D.G.J., 2019. A conceptual framework for the environmental surveillance of antibiotics and antibiotic resistance. Environ. Int. 130, 104880. https://doi.org/10.1016/j.envint.2019.05.074

Karkman, A., Do, T.T., Walsh, F., Virta, M.P.J., 2018. Antibiotic-Resistance Genes in Waste Water. Trends Microbiol. 26, 220–228. https://doi.org/10.1016/j.tim.2017.09.005

Kohanski, M.A., DePristo, M.A., Collins, J.J., 2010. Sublethal Antibiotic Treatment Leads to Multidrug Resistance via Radical-Induced Mutagenesis. Mol. Cell37, 311–320. https://doi.org/10.1016/j.molcel.2010.01.003

A DNA-based reference material for pathogen detection via metagenomic next-generation sequencing (mNGS)

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NIST has developed a 20-component bacterial DNA-based reference material (RM 8376) to assess the analytical performance of mNGS analyses. Because mNGS includes many steps, each of which contributes bias to the results, it is critical that those biases are identified for optimizing performance. This RM was designed to assess sequencing and informatics. The components include many wastewater-based pathogens (see Table), providing relevant controls for evaluating analytical performance. Further, because these materials are DNA, they may be employed in other diagnostics such as qPCR.

The RM includes a wide range of known pathogens, including Gram positive/negative, high/low G+C content, genome size, and near neighbors. Each chromosome was assembled into a circular contig and is available for use. To quantify the chromosomal copy number concentration, droplet digital PCR assays were developed for each component. The homogeneity and stability of each component assessed over several months, with each genome at approximately 50 ng/□L, or 10^7 copy/□L.

Keywords: bacterial DNA standards, metagenomics, pathogen detectionEmail:
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Table – NIST RM 8376 components, including source material, identity, assembled chromosome sizes, and copy number concentration with uncertainty.

ATCC ID	Part	Organism	Chr Size(s)	Plasmids	Chromosomal copy number concentration $\times 10^6$ (copy/□L)[(a)]
43895	A	*Escherichia coli* O157:H7	5564632	2	8.84 □ 0.38
BAA 2309	B	*Escherichia coli* O104:H4	5302905	1	8.89 □ 0.28
700720	C	*Salmonella enterica* enterica	4857492	1	9.72 □ 0.38
12324	D	*Salmonella enterica* arizonae	4482096	0	10.84 □ 0.52
BAA 44	E	*Staphylococcus aureus*	2964115	1	16.49 □ 0.76
12600	F	*Staphylococcus aureus*	2755072	1	17.38 □ 0.68
12228	G	*Staphylococcus epidermidis*	2504458	3	15.99 □ 0.60
BAA 47	H	*Pseudomonas aeruginosa*	6263669	0	8.27 □ 0.34
19606	I	*Acinetobacter baumannii*	3980879	0	12.01 □ 0.56
13077	J	*Neisseria meningitidis*	2181327	0	21.67 □ 0.94
12344	K	*Streptococcus pyogenes*	1914863	0	22.55 □ 0.86
19433	L	*Enterococcus faecalis*	2866948	0	14.75 □ 0.50
27061	M	*Achromobacter xylosoxidans*	6813185	0	7.28 □ 0.36
35654	N	*Aeromonas hydrophila*	4733720	0	9.97 □ 0.34
13883	O	*Klebsiella pneumoniae*	5303036	4	7.68 □ 0.36
25931	P	*Shigella sonnei*	4917056	0	9.67 □ 0.36
35016	Q	*Vibrio furnissii*[(b)]	3275680, 1641536	1	9.70 □ 0.36
19115	R	*Listeria monocytogenes*	2950983	0	17.39 □ 0.64
33152	S	*Legionella pneumophila*	3409194	0	13.63 □ 0.46
GM24385[(c)]	T	*Homo sapiens*			0.0323 □ 0.0015

(a) The values are expressed as x □ $2u(x)$, where x is the value and $u(x)$ is the standard uncertainty of x. The standard uncertainty combines reaction volume, unit, and repetition. While the best estimate value lies within the interval x □ $2u(x)$, this interval may not include the true value.
(b) Component Q has 2 chromosomes
(c) Sourced from Coriell

An organism-centric approach to performance metrics for metagenomic NGS-based diagnostics

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Complex Microbial Systems Group, NIST

Metagenomic Next-Generation Sequencing (mNGS) analyses applied to wastewater has potential for population-level characterization and tracking. Sequencing is generally agnostic to the DNA/RNA source, allowing the same data to be analyzed for >10k organisms (virus, bacteria, etc.) using different computational tools. The benefits are clear—outbreaks like SARS-CoV-2 could be retrospectively tracked, and health officials could respond quickly to rising levels of potential pathogens such as *Listeria*. However, mNGS-based diagnostics are also prone to false positives and negatives, and those can degrade confidence in the technology and public health administration in general.

We have proposed evaluating mNGS performance on a per organism basis. This *organism-centric* approach has particularly strong potential for wastewater because it allows end users to know the technical limitations and indicate how to address them, especially when distinguishing species or strains; the organism load is low; and the results of an analysis can be actionable. Further, the technology can provide indications of novel pathogens in need of a closer look, and this organism-centric approach can be scaled out to incorporate new taxa as needs evolve.

Keywords: metagenomics, sequencing, pathogen detection

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Table 1 Performance metrics by taxon can reveal fine-grain information, especially since performance varies by organism. Here, 10 hypothetical samples were compared by taxon, revealing poor performance for some taxa. Summarizing performance across all possible taxa (sample-centric) hides those potential deficiencies and overstates performance. When only a subset of the taxa are of interest,

Analysis Results						*Performance Metrics*					
Taxon	TP	FP	TN	FN	n	Sens	Pr	Spec	Acc	F1	DOR
Ak	**3**	**4**	**1**	**2**	10	**0.6**	**0.43**	**0.2**	**0.4**	**0.5**	**0.4**
Bl	**2**	1	4	**3**	10	**0.4**	**0.67**	0.8	**0.6**	**0.5**	2.7
Cm	**3**	1	4	**2**	10	**0.6**	**0.75**	0.8	**0.7**	**0.67**	6.0
Dn	4	1	4	1	10	0.8	0.8	0.8	0.8	0.8	16.0
Eo	5	1	4	0	10	1	0.83	0.8	0.9	0.91	26.7
Fp	5	0	5	0	10	1	1	1	1	1	100
Gq	5	0	5	0	10	1	1	1	1	1	100
Hr	5	0	5	0	10	1	1	1	1	1	100
Is	5	0	5	0	10	1	1	1	1	1	100
Jt	5	0	5	0	10	1	1	1	1	1	100
Sample-centric Results						Sen	Pr	Spec	Acc	F1	DOR
TOTAL	42	8	5042	8		0.84	0.84	1.00	1.00	0.84	100

Targeted NGS for Sensitive Detection of SARS-CoV-2 Genomes

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[1]Integrated DNA Technologies, Ann Arbor, MI

Discovery and detection of pathogenic genomes is of increasing importance. Next Generation Sequencing (NGS) is a sensitive and thorough way to surveil a wide variety of samples. Sequencing also provides insight into the strains that are circulating in a population, including novel strains. Targeted sequencing enriches for sequences of interest from the sample background, increasing sensitivity and saving on sequencing costs.[1] Swift Biosciences (now part of IDT) has developed the Swift Normalase™ Amplicon Panels (SNAP) workflow to enable fast and efficient preparation of targeted libraries for NGS sequencing. The SNAP workflow takes approximately 2.5 hours from cDNA to library and consists of two PCR steps. This one tube workflow is compatible with a wide variety of sample types and capable of sequencing large target regions using overlapping amplicons to achieve continuous coverage. The SNAP SARS-CoV-2 Additional Genome panel covers 99.7% of the SARS-CoV-2 genome using 345 amplicons.The SNAP technology performs well with low levels of target DNA/cDNA and with damaged samples. This panel has been used to determine the lineage of SARS-CoV-2 in wastewater samples.[2] Herein, we demonstrate the efficiency of the SARS-CoV-2 assay atlow viral titers and show that this kit is capable of discovery of novel mutations while maintaining high genomic coverage. This technology is not limited to SARS-CoV-2, but also able to target any sequence of interest.

1 Spurbeck et al. Science of the Total Environment. 789 (2021) 147829

2 Fontenele el al. https://doi.org/10.1101/2021.01.22.21250320

NGS: Targeted Sequencing; SARS-CoV-2; wastewater; amplicon

Standards and control materials used: SARS-CoV-2 standards from Twist Biosciences and BEI were used for input titration experiments.

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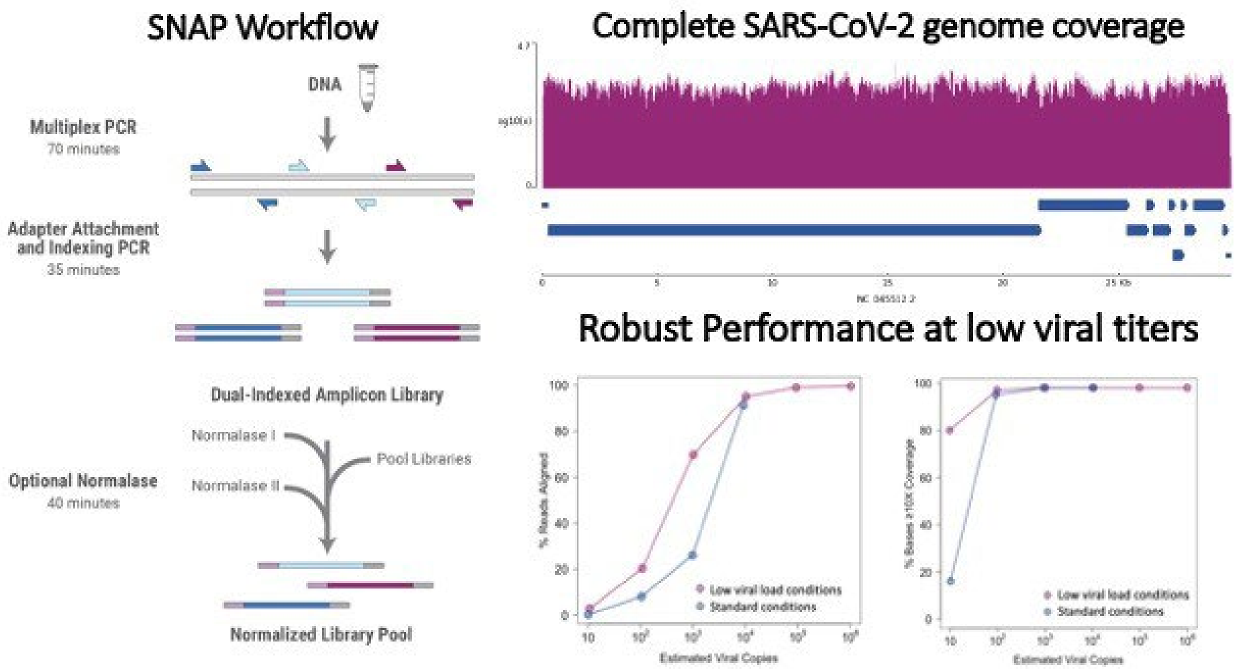

Figure 1. The SNAP Workflow diagram showing the one tube, two step process for library creation. The optional Normalase steps are also show. The SARS-CoV-2 genome coverage attained from the assay is visualized in IGV and show in purple. The performance metrics (% Reads aligned and % Bases >10X coverage) are shown for inputs of 10-10^6 viral copies.

Sources Of Variability In Methods For Processing, Storing, And Concentrating SARS-CoV-2 In Influent From Urban Wastewater Treatment Plants

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During the COVID-19 pandemic, wastewater surveillance of SARS-CoV-2 RNA has emerged as a way to track the virus in a broad population without the drawbacks of testing individuals. Understandably the emphasis has been on rapidly increasing the rate and extent of measuring SARS-CoV-2 RNA in wastewater to gain insight into outbreaks in communities globally. This has resulted in many different methods being used for sample collection, storage, virus capture, and quantification with few studies investigating the variability caused by these methodological differences and the impact of this variability on comparisons of wastewater surveillance data. In this study, controlled experiments were performed to test methods used to store wastewater samples, to inactivate virus in wastewater, to capture and concentrate virus in wastewater, and to extract and measure the viral RNA. We found the highest variability was caused by heat inactivation of the viruses (a 1-3 log decrease) and freezing of influent prior to concentration (1-4 log decrease), with impacts dependent on sample processing method. Sampling frequency, sampling strategy, concentration vs direct extraction, and PCR platform were also minor sources of variability. In contrast, the nucleocapsid gene target had nearly no effects. We found viral capture by membrane adsorption to be robust to changes in SARS-CoV-2 concentration and freeze-thaw variability. Pepper mild-mottle virus was much less sensitive to these methodological differences than was SARS-CoV-2, which challenges its use as a population-level control among studies using different methods. We applied the membrane adsorption method to monitor wastewater from a large wastewater treatment plant in Los Angeles County, California from April 2020-April 2021 and found a high correlation (r=0.78) to SARS-CoV-2 case counts. Wastewater based surveillance holds promise to efficiently measure the prevalence of SARS-CoV-2 in a larger, pooled population sample and has potential to serve as an early warning of future outbreaks. However, the diversity of methods, high variability reported among methods, and a lack of standardization make it difficult for municipalities and public health agencies to be able to interpret the SARS-CoV-2 concentrations from wastewater. Better characterizing the variability associated with methodological choices, in particular the limits of sensitivity of the methods, will aid decision makers in following the effects of vaccination campaigns, early detection of future outbreaks, and, potentially, monitoring the appearance of SARS-CoV-2 variants in the population.

Key words: droplet digital PCR, HA electronegative virus capture, magnetic bead extraction

Standards and control materials used: *Bovine Coronavirus vaccine, Asuragen armored HepG*

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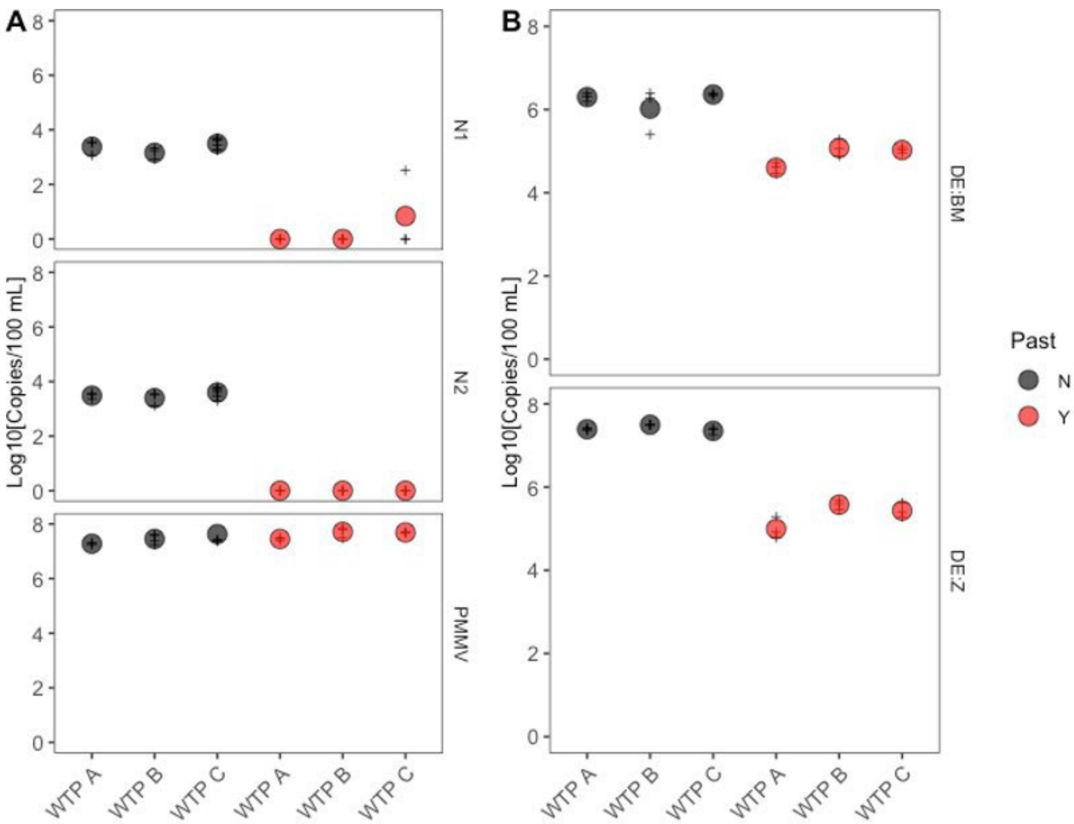

Figure 1. Concentrations measured with and without pasteurization. Circles represent average concentration for the three WTPs and faint crosses represent results from the individual plants. Black circles indicate samples not pasteurized; red circles indicate pasteurized samples; (A) SARS-CoV-2 N1 (top row), SARS-CoV-2 N2 (middle row), and PMMV (bottom row) levels for samples processed by membrane concentration (HA) B) BoCoV levels by direct extraction methods (DE:BM & DE:Z).

Challenges with Standardizing the Measurement of SARS-CoV-2 Recovery Efficiency in Wastewater

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A wide range of methods are currently used to quantify SARS-CoV-2 in wastewater and sludge, involving different concentration and extraction methods and variations on PCR. Quantifying the recovery efficiency of SARS-CoV-2 by different methods would be useful for comparing results, and would ideally offer the ability to correct for different recovery efficiencies. The most common approach to quantify recovery efficiency is to use a proxy virus that is spiked into the sample prior to processing, and is intended to model the behavior of SARS-CoV-2. Proxy viruses that have been used to date include bovine coronavirus, bovine respiratory syncytial virus, murine hepatitis virus, and human coronavirus OC43. However, there are a number of factors that affect the recovery efficiency and may manifest differently depending on sample characteristics, concentration method, and the method used to quantify the initial stock solution. These factors can lead to comparison of "apples and oranges". For example, if the proxy virus and SARS-CoV-2 have different association with solids, the proxy virus will not adequately quantify the differences between methods that capture signal from wastewater solids and those that do. Similarly, if the proxy virus is added as intact virus, and SARS-CoV-2 RNA is present in intact viruses and as free RNA (Wurtzer et al. 2021), the proxy virus will not adequately quantify differences between methods that capture signal from intact viruses and free RNA, and the biases will vary from sample to sample. An early study compared 36 methods used in different laboratories and reported recovery efficiencies varying over seven orders of magnitude (Pecson et al. 2020). A strength of this study is that samples were prepared by a single laboratory andspiked with a proxy virus (betacoronavirus OC43), such that the recovery efficiency could be calculated relative to the same initial concentration and spike-in method. However, in current practice, every laboratory is preparing their own proxy virus and quantifying it differently so that recovery efficiencies cannot be directly compared across methods and labs.

Key words: recovery efficiency, virus proxy

Standards and control materials used: Virus proxy (spike-in) to measure SARS-CoV-2 recovery

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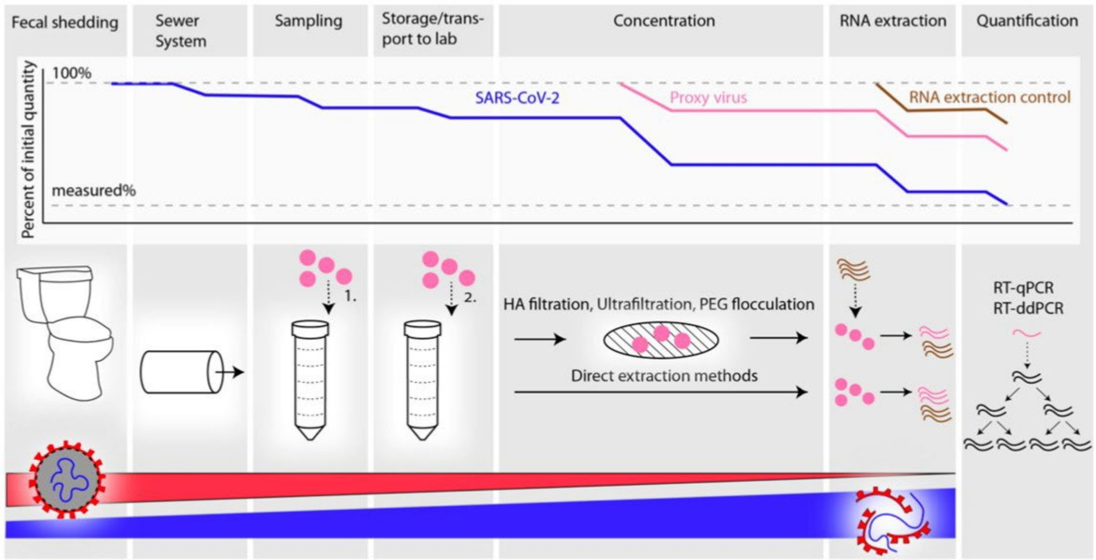

Figure 1. Factors affecting quantification of SARS-CoV-2 from wastewater. SARS-CoV-2 likely exists in wastewater along a continuum of intact (red) and nonintact (blue) viruses, and the ratio of these forms, and their association with solids, changes during transport of the sewage, sampling, and sample processing. For a sludge sample, there may also be loss of signal during primary settling. Spike-in proxy virus controls (pink) can be added 1) at the point of sampling prior to storage or 2) after storage at the beginning of sample processing. Proxy virus controls can account for degradation during storage and loss of signal due to incomplete recovery during concentration (pink line). A second control would be required to independently quantify the loss of signal during RNA extraction (brown line) because the spike control is affected by loss during RNA extraction. (Figure from Kantor et al. 2021).

References

Kantor, R.S., K.L. Nelson, H.D. Greenwald, and L.C. Kennedy. (2021) “Challenges in Measuring the Recovery of SARS-CoV-2 from Wastewater.” *Environmental Science & Technology*. 55(6): 3514-3519. https://doi.org/10.1021/acs.est.0c08210.

Pecson, B.M. et al. (2021) “Reproducibility and Sensitivity of 36 Methods to Quantify the SARS-CoV-2 Genetic Signal in Raw Wastewater:Findings from an Interlaboratory Methods Evaluation in the U.S.” *Environmental Science: Water Research & Technology*. https://doi.org/10.1039/D0EW00946F.

Wurtzer, S., P. Waldman, A. Ferrier-Rembert, G. Frenois-Veyrat, J. M. Mouchel, M. Boni, Y. Maday, V. Marechal, and L. Moulin. “SeveralForms of SARS-CoV-2 RNA Can Be Detected in Wastewaters: Implication for Wastewater-Based Epidemiology and Risk Assessment.” *Water Research* 198 (June 15, 2021): 117183. https://doi.org/10.1016/j.watres.2021.117183.

SARS-CoV-2 Wastewater Surveillance in a remote municipality in the Aleutians Islands of Alaska

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The City of Unalaska is a municipality located in the Aleutian Island chain approximately 800 miles southwest of Anchorage, Alaska. The City owns and operates a chemically enhanced primary treatment sewage facility which screens and disinfects an average of 0.483 million gallons of domestic wastewater per day.
Dutch Harbor is the largest commercial fishing port in the Pacific and is number one in the US for volume of fish. There are approximately 4,768 full time residents on the island and during peak seasons of the year, an additional 5-6,000 people are added to this population. The community's health care services are provided by a local clinic operated by Iliuliuk Family & Health Services, Inc. (IFHS). The clinic obtained two types of "rapid" COVID-19 testing devices at the beginning of the Covid 19 Pandemic, the Abbott IDNow system and the Cepheid GeneXpert IV-2 Molecular system and to date have performed 8,890 local clinical tests.
Wastewater-based epidemiology (WBE) began being explored as a new tool to track the spread of COVID-19 from the onset of the pandemic (Medema et al. 2020). Many studies report viral detection in sewage across the world. Detection of SARS-CoV-2 RNA in wastewater has been shown to be a valuable tool in early detection and informing public health decisions (Wu et al. 2020).
Weekly samples were collected at the Unalaska WWTP Influent and two lift stations from July 2000 until the present. Two methods were used to quantify SARS-CoV-2 in raw sewage. We developed a sensitive, consistent and reliable method to allow for pooled surveillance of wastewater which now serves as an early warning system.

Key words: SARS-CoV-2 Wastewater Surveillance**,** Method Development, Congregate living settings, Remote and rural environments

Standards and control materials used: The primers (N1 and N2) and DNA standards of SARS-CoV-2 nucleocapsid gene were used to quantify the titers of SARS-CoV-2 (Integrated DNA Technologies (IDT) 2019-nCoV CDC EUA qPCR Probe Assay primer/probe mix and 2019-nCoV_N Positive Control plasmid). Two replicates were performed. Positive and negative controls were included in each run as well as internal standardization control Pepper Mild MottleVirus (PPMoV) and the Seracare AccuPlex SARS-CoV-2 Verification Panel (0505-0168) was used as an extraction control.

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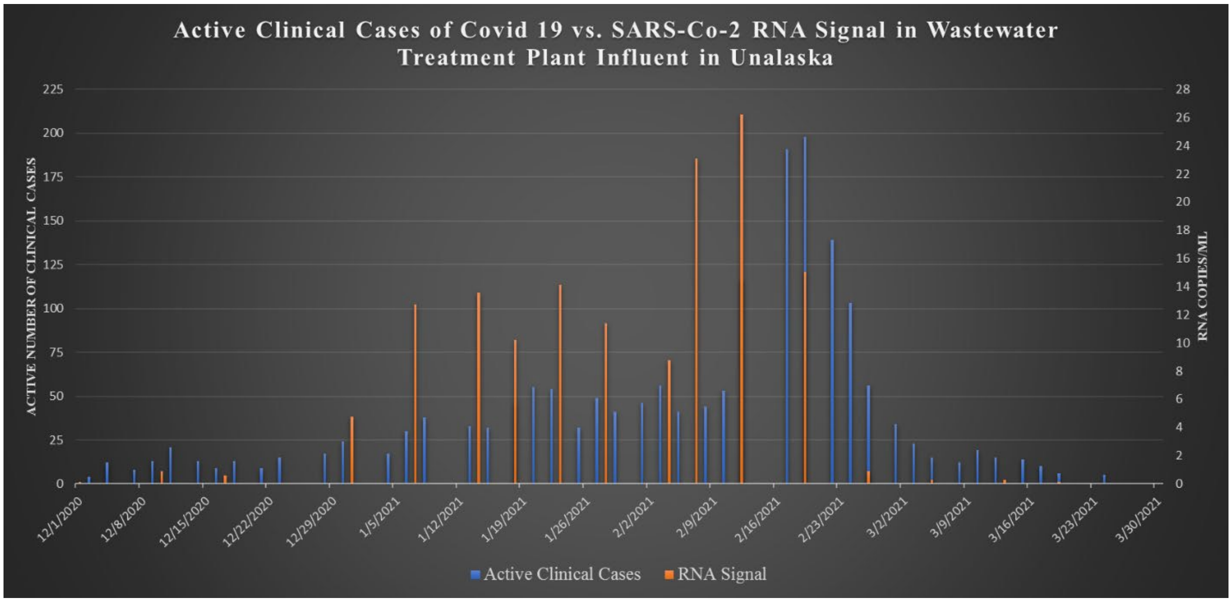

Figure 1. *Isolated cases on vessels kept quarantined and not connected to the city collection system were not included in this graph. The City remained insulated from the pandemic until December of 2020 at which time it experienced two outbreaks, the first at Unisea in Jan. 2021 and the second and more significant at Alyeska in Feb. 2021. Community spread remained low during the year and cases dropped off after vaccinations became widely available. Viral detection began at as low as ten reported active clinical cases. Viral load proceeded clinical cases by several days and followed clinical cases throughout the pandemic.*

References

Medema G, Heijnen L, Elsinga G, Italiaander R, Brouwer A. Presence of SARS-Coronavirus-2 RNA in Sewage and Correlation with Reported COVID-19 Prevalence in the Early Stage of the Epidemic in The Netherlands. Environ Sci Technol Lett. 2020;acs.estlett.0c00357. Published 2020 May 20. doi:10.1021/acs.estlett.0c00357

Fuqing Wu, Amy Xiao, Jianbo Zhang, Katya Moniz, Noriko Endo, Federica Armas, Richard Bonneau, Megan A Brown, Mary Bushman, Peter R Chai, Claire Duvallet, Timothy B Erickson, Katelyn Foppe, Newsha Ghaeli, Xiaoqiong Gu, William P Hanage, Katherine H Huang, Wei Lin Lee, Mariana Matus, Kyle A McElroy, Jonathan Nagler, Steven F Rhode, Mauricio Santillana, Joshua A Tucker, Stefan Wuertz, Shijie Zhao, Janelle Thompson, and Eric J Alm. 2020. "SARS-CoV-2 titers in wastewater foreshadow dynamics and clinical presentation of new COVID-19 cases." medRxiv.

Direct RT-qPCR assay for SARS-CoV-2 variants of concern (B.1.1.7 and B.1.351)detection and quantification in wastewater

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Less than a year following the SARS-CoV-2 outbreak, variants of concern have emerged in the form of variant B.1.1.7 the British variant and B.1.351 the South Africa variant. Due to their high infectivity and morbidity, it has become clear that it is crucial to quickly and effectively detect these and other variants. Here, we report improved primers-probe sets for RT-qPCR for SARS-CoV-2 detection including a rapid, cost-effective, and direct RT-qPCR method for detection of the two variants of concern (B.1.1.7 and B.1.351). All the developed primers-probe sets were fully characterized, demonstrating sensitive and specific detection. These primer-probe sets were also successfully employed on wastewater samples aimed at detecting and even quantifying new variants in a geographical area, even prior to the reports by the medical testing. The novel primers-probe sets developed and presented here have important implications; it will promote proper responses and pandemic containment, and may provide a basis for developing tools for the detection of additional variants of concern.

Key words: Real-Time Polymerase Chain Reaction; Wastewater-Based Epidemiological Monitoring; SARS-CoV-2; Molecular Probes; Variants of concern

Standards and control materials used: This study use synthetic genes for SARS-CoV-2 RT-qPCR detection calibration and wastewater for field proof of concept experiments.

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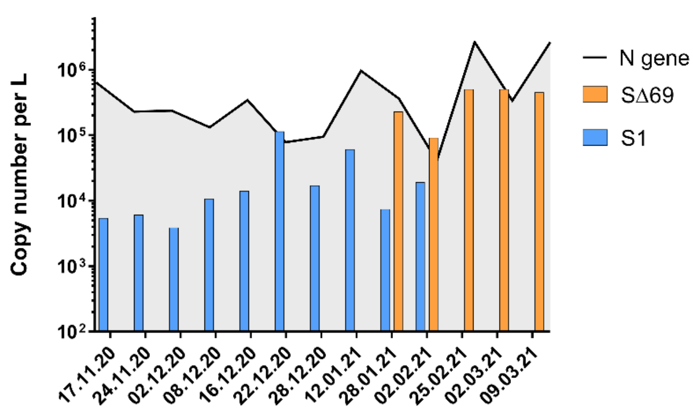

Figure 1. B.1.1.7 variant detection in Beer- Sheva wastewater (WWTP) over time. Samples collected between November 2020 and March 2021 were tested for N gene, S1 (Original lineage) and S�69 (British B.1.1.7 lineage) detection.

Assessing the representativeness of Covid-19 testing in different communities through wastewater surveillance

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We highlight the results of wastewater surveillance employed in the following communities to track the prevalence of Covid-19 infections therein.

In a sewershed that covers roughly 1 million residents in the greater New York City region, surveillance of SARS-CoV-2 concentrations is being conducted at different locations including the influent to different wastewater treatment plants. Based on the time-series-trends, concentrations of SARS-CoV-2 in the sewage streams have led the reported clinical case data by up to eleven days and in general have tracked the different waves of Covid-19 in the community.

In smaller residential populations (building-scale) in New York City, where clinical testing has been more frequent, wastewater testing has largely corresponded well with clinical data and has also been used to detect non-compliance with testing as well as the impact of any external interactions with the target community.

Data analysis and interpretation. The equivalence between the SARS-CoV-2 concentrations measured in wastewater and the corresponding fraction of the population infected is site-specific, as expected. Nevertheless, such equivalence calculations can be used to determine the severity of community infections as well as the impact of interventions such as vaccine administration.

Importantly, from a social-perspective, based on sewage surveillance, the progressive non-representativeness of clinical testing within some communities is also revealed.

Key words: wastewater; sewage; surveillance; population equivalence

Standards and control materials used: *(short answer)*: Standards for quantifying target virus biomarkers copy numbers in samples; wastewater flow and load information to attempt virus concentration-population equivalence determination.

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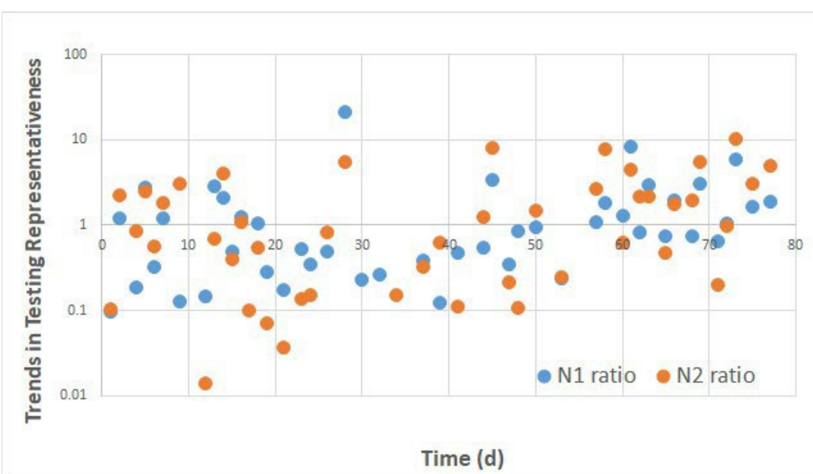

Figure 1. Shifting trends in the representativeness of clinical testing as inferred through wastewater surveillance. Values higher than 1.0 reflect increasing degree of non-representativeness.

2021 Standards for Wastewater Surveillance

Wastewater Surveillance's Role as Part of a Whole-of-University Response in Campus Protection

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During the last year, campuses of higher education have struggled with how to protect their communities in the face of the SARS CoV-2 pandemic. Initial strategies included massive routine testing of students, faculty, and staff to no testing at all[1]. At Oregon State University, selective COVID-19 testingof students, faculty and staff coupled with wastewater sampling and analysis, including RNA sequencing, across multiple campuses was used to inform the university's COVID-19 Continuity Management Team[2].Health authorities in the relevant counties were also kept advised of the progression of the disease within the campus community, and the presence of variants of concern. While the testing strategy, of necessity, evolved over the course of the pandemic, wastewater remained a consistent, and initially under-appreciated tool for providing insight on locations and pockets of the disease within the college community. Results from routine wastewater sampling across multiple campuses were used by university decision makers in directing increased testing, and subsequently quarantining of on campus residents. The data were also used in crafting website and email messaging for off campus students.

The net result was that information from wastewater surveillance informed health staff, underpinned rapid response testing of students, faculty, and staff, and help mitigate the spread of COVID-19 to the larger community.

[1] https://www.forbes.com/sites/jemimamcevoy/2020/09/11/19-of-the-25-worst-us-coronavirus-outbreaks-are-in-collegetowns/?sh=13d0f4491df7

[2] https://trace.oregonstate.edu/

Key words: Surveillance; COVID-19; wastewater sequencing; TRACE.

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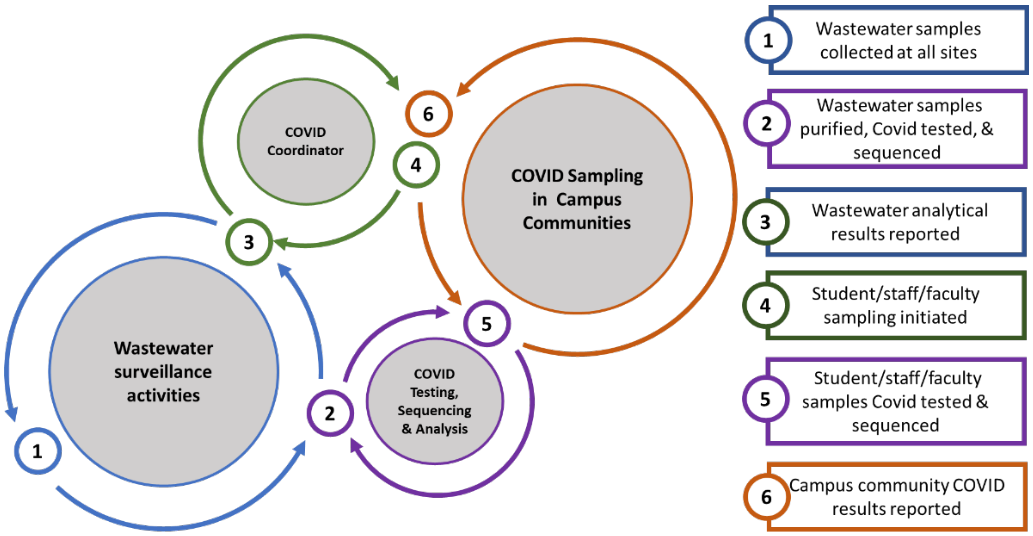

Figure 1. Process followed to collect, analyze, assess results, and direct additional sampling on the OSU campuses.

Towards Standardizing Antimicrobial Resistance Surveillance of Water Systems: AnExpert Survey and Workshop

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Monitoring sewage for antibiotic resistant bacteria and antibiotic resistance genes has the potential to inform the status of antimicrobial resistance (AMR) in human populations, while evaluation of treated effluent and recycled water can provide insight into the efficacy of treatment processes for reducing risk of spread in receiving environments (Hendrikson et al., 2019) and monitoring of surface water can reveal concerns for exposure via ingestion or recreation (Nappier et al., 2020). However, standardized methods for monitoring AMR in wastewater, recycled water, and surface water are needed in order to ensure that the data collected are meaningful and comparable across studies and surveillance efforts. While there have been numerous calls for standardization of methods for environmental AMR monitoring (Berendonk et al., 2015; Pruden et al., 2018; Hujibers et al., 2019), a key step towards achieving this goal will be agreement upon the purpose of such monitoring and prioritization of specific monitoring targets for achieving this purpose. To this end, we conducted a survey of 105 experts spanning the fields of academia and research, state and federal government, consulting, and water/wastewater utilities to obtain their recommendations regarding potential AMR targets. Following the online survey, we conducted a 4-day workshop attended by experts to discuss the state of the science, assess existing levels of standardization and feasibility, and obtain a final ranking of priorities for AMR monitoring in the U.S. water industry and regulatory community. Specifically, culture-based, quantitative polymerase chain reaction (qPCR)-based, and metagenomic-based methods were evaluated. Of special consideration was the prioritization of variables that consider overall potential to achieve monitoring objectives and address key research questions, for a wide-variety of stakeholders. A decision-tree framework was developed to assess which methods and targets are most appropriate based on specific monitoring objectives. Without such an effort, AMR research and surveillance will continue in silos and there will be a loss of opportunity to compare spatial and temporal trends, which will be critical to assessing the local and global rates of evolution and spread of AMR, assuring comparability of data and assessing the efficacy of mitigation measures. This perspectives study takes an important step towards identifying suitable targets for AMR monitoring standardization within water and wastewater systems in the U.S. and beyond.

Key words: Antibiotic Resistance; Wastewater Surveillance; Standardized Methods.

Standards and control materials used: *not applicable.*

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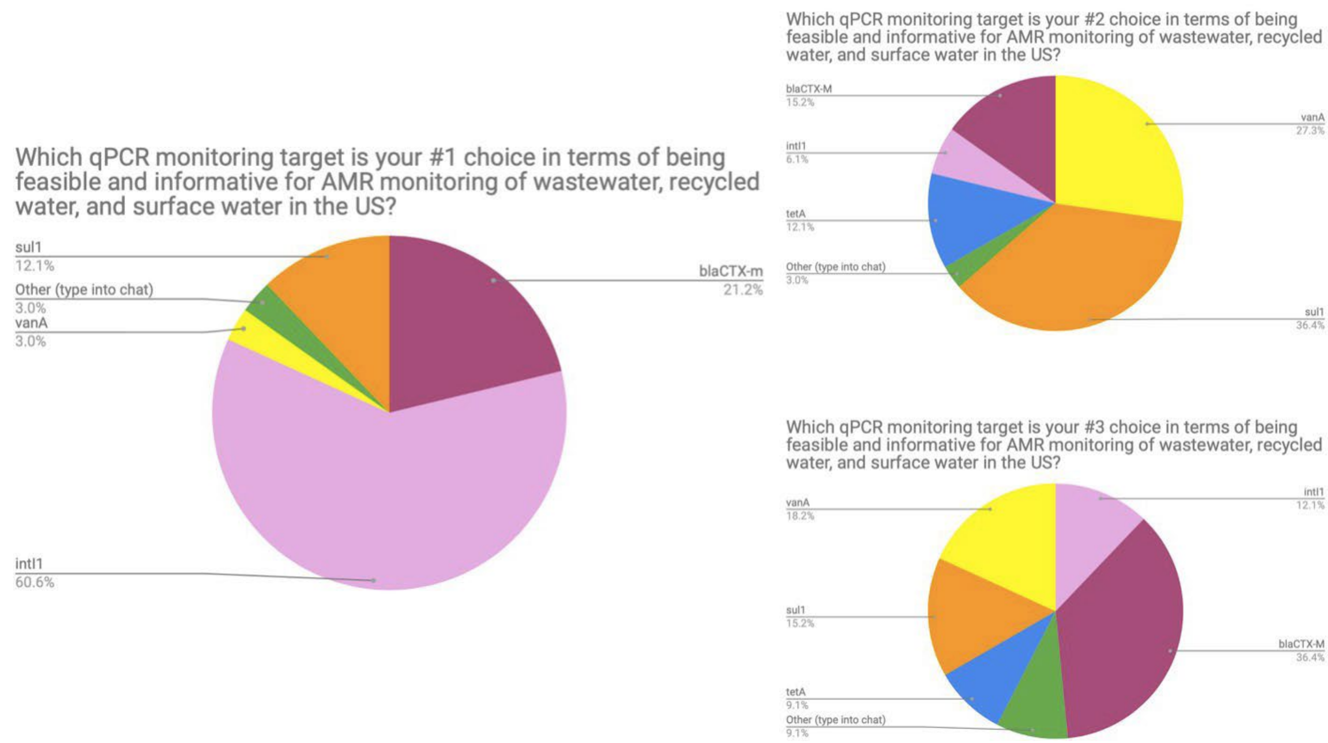

Figure 1. Workshop Experts rank their first, second, and third choices for a feasible and informative qPCR target for AMR monitoring in water for the US water industry.

References:

Berendonk, Thomas U., Célia M. Manaia, Christophe Merlin, Despo Fatta-Kassinos, Eddie Cytryn, Fiona Walsh, Helmut Bürgmann, Henning Sørum, Madelaine Norström, Marie Noëlle Pons, Norbert Kreuzinger, Pentti Huovinen, Stefania Stefani, Thomas Schwartz, Veljo Kisand, Fernando Baquero, and José Luis Martinez. 2015. “Tackling Antibiotic Resistance: The Environmental Framework.” Nature Reviews Microbiology 13(5):310–17.

Hendriksen, R.S., Munk, P., Njage, P. et al. Global monitoring of antimicrobial resistance based on metagenomics analyses of urban sewage. Nat Commun 10, 1124 (2019). https://doi.org/10.1038/s41467-019-08853-3

Huijbers PMC, Flach CF, Larsson DGJ. 2019. A conceptual framework for the environmental surveillance of antibiotics and antibiotic resistance. Environ Int 130:104880.

Nappier SP, Liguori K, Ichida AM, Stewart JR, Jones KR. Antibiotic Resistance in Recreational Waters: State of the Science. International Journal of Environmental Research and Public Health. 2020; 17(21):8034. https://doi.org/10.3390/ijerph17218034

Pruden, A., Alcalde, R. E., Alvarez, P. J., Ashbolt, N., Bischel, H., Capiro, N. L., ... & Zhou, Z. (2018). An environmental science and engineering framework for combating antimicrobial resistance. Environmental Engineering Science, 35(10), 1005-1011.

First detection of SARS-CoV-2 proteins in wastewater samples by mass spectrometry

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On March 12, 2020, the World Health Organization (WHO) declared COVID-19 as a global pandemic. COVID-19 isproduced by a novel �-coronavirus known as Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV2) [1]. Several studies have detected SARS-CoV-2 RNA in urine, feces, and other biofluids from both symptomatic and asymptomatic people with COVID-19 [2], suggesting that SARS-CoV-2 RNA could be detected in human wastewater [3]. Thus, wastewater-based epidemiology (WBE) is now used as an approach to monitor COVID-19 prevalence in many different places around the world [4-10]. Reverse transcription quantitative polymerase chain reaction (RT-qPCR) is the most common SARS-CoV-2 detection method in WBE, but there are other methods for viral biomolecule detection that could work as well. The aim of this study was to evaluate the presence of SARSCoV-2 proteins in untreated wastewater (WW) influents collected from two wastewater treatment plants (WWTPs), fromDurham Region, Ontario, Canada, using a LC-MS/MS-based proteomics approach. Twenty-four-hour composite influent samples from each of the two wastewater treatment plants (WWTP) were obtained over the course of 15 weeks – for a total of 171 samples across all sampling times and locations. A cross correlation was performed firstwith all the 160 pairs of data (x: Protein pp1ab 7-day midpoint mean viral signal, y: 7-day midpoint mean in reportednew cases of COVID-19 by onset date) and with lags between -15 to 15 as the maximum (cross correlation in timeseries). The lag with the highest value of r was selected and was used to perform a Pearson correlation analysis (one-tailed with a confident interval of 95%). We identified many SARS-CoV-2 proteins in these wastewater samples, with peptides from pp1ab being the most consistently detected and with consistent abundance (protein related to the COVID-19 infection).

Key words: SARS-CoV-2, COVID-19, liquid-chromatography mass spectrometry, wastewater, proteomics

Standards and control materials used: N/A

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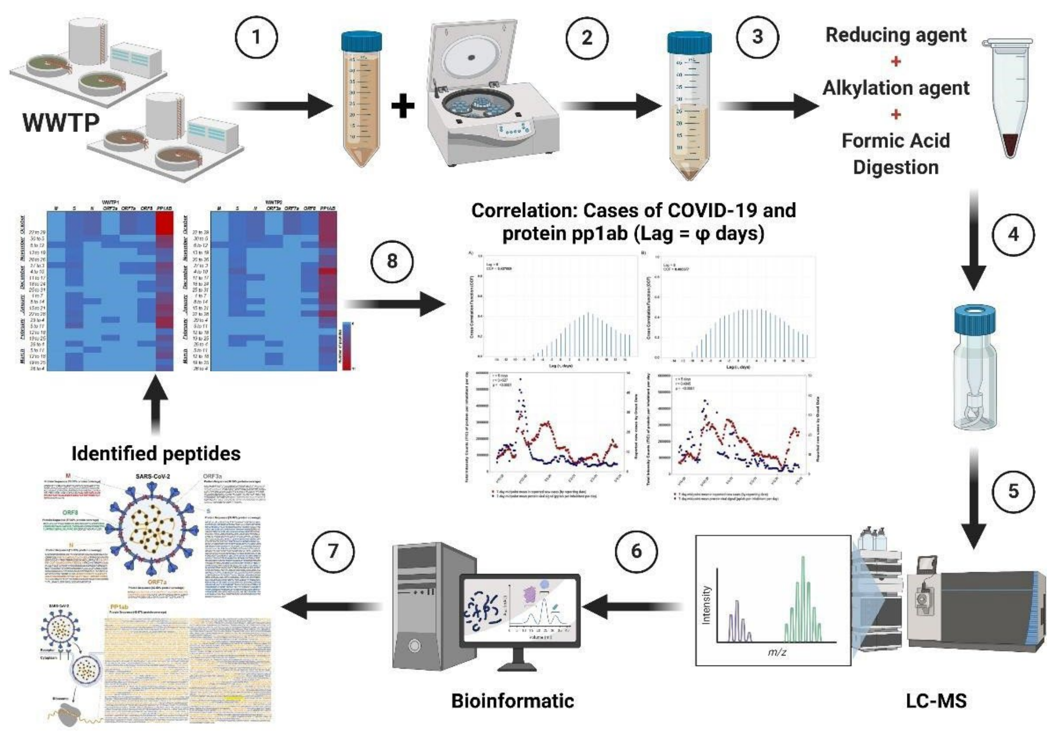

Figure 1. Workflow for protein identification by LC-MS and the correlation of COVID-19 cases vs protein pp1ab.

References

1. Li, Q., et al., *Early Transmission Dynamics in Wuhan, China, of Novel Coronavirus-Infected Pneumonia.* N Engl J Med, 2020. **382**(13): p. 1199-1207.
2. Wang, W., et al., *Detection of SARS-CoV-2 in Different Types of Clinical Specimens.* JAMA, 2020. **323**(18): p. 1843-1844.
3. Kitajima, M., et al., *SARS-CoV-2 in wastewater: State of the knowledge and research needs.* Sci TotalEnviron, 2020. **739**: p. 139076.
4. Ahmed, W., et al., *First confirmed detection of SARS-CoV-2 in untreated wastewater in Australia: A proofof concept for the wastewater surveillance of COVID-19 in the community.* Sci Total Environ, 2020. **728**: p. 138764.
5. D'Aoust, P.M., et al., *Catching a resurgence: Increase in SARS-CoV-2 viral RNA identified in wastewater48 hours before COVID-19 clinical tests and 96 hours before hospitalizations.* 2020.
6. D'Aoust, P.M., et al., *Quantitative analysis of SARS-CoV-2 RNA from wastewater solids in communitieswith low COVID-19 incidence and prevalence.* 2020.
7. Sherchan, S.P., et al., *First detection of SARS-CoV-2 RNA in wastewater in North America: A study in Louisiana, USA.* Sci Total Environ, 2020. **743**: p. 140621.
8. Westhaus, S., et al., *Detection of SARS-CoV-2 in raw and treated wastewater in Germany - Suitability forCOVID-19 surveillance and potential transmission risks.* Sci Total Environ, 2021. **751**: p. 141750.
9. Randazzo, W., et al., *SARS-CoV-2 RNA in wastewater anticipated COVID-19 occurrence in a low prevalence area.* Water Res, 2020. **181**: p. 115942.
10. Lodder, W. and A.M. de Roda Husman, *SARS-CoV-2 in wastewater: potential health risk, but also datasource.* The Lancet Gastroenterology & Hepatology, 2020. **5**(6): p. 533-534.

WBE Data Sharing, Ethics, and Community Outreach

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Key words: wastewater based epidemiology, COVID-19, SARS-Cov-2, environmental monitoring, research ethics, community engagement

Standards and control materials used: n/a

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Abstract: Issues of privacy or stigmatization pose potential concerns for research, data collection, and analysis of wastewater. However, through the assurance of anonymous results, the protection of identifiable information, and diversity of selected sampling sites, the Co-Immunity Project has incorporated ethical approaches to their work which safeguard the safety and well-being of individual privacy in effective ways. **By developing strategies for transparency around scientific findings and research outcomes**, as well as plans for crafting community agreements that incorporate the input of our community members, **ethical research methods have become a major component of the Co-Immunity wastewater study.** As further knowledge and insights regarding the ethics of wastewater testing continue to emerge and evolve, we are committed to adjusting our own principles and adopting new guidelines into our studies with the intent of promoting equity and inclusivity in the realms of medicine and public health.

Four strategies have emerged that create opportunities to increase transparency and establish bi-directional communication with the public around our wastewater monitoring research:

1. Online dashboard: This dashboard is updated weekly and allows anyone to see current and past levels of SARS-CoV-2 in Louisville's wastewater samples.
2. WBE ethics-centric website: This website hosts facts and frequently asked questions about wastewater work, a history of water monitoring for public health, a link to the online dashboard, and a gallery detailing how wastewater samples are collected.
3. Office Hours: Beginning on Thursday, June 10, 2021 the research team will host an open office hour via Zoom and Facebook Live to make wastewater researchers available to the public, to learn what people think about wastewater monitoring, and hear about possible concerns.
4. Community Survey: The next round of Co-Immunity's Community Testing will run from June 17 through June 23. This round will include a survey about our wastewater monitoring work. This survey will collect the public's level of knowledge about wastewater monitoring, how supportive people are of this type of work, and about possible concerns.

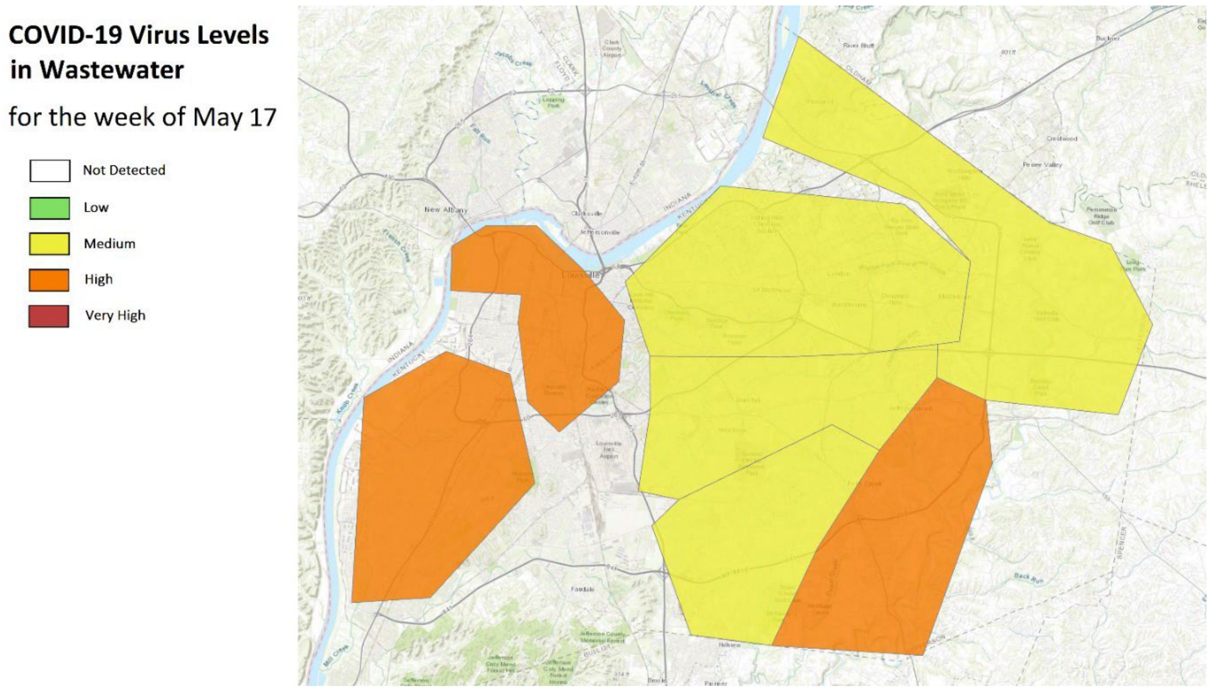

Figure 1. UofL Envirome Institute Wastewater Dashboard Map, May 17, 2021. Access via https://louisville.edu/envirome/thecoimmunityproject/dashboard

Figure 2. Co-Immunity Project's Wastewater Research Office Hour Flyer for June 10, 2021

Systematic review and meta-analysis of correlations for time-lagged COVID-19cases and viral load in wastewater

Michael Austin

San Diego State University

Early detection of cases by the diagnostic testing of symptomatic persons and regular screening, isolation of presumed and confirmed cases and contact tracing are the key to controlling an infectious disease outbreak. However, the standard public health approach to an outbreak requires augmented ancillary surveillance mechanisms to successfully control COVID-19. Amid the pandemic, numerous studies have reported a positive correlation between COVID-19 epidemic indicators and the normalized viral load of SARS-CoV-2 in wastewater. Additionally, studies report that the viral load in wastewater is a leading indicator of confirmed cases within a population by days to weeks. Yet, significant methodological questions remain to robustly trend community cases with the viral signal in wastewater utilizing the technique known as wastewater-based epidemiology (WBE). The aim of this study is to systematically review the literature on WBE for studies trending normalized viral load of SARS-CoV-2 with disease indicators and to compare the effects observed at baseline with those that include a timelag. We also aim to pool results for studies reporting common effect sizes and outcomes to delineate study-level heterogeneity.

Key words: N/A

Standards and control materials used: N/A

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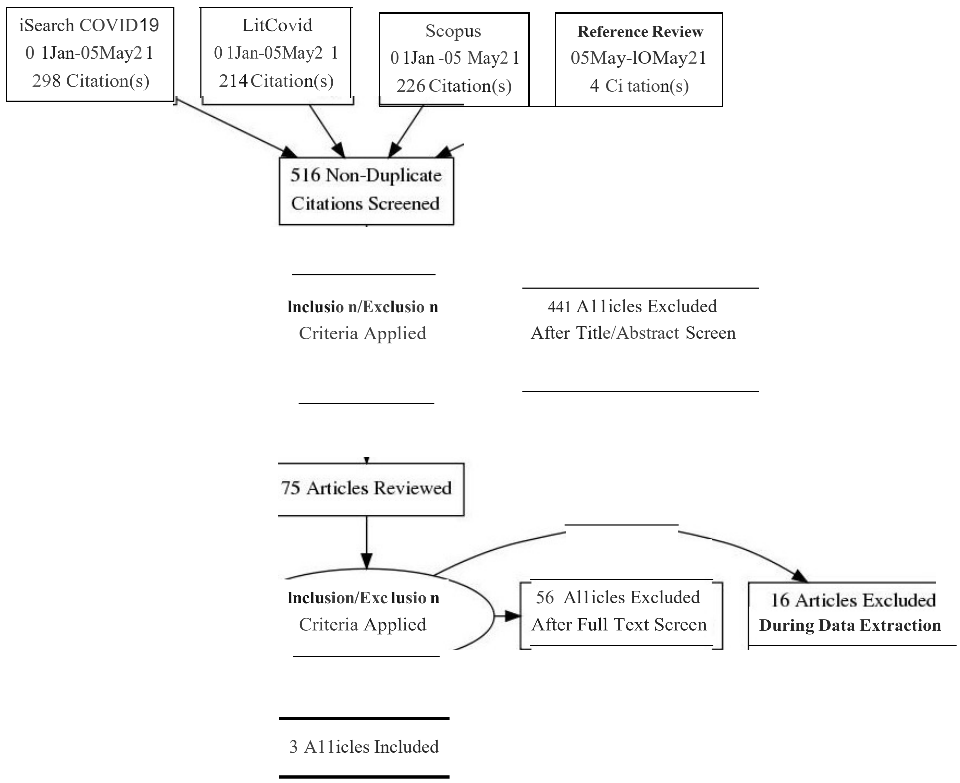
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Nucleic Acid Extraction with Microbubbles from Wastewater Produces Simple andHigh-Yield Workflows

M. Maloney[1], C. Livi[1], J. Roussey[1], C. Wegner[1],
[1]Akadeum Life Sciences, Inc., Ann Arbor, MI

The COVID-19 pandemic has highlighted the need for simple but sensitive technologies and simple workflows to enable environmental monitoring for communicable disease agents. Nearly everything about human behavior leaves a signal in urine, stool, or blood – this makes sewage an efficient pooled-sample source to monitor circulating infectious agents in a community. Novel approaches to surveillance will enable sensitive, specific and timely response to infections.

Akadeum's microbubbles are overcoming barriers to rare target capture from complex matrices such as wastewater utilizing an innovative approach: field-deployable, buoyant, functionalized microbubbles. In this presentation we demonstrate the utility and benefits of floatation-based capture reagents and associated workflows. Here proprietary microbubbles are specifically functionalized to capture nucleic acids. Unlike the inherent limitations of magnetic bead-based separation, microbubbles do not have the same volume and equipment restrictions. Using microbubbles for nucleic acid extraction employs a positive selection protocol in which the microbubbles bind to the desired target – in this case, total RNA including SARS-CoV-2 if present – isolating and enriching the analyte for downstream genomic analysis.

First demonstrated in saliva samples, the microbubbles were able to interrogate large fluidic volumes, which enables a pooled sample approach desirable in community surveillance of viral infection. In fact, microbubble extraction delivered 25x higher sensitivity in detecting SARS-CoV-2 RNA based on qPCR detection compared to a magnetic isolation kit. Translating this workflow to wastewater samples, the microbubble nucleic acid extraction can directly interrogate large fluidic volumes of unfiltered sewage samples to increase RNA capture for downstream processing. A sensitivity of at least 100 copies per mL was observed with a protocol that avoids preprocessing steps such as the removal of nucleic acid-rich solids.

Akadeum offers a simplified and cost-effective performance platform for the isolation of cells, proteins and nucleic acids that can be seamlessly integrated into existing workflows. Akadeum is seeking partnerships for applications of the nucleic acid microbubble in diagnostics and community surveillance including cell, protein and nucleic acid-based analyte enrichment for testing.

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	Ct		Copy #	
Condition	N Gene	ORF1ab	N Gene	ORF1ab
Naked RNA	30.7	36.3	2137	46.2

Figure 1. Akadeum microbubble-based RNA extraction technology enables a simplified workflow that avoids preprocessing cleaning steps such as the removal of solids. This increases sensitivity by retaining the RNA that would otherwise be lost by preprocessing. Here we demonstrate detection of covid RNA in a wastewater sample (n = 3) spiked with 100 cp/mL of Sars-CoV-2 RNA.

Single Particle ICP-MS: A Powerful Tool for Characterization of Nanomaterials in Wastewater Surveillance

Monique E. Johnson[1], Karen E. Murphy,[1] Antonio R. Montoro Bustos[1], and Michael Winchester[1]

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Nanomaterials, defined as having at least one dimension falling in the range from 1 nm to 100 nm, have been identified as contaminants of emerging concern by the Environmental Protection Agency [1]. Similar to traditional chemical contaminants, some synthetic nanomaterials have been shown to be toxic to microbes, plants, and animals. The ever-increasing incorporation of nanomaterials into consumer products results in many routes of environmental exposure, including wastewater.

Analytical methods, critical for the detection and characterization of nanomaterials in wastewater, can address current gaps in knowledge. In particular, single particle inductively coupled plasma mass spectrometry (spICP-MS) is an emerging technique that enables measurements of size distributions and number concentrations of nanoparticles suspended in liquids. Special characteristics of spICP-MS that make it attractive for wastewater surveillance include extremely good nanoparticle detection capability down to truly environmentally relevant levels and extremely rapid analysis. The use of spICP-MS for wastewater surveillance has been outlined in recent reviews [2,3] and demonstrated in publications describing the detection and sizing of TiO_2 nanoparticles in municipal sewage treatment plants [4] and the size characterization of silver nanoparticles in wastewater [5,6]. However, the technique presents challenges and limitations that researchers have highlighted and are striving to overcome, *particularly with respect to the lack of reference materials and standardization of spICP-MS methodologies*. Provision of suitable reference materials and standard methods would enable widespread effective and efficient application of spICP-MS for wastewater surveillance to ensure that human health is not affected detrimentally by nanomaterials.

The goal of our presentation is to highlight a few examples of the work that has been done within the NIST Inorganic Chemical Metrology Group (646.01) toward the advancement of spICP-MS as a mature technique. This work has provided a foundation for developing the standards and reference materials that are necessary to enable routine wastewater surveillance for nanoparticles. In the past five years, our group has engaged in several activities to build a robust nanomaterial spICP-MS measurement infrastructure, including:

- validation of spICP-MS for routine characterization of gold nanoparticles of different sizes, coatings, and surface charge at environmentally relevant concentrations in water;
- analysis of silver and titanium dioxide nanoparticles in water;
- characterization of silicon dioxide food additive materials in aqueous media;
- the characterization of the uptake of gold nanoparticles in *Caenorhabditis elegans*; and
- the potential application of spICP-MS for the characterization of nanoplastics suspended in water.

Key words: Nanomaterials, single particle ICP-MS, environmentally relevant concentration, reference materials, standards.
Standards and control materials used: NIST RM 8012 and 8013 gold nanoparticles; NIST RM 8017 silver nanoparticles; NIST SRM 1898 TiO_2; gold, silver, silicon, and titanium ionic standard solution calibrants

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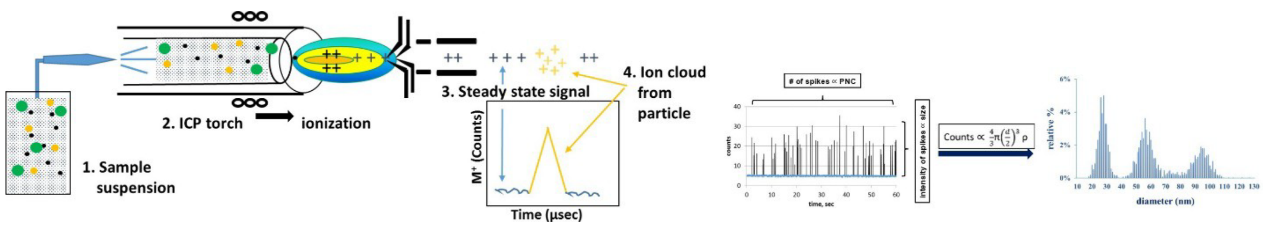

Figure 1. Schematic representation of spICP-MS operation principle and simultaneous information provided.

References
[1] https://www.epa.gov/sites/production/files/2014-03/documents/ffrrofactsheet_emergingcontaminant_nanomaterials_jan2014_final.pdf
[2] Nano/microplastics in water and wastewater treatment processes – Origin, impact, and potential solutions. https://doi.org/10.1016/j.watres.2019.06.049
[3]https://pubs.rsc.org/en/content/articlehtml/2020/ja/c9ja00206e. A critical review of single particle inductively coupled plasma mass spectrometry – A step towards an ideal method for nanomaterial characterization.
[4] https://link.springer.com/article/10.1007/s00128-017-2031-8. Detection of TiO2 Nanoparticles in Municipal Sewage Treatment Plant and Their Characterization Using Single Particle ICP-MS.
[5] https://www.azonano.com/article.aspx?ArticleID=4232. Particle Size Measurements on Silver Nanoparticles in Waste Water with Single Particle ICP-MS.
[6] https://doi.org/10/1016/j.watres.2019.03.031 Incidence and persistence of silver nanoparticles throughout the wastewater treatment process.

A Direct Nucleic Acid Capture Method for Purification and Detection of SARS-CoV-2 Genetic Material from Wastewater

Nathan Feirer[1] and Subhanjan Mondal[1]

[1]Promega Corporation, 5430 E Cheryl Pkwy, Madison, WI 53711

Early in the COVID-19 pandemic, scientific studies demonstrated that the genetic material of SARS-CoV-2, an enveloped RNA virus, could be detected in wastewater. This finding mobilized investigation of whether wastewater-based epidemiology (WBE) monitoring the genetic signal of SARS-CoV-2 could be used to track 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 for sensitive detection and utility in WBE. We hypothesized that a direct capture method that renders total nucleic acid conducive to binding to affinity resin may be able to overcome cumbersome, non-standardized, and variable 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 to remove co-purifying PCR inhibitors, and then eluted with water. The eluted nucleic acid can then be further processed in a second step with either a spin column or using an automated nucleic acid purification system, like the Maxwell RSC®.

In parallel, we formulated RT-qPCR enzyme mixes that demonstrate resistance to PCR inhibitors commonly found in wastewater. RT-qPCR assays were developed to detect N1, N2 (nucleocapsid) and E (envelope) gene fragments of SARS-CoV-2 as part of multiplexed assays. Pepper Mild Mottle Virus (PMMoV), a fecal indicator RNA virus present in wastewater,and an exogenous inhibition control were included in all PCR reactions for quality control. The workflow has a limit of detection of 1.6 GC/ ml with 40 ml of raw wastewater sample. Using this workflow, we monitored wastewater samples from three WWTP in Dane County, Wisconsin, serving a combined population of over 300,000 people, for four months covering the peak of community prevalence. Our analysis was compared to the COVID-19 cases declared by the municipalities, demonstrating strong correlation between the SARS-CoV-2 viral load present in wastewater and clinical cases.

Key words: SARS-CoV-2, Wastewater, RT-qPCR, COVID-19, Direct Capture

Standards and control materials used: *Processing/Testing Methods*

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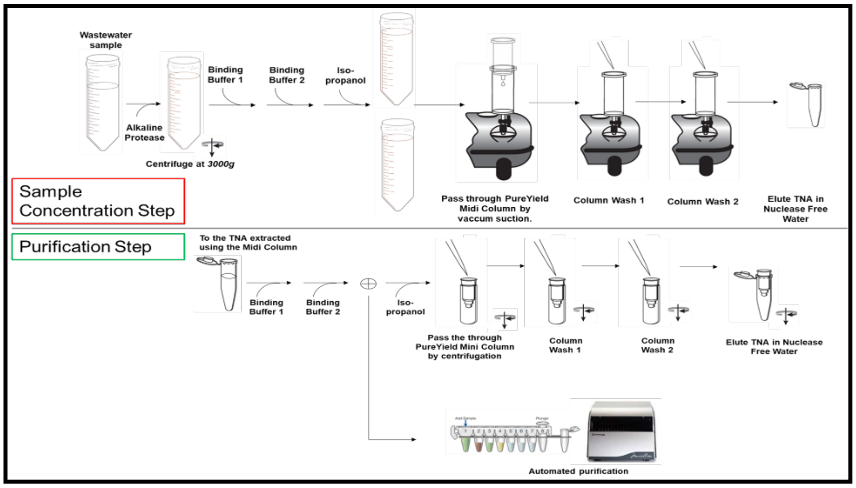

	20X SARS-CoV-2 Primer / Probe
SARS CoV-2	**N1** or **N2** or **E** forward primer
	N1 or **N2** or **E** reverse primer
	N1 or **N2** or **E** Probe (FAM)
Human fecal & Matrix recovery	**PMMoV** forward primer
	PMMoV reverse primer
	PMMoV Probe (Cy5)
Inhibition Assessment	**IAC** control forward primer
	IAC control reverse primer
	IAC Control Probe (HEX)
	IAC 435bp DNA-template
	100X CXR (ROX)

Figure 1. **Workflow of the direct nucleic acid capture method for purification and detection of SARS-CoV-2 genetic material fromwastewater**. Image on the left details the direct capture and purification procedure. Table on right demonstrates components of multiplex RT-qPCR reaction.

Using municipal data to develop geographically-precise sampling frame standards

R. Yeager[1,2], R. H. Holm[1], K. Saurabh[3,4], D. Talley[7], A. Bhatnagar[1] and T. Smith[1]

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Wastewater monitoring for SARS-CoV-2 within communities can be utilized as a powerful to surveil and respond to the current COVID-19 pandemic, variants of concern, and future outbreaks with pandemic potential. However, there are currently no evidence-based and widely-accepted standards for urban sampling site identification and assessment. To inform development of such standards, we describe a protocol for developing a geographically-resolved wastewater sampling methodology in Jefferson County, Kentucky, and describe our preliminary results. We utilized this site selection protocol to identify 17 sample locations, based on municipal sewer data, representing distinct wastewater catchment areas. We collected samples (n = 285) at these locations from September 8 to October 30, 2020 from one to four times per week. We then compared SARS-CoV-2 testing results with contemporaneous and geographically-matched clinical testing-based COVID-19 rates. We found that SARS-CoV-2 RNA was consistently present in each catchment area with significant spatial and temporal variation. We also observed substantial differences between trends in wastewater-based detection and clinical-testing. These findings verify that our site selection protocol is an efficacious approach, which may be used to inform development of a standardized best-practice approach of geographically-resolved SARS-CoV-2 wastewater sample site selection in urban areas.

Key words: Wastewater, Geographic, GIS, sewershed, catchment

Standards and control materials used: *Municipal data and preliminary monitoring results*

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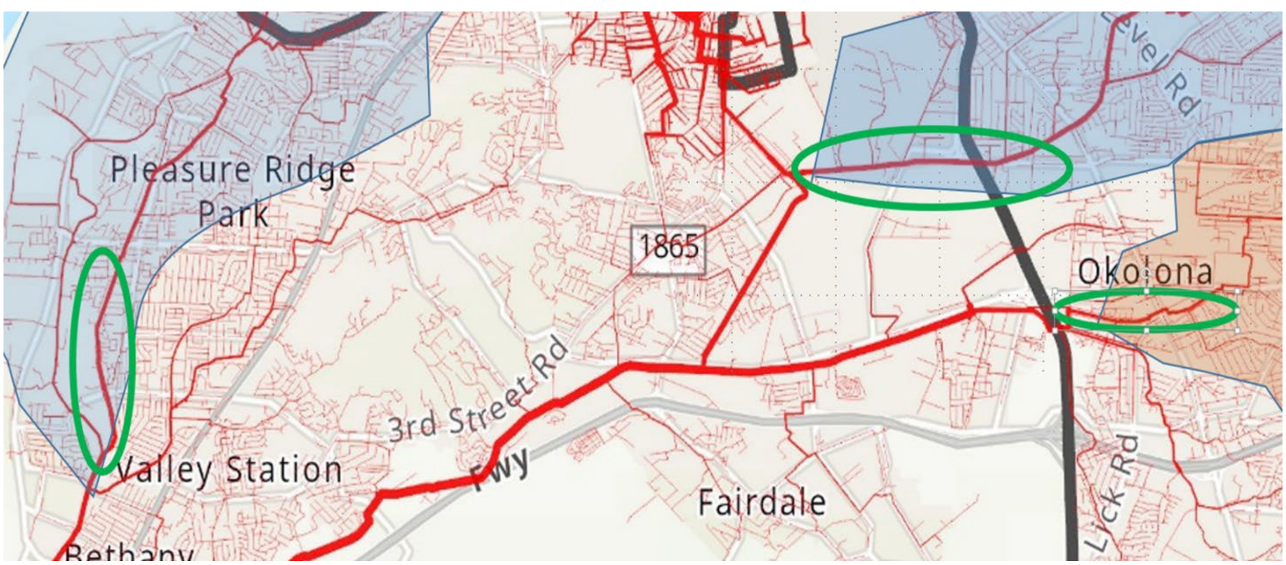

Figure 1. Visualization of geographically-resolved site selection process.

Rapid Capture of SARS-CoV-2 Using Nanotrap® Magnetic Virus Particles

Alex Barclay[1], Smruthi Karthikeyan[2], Ben Lepene[1], Patrick Andersen[1], Daniel Goldfarb[1], Kevin Kolb[1], Joshna Seelam[1], Tara Jones-Roe[1], Rob Knight[2], Robbie Barbero[1]
[1]Ceres Nanosciences, Inc.
[2]University of California San Diego

SARS-CoV-2 is the causative agent of COVID-19, a disease that has caused a worldwide pandemic and claimed over 3 million lives. SARS-CoV-2 is shed in feces, enabling wastewater-based surveillance of the disease, which can allow for monitoring of infection rates in communities as well as to help identify SARS-CoV-2 variants of concern that may occur in an area. Methods currently employed to isolate SARS-CoV-2 from wastewater are long, arduous, and are not suitable for high throughput testing. Here, we offer an alternative method for viral isolation, use of Ceres Nanosciences' Nanotrap® Magnetic Virus Particles, which capture and concentrate whole virus from wastewater. This new method gives comparable results to PEG/ultracentrifugation and HA filtration across a range of viral titers despite using 80% less initial volume than either while also reducing processing time by several hours. Here we demonstrate that this method is compatible with multiple viral RNA extraction kits and that it can readily be automated, allowing for high-throughput testing. Overall, the Nanotrap® Magnetic Virus Particles offer a shorter, more user-friendly workflow than competing virus isolation methods while maintaining comparable sensitivity.

Key words: viral enrichment, viral concentration, wastewater processing
Standards and control materials used: *Heat-inactivated SARS-CoV-2 (ATCC)*
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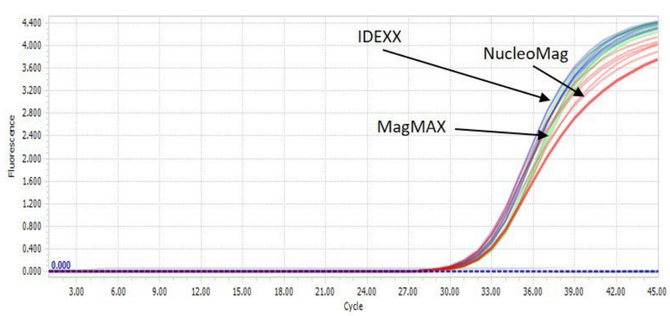

Sample	Ct
NT + IDEXX	29.56
NT+ MagMAX	29.95
NT+ NucleoMag	29.54

Figure 1. Wastewater spiked with heat-inactivated SARS-CoV-2 at 1000 copies/mL. Virus concentration performed using Nanotrap® Magnetic Virus Particles and compatibility with three standard RNA extraction kits demonstrated using the CDC nCoV-2019 N1 RT-PCR assay.

Equity in standardized collection of pooled community stool for pathogen detection: Moving from pit latrines to sewered systems globally

Rochelle H. Holm[1], and Ted Smith[2]

[1]Centre of Excellence in Water and Sanitation, Mzuzu University, Mzuzu, Malawi; [2]Christina Lee Brown Envirome Institute,University of Louisville, Louisville, Kentucky, United States of America

Sustainable Development Goal (SDG) 3 covers health and 6 covers sanitation, and the COVID-19 pandemic has brought the research community interest in health monitoring using pooled community stool to the forefront. While 41% of the world's population uses a sewered connection, an equal 41% uses a non-sewered sanitation system such as septic tanks, pit latrines and other improved on-site systems (UNICEF and WHO, 2019). Both sewered and non-sewered systems can track pathogens (Capone et al., 2021; Yeager et al., 2021). Yet, sample collection standards for both sewered and non-sewered systems use similar equipment and human capacity; though existing pit latrine sampling utilizing fecal sludge management approaches for pathogen detection are more similar than wastewater sampling approaches for environmental compliance. The pandemic has brought more equitable sharing of sample collection methods globally through avenues such as the National Science Foundation Research Coordination Network webinar series, a wastewater focused Slack channel, and journals waiving publication fees for COVID-19 research. The pandemic has also brought attention to value new professionals entering the sanitation field, where researchers from the global south with experience looking at pathogen detection in pit latrines have unique experience to share with researchers pivoting other research interests (including graduate students, post-docs and early career professionals) in the global north. Post- pandemic, instead of a 'brain drain' of sanitation professionals leaving the global south there could be greater incentive to continue their health monitoring using pooled community stool. The equity in pooled community stools for pathogen detection has uniquely brought about collaboration and cooperation during the pandemic for standardized sample collection that can only help global sanitation capacity building with practical skills across the global south and north to meet the SDGs and support a better post-pandemic world.

Key words: sample collection; capacity building; pit latrines; sewered sanitation systems

Standards and control materials used: Sample collection standards for both sewered and non-sewered systems use similar equipment and human capacity.

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Figure 1. Sample collection methods for pathogens from pit latrines (left) and sewered systems (right).

First detection of SARS-CoV-2 RNA in wastewater in North America: A study in Louisiana, USA

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We investigated the presence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) RNA in wastewater samples in southern Louisiana, USA. Untreated and treated wastewater samples were collected on five occasions over a four-month period from January to April 2020. The wastewater samples were concentratedvia ultrafiltration (Method A), and an adsorption–elution method using electronegative membranes (Method B). SARS-CoV-2 RNA was detected in 2 out of 15 wastewater samples using two reverse transcription-quantitative polymerase chain reaction (RT-qPCR) assays (CDC N1 and N2). None of the secondary treated and final effluent samples tested positive for SARS-CoV-2 RNA. To our knowledge, this is the first study reporting the detection of SARS-CoV-2 RNA in wastewater in North America, including the USA. However, concentration methods and RT-qPCR assays need to be refined and validated to increase the sensitivity of SARS-CoV-2 RNA detection in wastewater.

Keywords
Wastewater-based epidemiology
SARS-CoV-2
Surveillanc
eCOVID-
19 RT-
qPCR
Wastewater

Standards and control materials used: *phi6*

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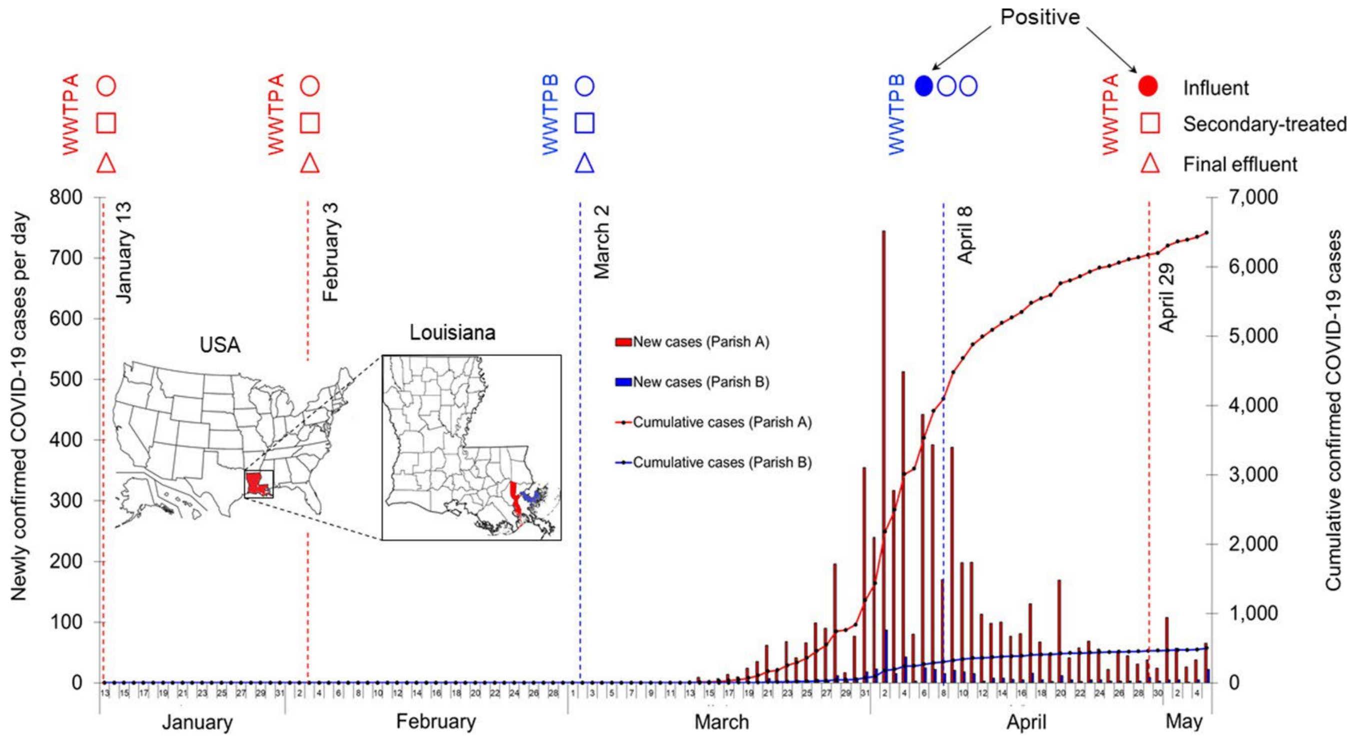

Fig. 1. SARS-CoV-2 RNA detection in wastewater and confirmed COVID-19 cases in southern Louisiana, USA. Circles, squares, and triangles represent sample types, i.e.,influent, secondary-treated, and final effluent, respectively. Red and blue symbols represent samples collected from WWTPs A and B, respectively. Closed and open symbols denote positive and negative SARS-CoV-2 RNA detections, respectively.

Whole Cell Reference Materials to Assess Microbial Detection in Wastewater

Sandra Da Silva[1], Joy P. Dunkers[1], Kirsten H. Parratt[1], Guilherme Pinheiro[1,2], Samuel T. Hailemichael[1,3], and Nancy J. Lin[1]
[1]Biosystems and Biomaterials Division, Material Measurement Laboratory, National Institute of Standards and Technology, Gaithersburg, MD; [2] Metrology Institute (INMETRO), Rio de Janeiro, Brazil;
[3]Montgomery College, Germantown, MD

Wastewater surveillance (WWS) holds great promise for monitoring public health concerns such as emerging pathogens (e.g., SARS-CoV-2), microbes (e.g., enteric bacterial pathogens), or antimicrobial resistance (AMR) genes. For example, a global effort is underway to monitor wastewater for AMR genes to assess the extent of AMR in communities.[1] When detecting microbes or their genes in wastewater, it is challenging to compare protocols and assess overall process efficiency due to high variability in samples and workflows across the country. Highly characterized whole cell reference materials (RMs) can normalize analytical workflows, enable method comparison, and challenge the entire process from sample collection to data analysis, ultimately increasing confidence in results. NIST is developing two viable whole cell RMs as surrogates for microbial (bacterial or fungal) targets of interest to assess workflow parameters, such as sampling efficiency, DNA extraction efficiency, limit of detection, and bioinformatics pipelines. The first consists of *Escherichia coli* NIST0056 cells characterized for total and viable cell number and genome copies/cell using flow cytometry and optical microscopy. The second, NIST RM 8230–*Saccharomyces cerevisiae* NE095 (target date Jan 2022), is characterized for total cell number and colony forming units and engineered to contain a noncoding chromosomal DNA target. This target eliminates false positives from near neighbors and can serve as a surrogate for AMR genes in molecular detection methods. These existing RMs could be applied directly to WWS or our capabilities could be translated to develop RMs using other relevant microorganisms. One could envision a whole cell RM based on non-replicating bacteria or fungi to safely spike into any point of the WWS workflow at a known concentration. RMs such as this could serve as internal references to assess the overall process, including operators, technologies, and protocols, or as tools for training, method comparison, or risk management exercises. These current and potential future whole cell microbial RMs could support WWS capabilities and ultimately improve assurance in the results provided to public health decision makers.

Keywords: antimicrobial resistance, *Escherichia coli*, flow cytometry, genome copy number, microbial detection, reference material, *Saccharomyces cerevisiae*, viable

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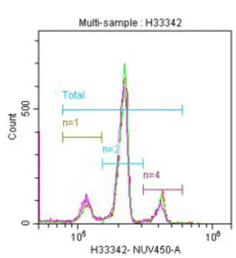

This *E. coli* RM under development will be characterized for total number of cells and genome copies/cell, two parameters that challenge our ability to confidently quantify microbial systems. Viability will also be reported. The characterization is done using flow cytometry (shown, where n is genome copy number) and optical microscopy. The final RM will be lyophilized pellets.

[1] Hendriksen, R.S., Munk, P., Njage, P. *et al.* Global monitoring of antimicrobial resistance based on metagenomics analyses of urban sewage. *Nat Commun* **10,** 1124 (2019). https://doi.org/10.1038/s41467-019-08853-3

NIST SRM 2917: A Plasmid DNA Standard for Molecular Recreational Water Quality Testing

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[2]Statistical Engineering Division, Information Technology Laboratory, National Institute of Standards and Technology, Gaithersburg, MD, USA;
[3]U.S. Environmental Protection Agency, Office of Research and Development, Cincinnati, OH, USA

Contamination of waterways with fecal material can lead to the spread of pathogens that can have serious impacts on human and environmental health. The U.S. Environmental Protection Agency (EPA) is responsible for protecting human and environmental health through the safeguard of recreational waters. Traditional monitoring practices involve time consuming cultivation procedures which can delay decision making. To accelerate this process molecular techniques, specifically quantitative polymerase chain reaction (qPCR) methods, for identifying host-associated genetic markers of fecal pollution and quantifying fecal indicator bacteria have been developed at EPA and by other researchers. National implementation of these methods requires rigorous proficiency testing by state and local public health agencies, as well as commercial entities that hope to adopt these methods. In a collaborative effort to support the adoption of these molecular methods, the EPA and NIST developed a plasmid DNA Standard Reference Material (NIST SRM 2917) for the purpose of implementing select qPCR assays for recreational water quality monitoring applications (figure 1). The material consists of 6 Levels of a linearized plasmid DNA containing 13 single-copy PCR targets selected by the EPA. The vector consists of a standard pUC plasmid with an ampicillin resistance gene, an origin of replication, and M13 universal priming sites flanking the target genetic marker construct. Dilution levels span approximately 5 to 500,000 plasmid copies per µL. Each tube of material contains approximately 200 µL of plasmid DNA in TE buffer (pH 8.0) with 10 ng/µL of RNA stabilizer (yeast tRNA) in a 1.5 mL low retention microcentrifuge vial. The material is stored at 4 °C and should not be frozen. Approximately 1,000 units were generated. The material was characterized by NIST using droplet digital PCR to establish homogeneity and stability of the material as well as assign an absolute copy number to each dilution level.

Key words: Reference material, qPCR, ddPCR, molecular detection methods, fecal indicator bacteria

Disclaimers: Information has been subjected to U.S. EPA peer and administrative review and has been approved for external publication. Any opinions expressed in this paper are those of the authors and do not necessarily reflect the official positions and policies of the U.S. EPA. Any mention of trade names or commercial products does not constitute endorsement or recommendation for use. Certain commercial equipment, instruments, or materials are identified in this paper to specify adequately the experimental procedures. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology; nor does it imply that the materials or equipment identified are necessarily the best for the purpose.

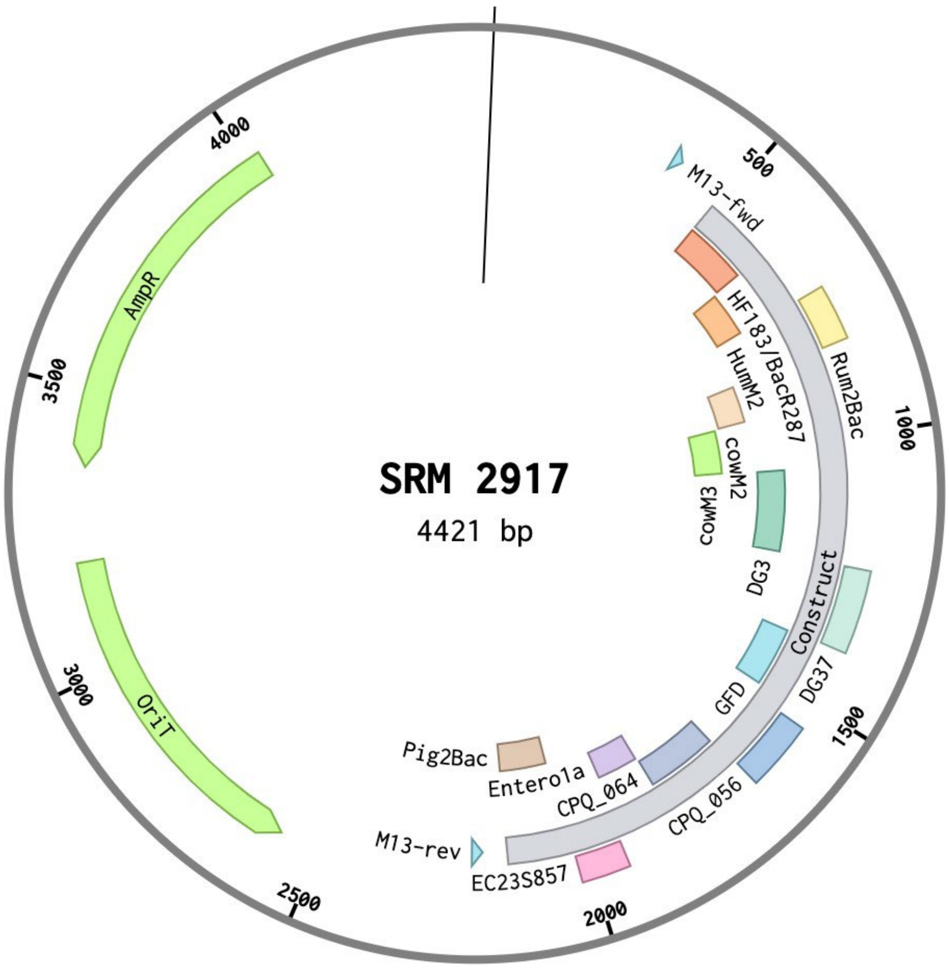

Figure 1. The 1.7 kb construct containing the 13 targets was synthesized *de novo* using the IDT gBlock technology. The 13 targets include genetic markers for human (HF183/BacR287, HumM2, CPQ_056, CPQ_064), ruminant (Rum2Bac), pig (Pig2Bac), cattle (CowM2, CowM3), dog (DG3, DG37), avian (GFD), Enterococcus (Entero1a), and *E. coli* (EC23S857) pollution sources for fecal indicator bacteria. The gBlock was then inserted into a pUCIDT vector (2752 bp) carrying an AMP selection marker (AMP^R) and an origin or replication (ORI) to produce the 4421 bp plasmid (shown circular, but final SRM is linearized).

Development *of RNA Standards for Quantification of SARS-CoV-2 and PMMoV by RT-qPCR for Wastewater Surveillance*

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Over the course of the COVID-19 pandemic, wastewater surveillance has emerged as a promising tool to monitor spread of the virus in a community. Wastewater is a complex matrix, so analytical methods like RT-qPCR needs to be designed with appropriate controls to quantitate and normalize viral levels for trend analysis. In this work we describe methods for designing and manufacture of quantification standards for SARS-CoV-2 and Pepper Mild Mottle Virus (PMMoV) RNA. PMMoV is a plant virus with a ssRNA genome and is a well-documented human fecal indicator. PMMoV RNA also serves as a useful process control and normalization tool for wastewater surveillance.

To generate quantitative standards, we first cloned the Envelope and Nucleocapsid (E/N) genes of SARS-CoV2 in pGEM3z vector. Likewise, a 362bp fragment of the PMMoV genome consisting the RT-qPCR target is also cloned in a pGEM3z vector. The two plasmids are then linearized by XbaI and in-vitro transcribed to generate RNA transcripts. Any remaining DNA in the reaction is eliminated by DNase treatment. RNA was then quantified with a fluorescent RNA dye (QuantiFluor RNA System) and by droplet digital PCR (ddPCR). The relationship of quantity of RNA (in pg) corresponding to copy number as determined by ddPCR is established. The RNA was then diluted at 4 million copies/ul corresponding to 20pg/ul and 7.5pg/ul for the SARS-CoV-2 (E/N) and PMMoV RNA respectively. Both the RNA was then tested with the primes/probe set prescribed by the US Centers for Disease Control (CDC) that target the nucleocapsid (N1 and N2) gene, or the envelope (E) gene of the SARS-CoV2 RNA and primers/probes for PMMoV for the PMMoV RNA. The quantitation standards were stored at -20°C and subjected to ten freeze-thaw cycles. No decrease in Ct values was observed implying that the quantification standards were stable. The standards were used to quantify SARS-CoV-2 and PMMoV RNA from three wastewater treatment plants in Dane county, WI. Normalization of SARS-CoV-2 RNA with PMMoV RNA resulted in better correlation with 7-moving average of new clinical cases a municipality served by a treatment plant.

Key words: RT-qPCR, quantitation standards, in-vitro transcribed RNA

Standards and control materials used: *In-vitro transcribed RNA*

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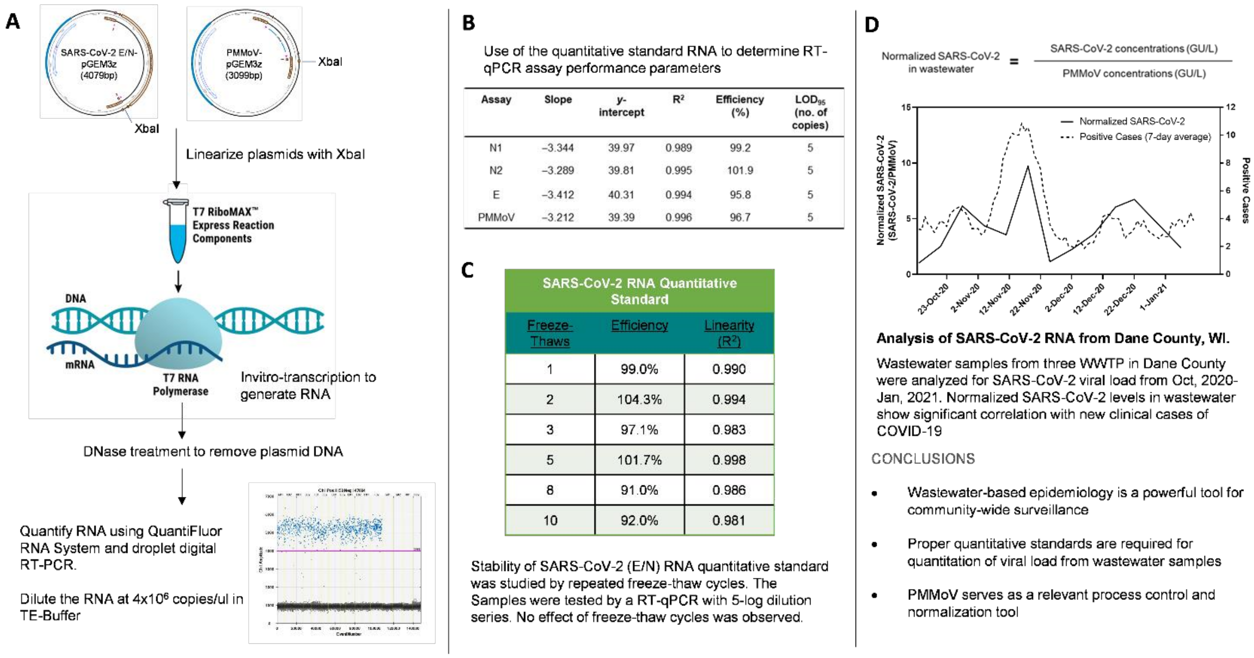

Assay	Slope	y-intercept	R^2	Efficiency (%)	LOD_{95} (no. of copies)
N1	−3.344	39.97	0.989	99.2	5
N2	−3.289	39.81	0.995	101.9	5
E	−3.412	40.31	0.994	95.8	5
PMMoV	−3.212	39.39	0.996	96.7	5

Freeze-Thaws	Efficiency	Linearity (R^2)
1	99.0%	0.990
2	104.3%	0.994
3	97.1%	0.983
5	101.7%	0.998
8	91.0%	0.986
10	92.0%	0.981

Figure 1. Development of RNA standards for quantification of SARS-CoV-2 and PMMoV by RT-qPCR for Wastewater Surveillance

Assessing Community Acceptance: Wastewater Monitoring Community Survey (WMCS)

Ted Smith[1], Kandi L. Walker[2], Joy L. Hart[2], Rochelle H. Holm[1], Rachel Keith[1], Lauren Anderson[1], Lauren Heberle[3], Dan Riggs[1], and Aruni Bhatnagar[1]

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Community wastewater monitoring can be a powerful early warning tool to protect public health. However, given the wide range of possible implementations, and the situational interpretation of the medical ethics issues present when conducted in a non-regulatory fashion, standards development should include a public perspective. The University of Louisville has developed a straightforward instrument to quickly assess both awareness and acceptance of wastewater monitoring, specifically focused on COVID-19 wastewater surveillance. We propose that standards development and implementation adopt a common feedback approach nationally which could inform regional sensitivities to public health surveillance acceptance as well as strategies for standard approaches to implementation. This seven-item questionnaire includes three items that assess awareness of this typeof public health surveillance and three items that focus on key aspects known to affect acceptance of specific implementations. For example, the instrument measures the importance of data sharing with the public and the size of catchment areas that a community member is comfortable having sampled. The instrument is being evaluated as a component of a larger public health community trial which will allow comparison with other data sources and present opportunities to increase its predictive validity.

Key words: Wastewater Surveillance; Ethics; Community

Feedback.Standards and control materials used: *NONE*

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University of Louisville Wastewater Monitoring Community Survey (WMCS)

1) Can the coronavirus that causes COVID-19 be detected in the city sewer system?
   Yes No I Don't know

2) Did you know that the amounts of the COVID virus in sewers reflect the general level of infection in the community ? Yes No

3) Did you know that UofL is working with Louisville Metropolitan Sewer District (MSD) to test whether measurements of coronavirus in wastewater could be used to determine the risk of COVID-19 across Louisville?
   Yes No

4) On a scale of 1 to 7, how much do you support monitoring sewage to better understand COVID infection levels in our community instead of only testing people?

1	2	3	4	5	6	7
Very Supportive	Moderately Supportive	Supportive	Indifferent	Opposed	Moderately Opposed	Very Opposed

5) On a scale of 1 to 7, how important is it to share the results of wastewater testing with the public?

1	2	3	4	5	6	7
Very Important	Moderately Important	Important	Neutral	Unimportant	Moderately Unimportant	Very Unimportant

6) Measuring at different sewer locations can help identify patterns of infection for different sized areas. Measuring coronavirus in samples from manholes or other equipment can help scientist understand what is happening in different areas of the city or even different neighborhoods. Please tell us which statement best describes the smallest number of households you support being measured:

1	2	3	4	5
Support Measuring Largest Areas (>50,000 households)	Support Measuring Smaller Sections (>30,000 households)	Support Measuring Neighborhoods (>5,000 households)	Neither Support or Oppose	Oppose Measuring Any Size

7) Optional: Please share any other information you'd like about your views on monitoring sewers for signs of COVID.

Figure 1. Wastewater Monitoring Community Survey (WMCS)

Comparison of Sampling Frequency and Concentration Methodology for the Detectionand Quantification of SARS-CoV-2 in Wastewater

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Wastewater-based epidemiology (WBE) has been implemented across the world to track the COVID-19 disease burden of a community through the quantification of SARS-CoV-2 (the virus responsible for COVID-19) in wastewater samples. Due to the rapid implementation of WBE programs in combination with supply chain issues and varying budget constraints, many different protocols for sampling and analysis have been developed. However, the effects of these various techniques on the interpretation of the reported detection and quantification of SARS-CoV-2 is limited. To that end, studies were conducted to evaluate a variety of sampling and viral concentration techniques. Two of the most common sampling techniques utilized for WBE, grab and composite sampling, were evaluated by comparing the SARS-CoV-2 concentrations in 24 hourly grab samples with their associated 24-h composite sample at four sewershed scales (city, neighborhood, city block and individual building). Additionally, the effects of sampling frequency on reported SARS-CoV-2 composite concentrations were evaluated at a low-flow building site with sampling frequency varying from 5 min to 1 h.
The results indicated that composite sampling should be utilized at every sewershed scale. However, at the city scale (*e.g.* wastewater treatment plant influent), grab samples may be acceptable if only attempting to determine the presence or absence of SARS-CoV-2. At the scale of city block and individual buildings, high- frequency composite sampling (*e.g.* 15 min sampling) is required to capture the temporal variation in viral signal. Another critical step to optimize is the concentration of SARS-CoV-2 from wastewater. Six common WBEconcentration methods were compared by quantifying the SARS-CoV-2 concentration from the same wastewater treatment plant influent sample. The best relative recovery was found in the methods that utilized electronegative membrane filtration. To supplement this knowledge, the different solid and liquid fractions of an influent were separated into the individual components (*i.e.,* liquid, settleable solids and non-settleable solids) to evaluate where SARS-CoV-2 RNA was partitioned in the wastewater. Of the SARS-CoV-2 captured by the electronegative membrane, roughly 90% was found in the non-settleable solid fraction. Thus, future optimization of concentration methods should focus on retaining the SARS-CoV-2 RNA from the non-settleable solids fraction of wastewater.

Key words: SARS-CoV-2; Wastewater-based Epidemiology; Composite Sample; Grab Sample; Concentration; Frequency; Filtration; Non-settleable Solids

Standards and control materials used: Positive SARS-CoV-2 controls containing the E, N, ORF1ab, RdRP, and S genes and human RNAse P RNA (EDX SARS-CoV-2 Standard, Exact Diagnostics, Fort Worth, TX) and negative controls containing certifiedSARS-CoV-2-free human RNAse P RNA (EDX SARS-CoV-2 Negative, Exact Diagnostics, Fort Worth, TX) were included in each extraction plate. Extraction blanks of phosphate buffered saline (PBS) were included with every run as an extraction contamination control. Bovine coronavirus (Bovine Rotavirus-Coronavirus Vaccine from Zoetis, NJ, USA), was selected as a process recovery control due to its morphological and structural similarity to SARS-CoV-2.

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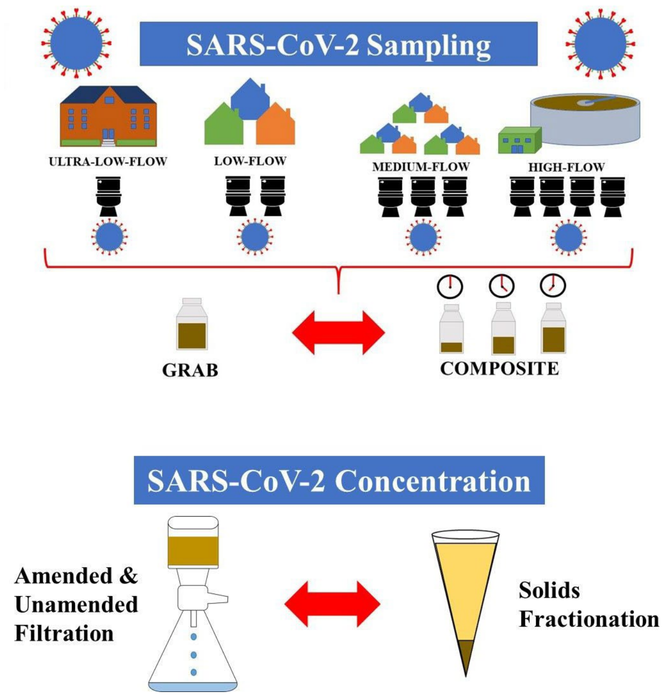


Figure 1. Overview of SARS-CoV-2 sampling frequency and SARS-CoV-2 concentration analyses

PiGx SARS-CoV-2 wastewater: A pipeline for reproducible wastewater sequencing analysis providing comprehensible geo-tagged time series reports

Authors: Altuna Akalin[1], Vic-Fabienne Schumann[1], Ricardo Wurmus[1], Miriam Faxel[1], Jan Dohmen[1], Rafael Cuadrat[1]

[1] Max-Delbrück Centrum, Berlin Institute for M edical Systems Biology

Setting up monitoring systems for the development of SARS-CoV-2 through wastewater sampling has become a crucial mission for many nations all over the world [1],[2] . Developing protocols for comparable sampling and sequencing on the one side but also developing robust and scalable analysis tools on the other side remains an ongoing challenge so far.

We present the **PiGx SARS-CoV-2 wastewater** sequencing pipeline [3] , a Bit by Bit reproducible pipeline using GuixHpC [4] which provides reports that are intuitive to use and easy to interpret. They combine visualisation, access to quality control reports and downloadable data tables for sharing and further processing. They grant geo-tagged visual time series overviews over the dynamics of single mutations and abundances of given variants of concern (VOC) which were derived by deconvolution. Under development right now are features in order to identify untracked mutations that show significant increase over time and also to track the relative abundance of SARS-CoV-2 in wastewater samples.

We aim to collaborate with many groups working with different strategies in order to make this pipeline as easily accessible as possible and applicable for data from various sequencing protocols. With **PiGx SARS-CoV-2 wastewater** we aimto develop a tool to support combined and global research efforts and make wastewater sequencing data comparable, reportable and reproducible.

[1] www.cdc.gov, “Developing a wastewater surveillance sampling strategy” (accessed: 21/06/06)

[2] www.dw.com, “Eu states must monitor sewage systems for covid” (accessed: 21/06/06)

[3] https://github.com/BIMSBbioinfo/pigx_sarscov2_ww (accessed: 21/06/06)

[4] https://hpc.guix.info/, (accessed: 21/06/06)

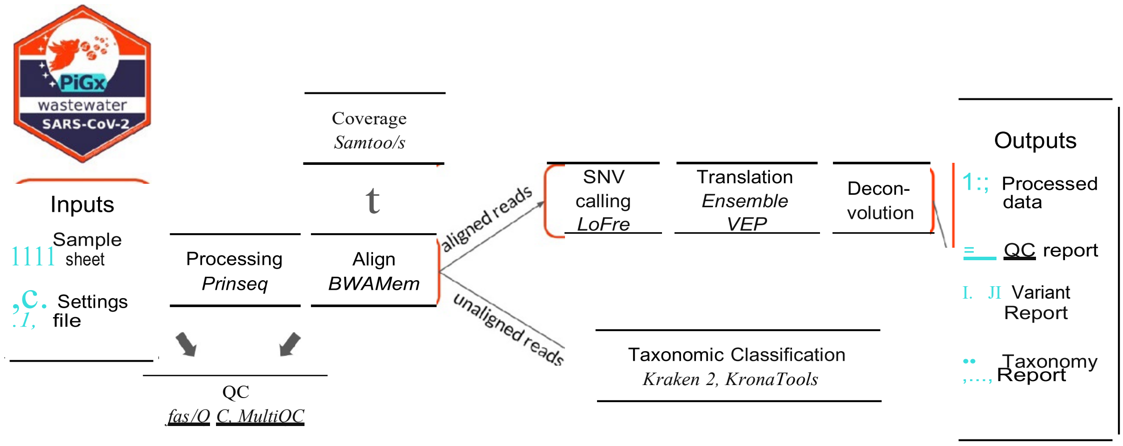
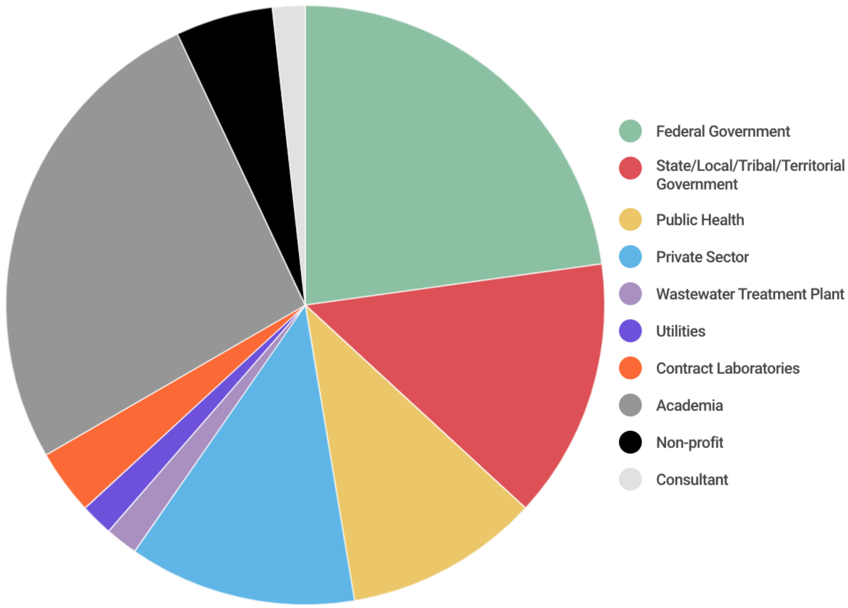


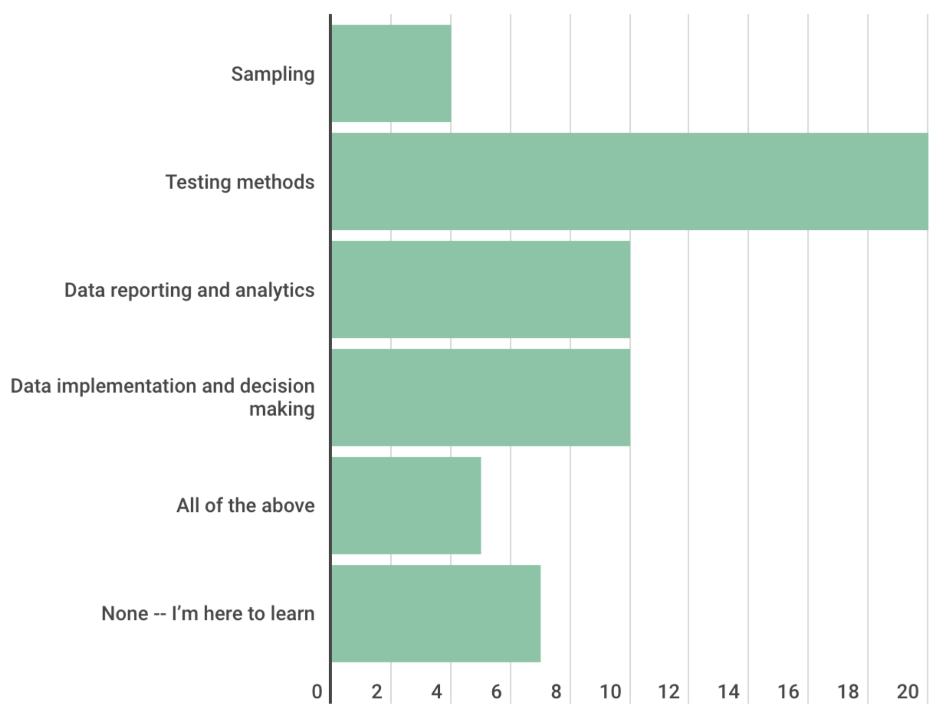
Figure 1: Workflow diagram of the PiGx SARS-CoV-2 wastewater sequencing pipeline

Appendix E: Workshop Poll Results

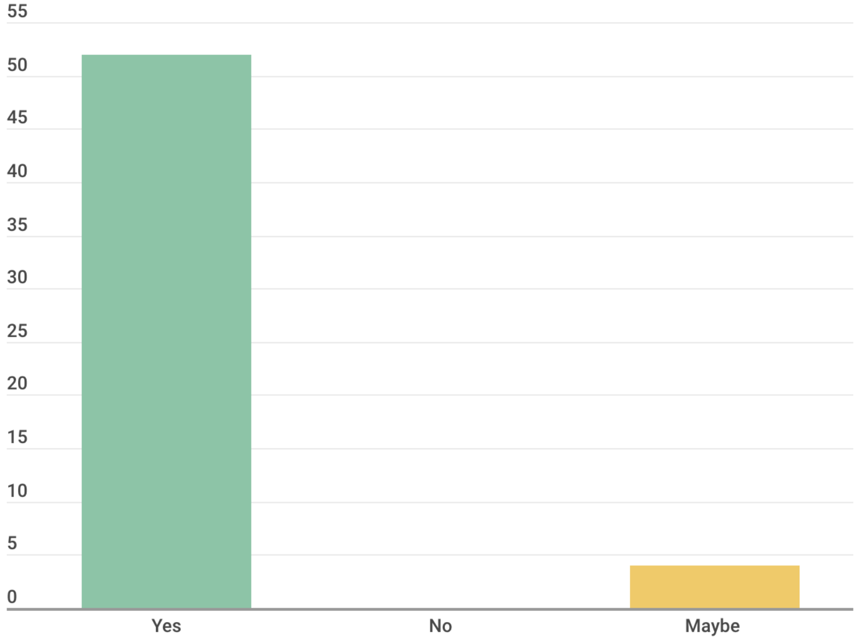
Q1- What type of organization do you represent? (select one) (57 respondents)



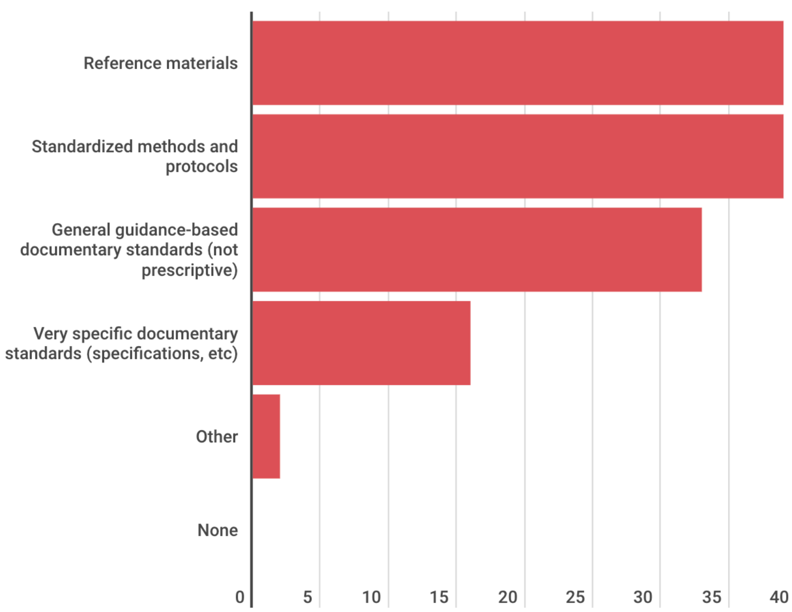
Q2- Which step(s) of the wastewater surveillance process do you work in and/or contribute to? (56 respondents)



Q3- Do you see a need for the development of standards (documents, guidance, reference materials, etc.) to support an enduring capability in wastewater surveillance? (56 respondents)

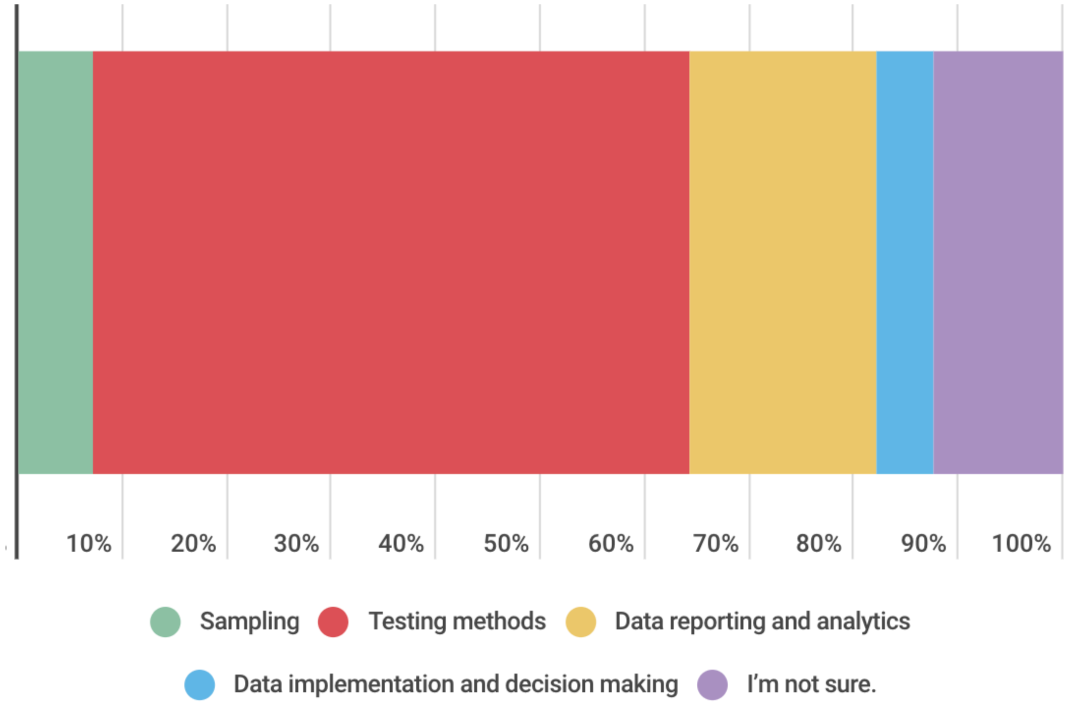


Q4- What type of standards would be useful, in your opinion? (select all that apply) (54 respondents)

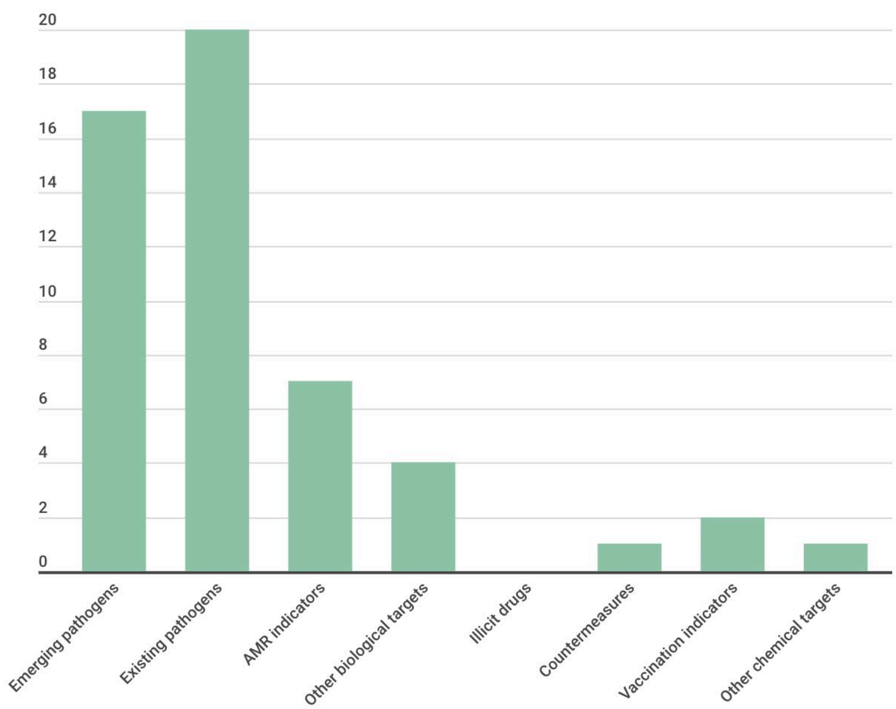


Other: Reproducible methods so results are comparable from one lab to another; Not sure, but there should be a methodical process to determine this; Precise biological culture standards and DNA and RNA standards

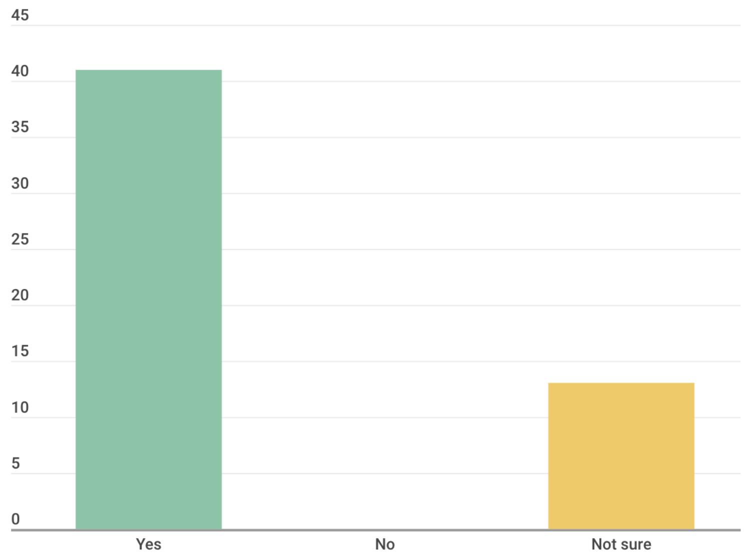
Q5- Which step in the wastewater surveillance workflow is most in need of standards? (56 respondents)



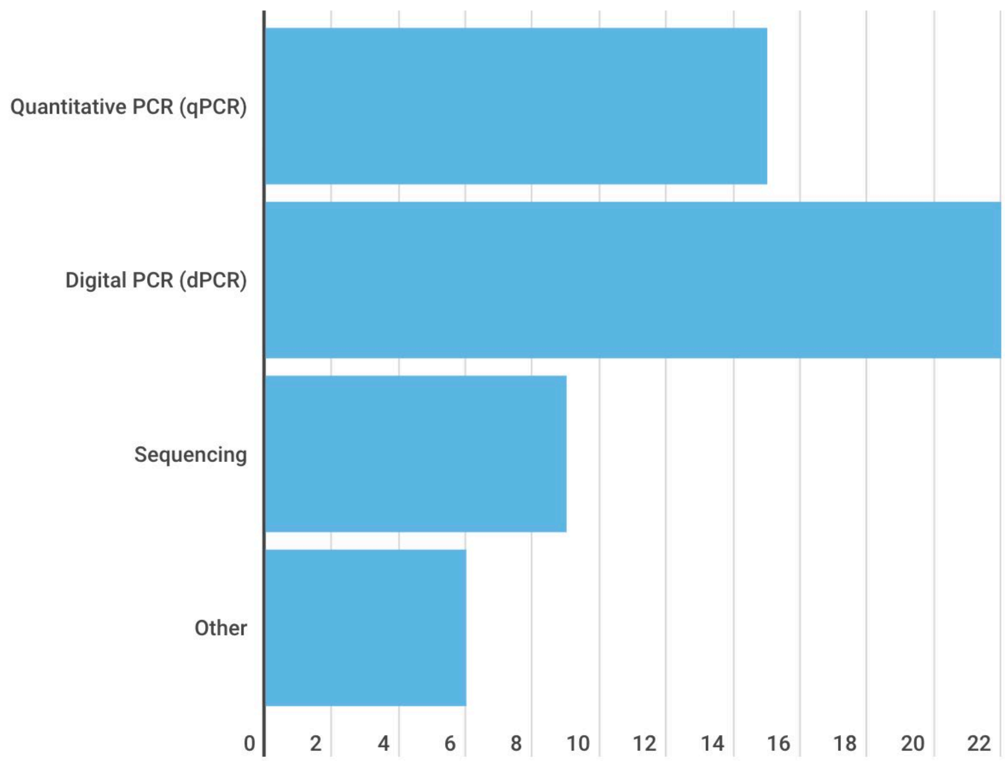
Q6- What screening targets should be considered to enable standards to be flexible and affordable to support multiple applications? (select top two) (52 respondents)



Q7- Should sampling design be co-produced with end-users of the data? (54 respondents)

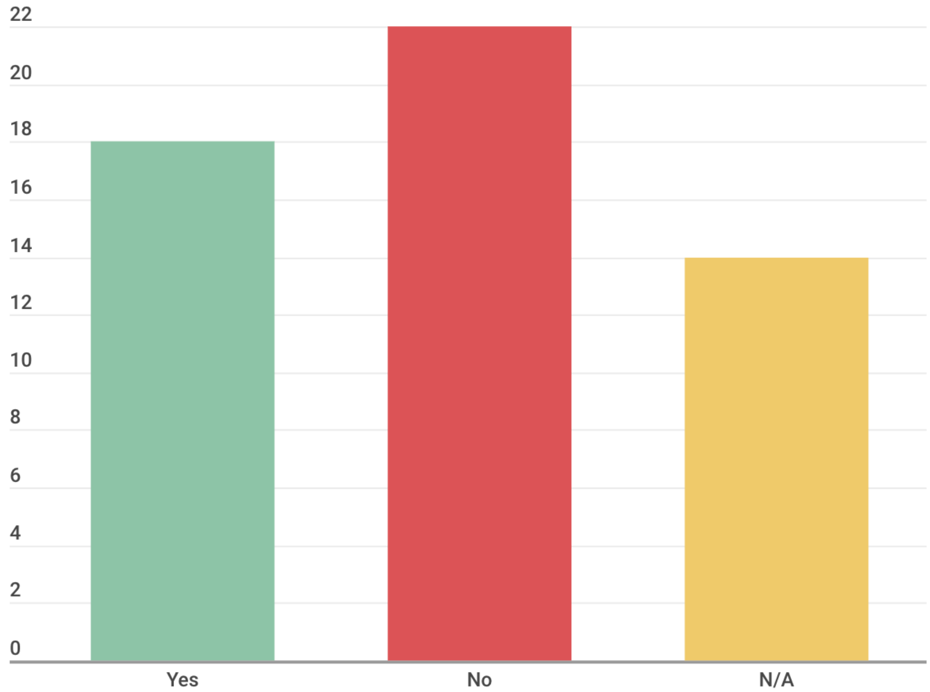


Q8- In your opinion, what is the most promising detection technology for biological targets? (52 respondents)

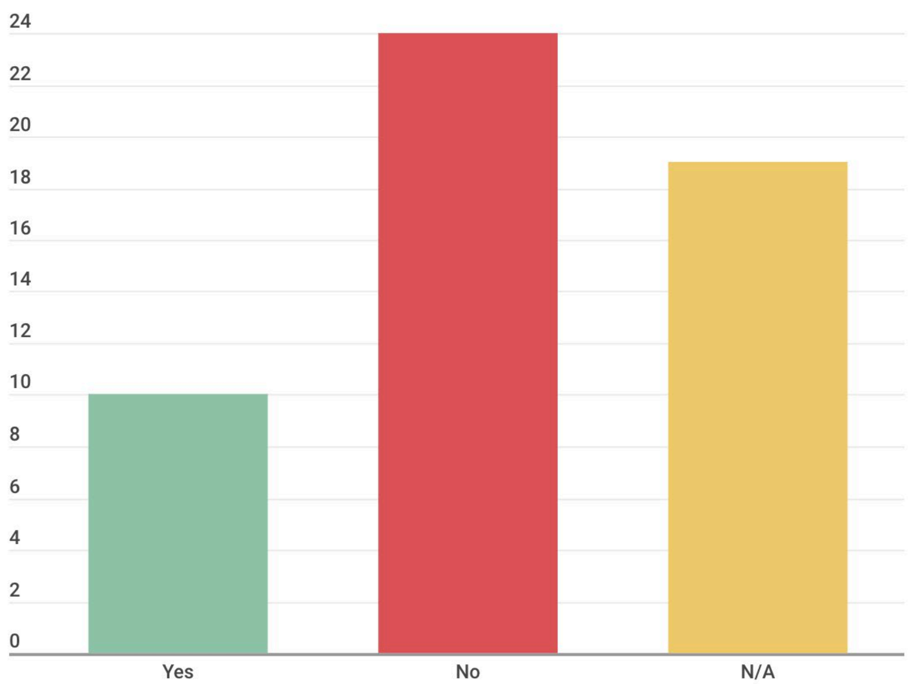


Other: All of the above. At this time, there are hopes that ddPCR or dPCR will be better than qPCR. But it remains to be studied in more details. As to sequencing, it is very important;
ddLAMP - more rapid and less expensive than ddPCR; Digital PCR and sequencing are the most promising at this time; Microarrays including multi-modal testing; digital PCR for existing targets, sequencing for emerging, unknown, or variants; not qualified to offer an opinion

Q9- Are the controls and reference materials you currently use sufficient? (54 respondents)

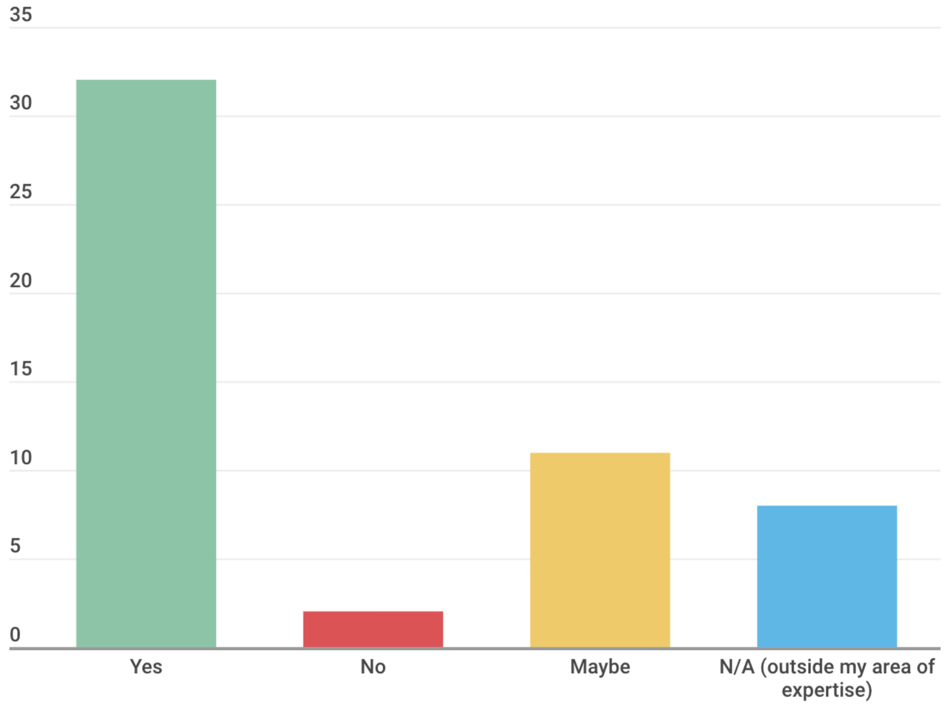


Q10- Do you have high quality control materials or reference materials readily and consistently available to you? (53 respondents)

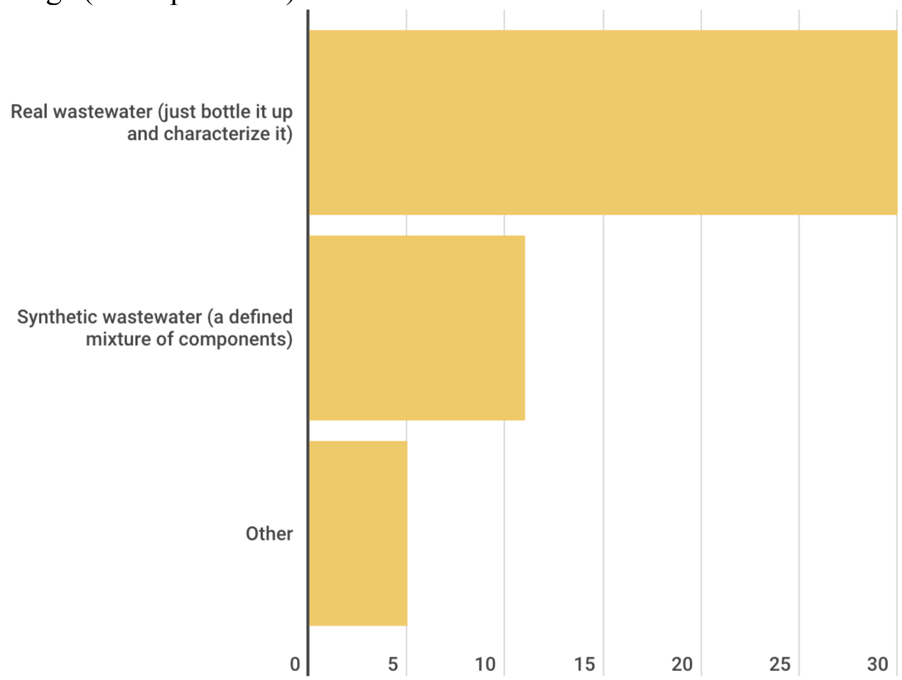


Q11- If you answered yes to question 10, please describe the materials here. (4 respondents): controls and reference materials are provided by University labs; prepare and QA/QC, OC43 stocks for recovery controls in-house; RNA Preps from BEI for SARS-CoV2 std curves; IDEXX Water test kits; In-house BSL3 facility on campus produces them; doesn't scale though.

Q12- Would a wastewater matrix reference material be a valuable control? (53 respondents)

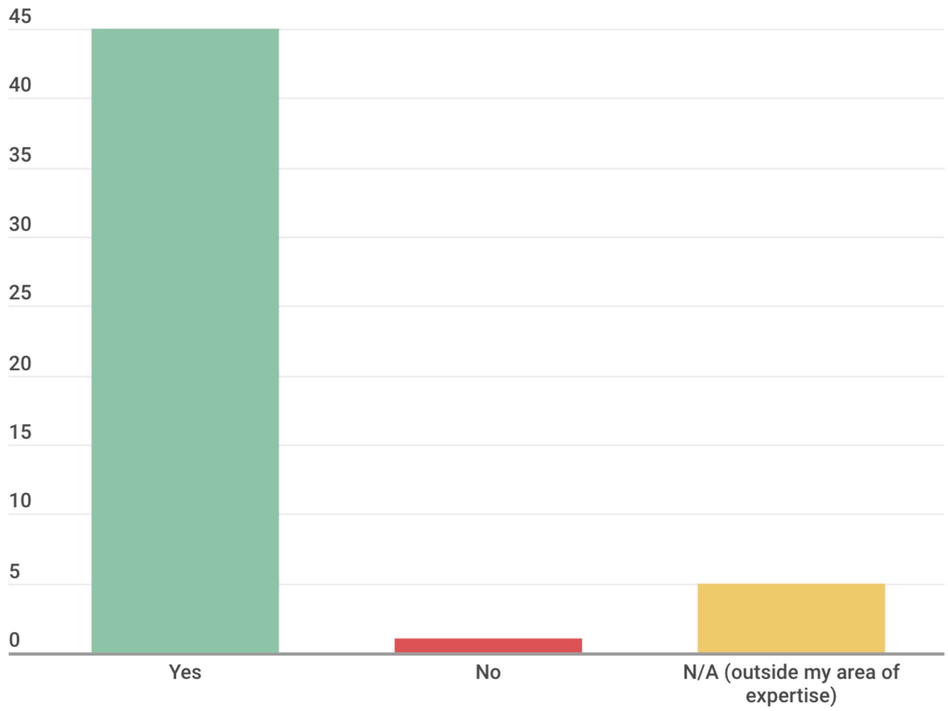


Q13- What matrix-type reference materials would best serve to provide QA/QC for wastewater testing? (46 respondents)

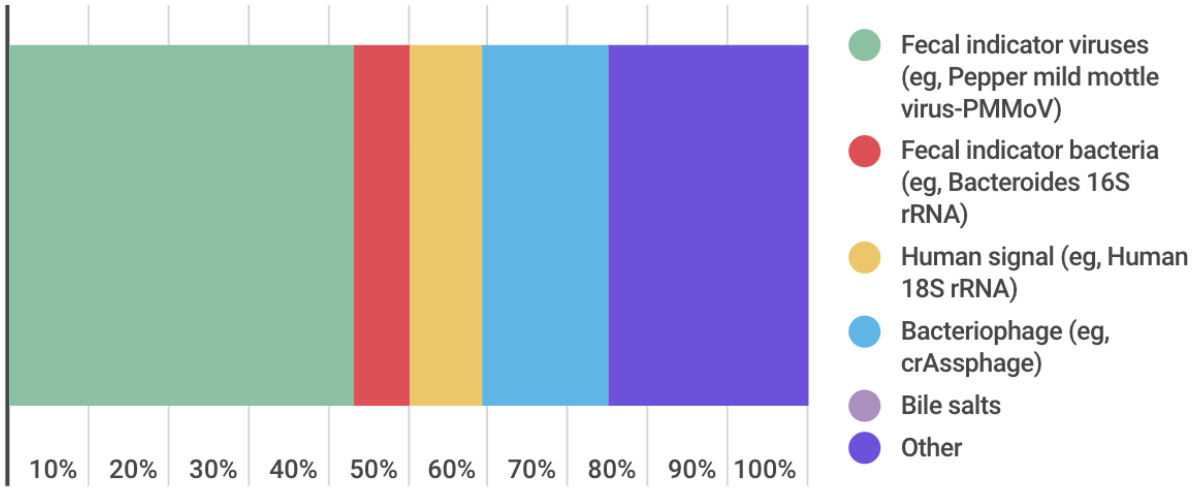


Other: Wastewater is so variable, I think you may need a range of different wastewater types, including industrial solvents, detergents, metals, etc; Wastewater is variable across the world so not sure?; multiple real wastewaters that span a range of characteristics (e.g. low and high total suspended solids); Real wastewater and sluge with spiked in controls; I don't know!

Q14- Would reference materials based on biological or chemical targets of interest be useful? (51 respondents)

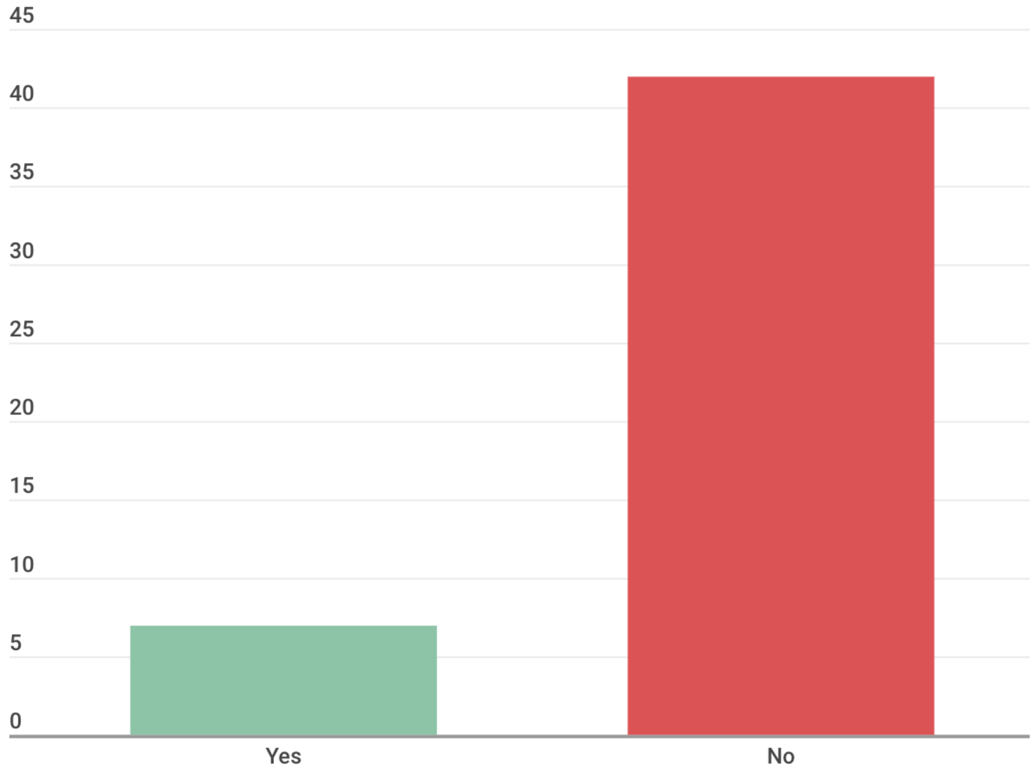


Q15- In your opinion, what is the best marker for normalizing with respect to the human fecal load in a wastewater sample? (44 respondents)



Other: It depends on what target you're trying to detect- virus, bacteria, etc; Nitrogen, BOD, TSS, creatine, 5-HIAA as examples; Hard to know if any of these work in a reproducible way in various locations; I think this can be fairly scenario specific. I like a combo of FIV and FIB. The key is to understanding their variability in your system. FIB (in particular E.coli) has the largest amount of reference data for between site comparison of wastewater strength.; I don't think we have enough information yet to determine which approach works best; Not enough quality data to determine at this time; I don't think we have sufficient information to decide between these and other markers, especially internationally -- more studies are needed to decide on the best marker; Would be great to have spike ins from different analyte classes to enable multi-omics analysis and check sample prep that enables this; I don't know!; outside my area of expertise

Q16- Are you aware of any documentary standards that support wastewater surveillance? (49 respondents)



Q17- If you answered "yes" to Question 16, please list the documentary standards here. (5 respondents):

1. CDC and APHL have sampling and analytical guidance
2. Not is US, but I believe EU now has documentary standards, Australia may as well
3. NWSS data reporting
4. Califórnia waste water standards for reuse
5. US EPA 1600

Q18- What hinders comparable data in wastewater surveillance? (30 respondents, open text)

1. local politics; lack of standardized controls
2. Method, Data security needs
3. different protocols for sample prep and totally absent provenance of samples as they move from collection to the lab (e.g. temperature changes in shipping)
4. Differing testing methods/protocols
5. No consensus method; No reference material (e.g., PT sample or round robin sample, of the type provided by WRF in August 2020)
6. Variability of treatment systems, collection system networks, uncertainty in sewershed area/population being measured.
7. Differences in sampling, processing, measuring the targets, differences in the amount of human fecal concentration, and differences in the wastewater matrix are all hurdles that need to be overcome to for comparable data.
8. Unknown variability in sewer systems.
9. Signal strengths are going to be different between laboratories because they are all using different methods.
10. everyone doing their own thing (different gene targets, RT-dPCR vs RT-qPCR, different concentration methods, different normalizations or no normalization, etc.)
11. lack of common normalization schemes and data analysis guidelines.
12. Lack of Sampling standards
13. Within a single facility, much comes down to consistency and documentation of methods and appropriate data quality checks/reviews, though there will always be changes in the underlying populations and what else is in the wastewater that can be difficult to get data on (i.e. travel, run off infiltration, etc.). Comparability between facilities is a much harder proposition, as not only will there be the same sorts of changes over time as above, but there are also very likely to be broader structural differences in the respective populations and wastewater streams that we currently have relatively little data on.
14. Low apparent recovery and large variability in recover of SARS-CoV-2 from wastewater
15. Sample inactivation, storage, analysis, and interpretation differences
16. High matrix variability.
17. Too many unvalidated or under-validated methods, no standard in data analysis and reporting. There is a real problem with calculations that involve adjustments based on various factors that are not well validated themselves.

18. Lab differences and/or lab methods differences. Given the lack of controlled inter-lab comparisons, it's impossible to say currently what the primary lab method components that contribute to the lack of comparability are
19. Standard sampling plan and methods to make WBE data comparable among different sampling areas.
20. differences in testing methods and recovery, as well as unknown impact of differences in wastewater matrices.
21. measuring recovery efficiency, different forms of SARS-CoV-2 (and other pathogens) in wastewater, See Kantor and Nelson et al 202 (https://pubs.acs.org/doi/abs/10.1021/acs.est.0c08210)
22. Differing methodologies
23. Different methods at every step, lack of data release policies.
24. Need educational opportunities for cross training so that Genomics specialists understand chemical analysis and vice-versa.
25. Differences in testing and using data.
26. variety of different sampling and testing methods, no standardization
27. Differing methods for detection as well as normalization
28. Multiple analytical methods and lack of standards
29. Too much variation is sample processing and reporting
30. lack of standardized test methods

Q19- What is the greatest challenge to be faced when developing a sustained wastewater surveillance capability? (29 respondents, open text)
Recurring Themes: (1) Need for funding and resources (labor and consumables); (2) Need for buy-in from public, officials, and utilities; (3) Need for reliable, comparable data; (4) Lack of understanding in data analysis and translation

1. Midwestern, small-town-minded **politics**
2. The **cost** and **availability of consumables** and capital purchases may be inhibitive
3. **Funding**
4. Keeping **value of effort clear** to the contributing WWTPs
5. The fact that public health labs do not have the nuanced academic experience to conduct these methods routinely, **analyze the results accurately, and translate** / implement them to public policy
6. In addition to **funding**, developing a standard set of methods which are sensitive and can enable comparison across facilities and populations is important.
7. **Buy in from leadership** (governmental, departmental, etc.), **resources** for staff time.
8. **Cost** - need a source of funding. The variety of different workflows will make it challenging to **analyze data** across locations and between laboratories.
9. **$$$**
10. uncertainties of sewer systems, and the behavior of RNA inside sewer lines.
11. **Capital costs** for BSL 2 instrumentation and space
12. If I had to pick just one, I'd say the current lack of well-supported best practices for laboratory methods (of all sorts, from the sample concentration and RNA extraction

methods to how the RNA is quantified). **How best to interpret the data** is big challenge currently
13. Continued cooperation of wastewater treatment facilities and funding
14. **Cost and data interpretation**
15. What question/**reason for surveillance**?
16. **Money**
17. sustained testing **capacity**
18. Fast testing, **reliable results**, information widely available to the public for decision making.
19. Picking a **standard method** that captures both the capacity need as well as the changing detection limit needs based on the phase of epidemic.
20. **funding**, getting the samples (and understanding the sewer network/area represented by the sample)
21. Local **funding** for wastewater surveillance.
22. That **funding** body enthusiasm will wane after the COVID-19 pandemic, just as enthusiasm for public health measures has waned after other pandemics.
23. **Cost** per sample
24. Differences in **funding** and facilities between wastewater groups.
25. **standardization**
26. Ability to incorporate new targets, **money** for sampling, detection limits of targets
27. lack of **standards** and standardized methods for sampling and analytical
28. The concentration process is incredibly **labor and time intensive**. This is the weakest link in our workflow. Increased technology here would significantly improve the process.
29. getting data that is **comparable and reliable**

Q20- If you could wish for one standard to be readily available for improving the measurement of SARS-CoV-2, what would that be? (26 respondents, open text)

Materials:
1. reporting the quantity of RNA copies per mL
2. Standards for Variants of Concern and Variants of Interest.
3. Matrix spikes and a
4. synthetic wastewater, sludge, etc
5. PMMoV
6. A modified SARS-CoV-2 virus for use as a spiked recovery control
7. Recovery controls
8. Quantitative standard for SARS-CoV-2 RNA
9. Matrix recovery control material.
10. independently quantified recovery control proxy virus - quantified with 100% extraction efficiency (or absolutely known extraction efficiency)
11. Reference wastewater with known levels of human and SARS-CoV-2 nucleic acid
12. Multiple analyte classes spiked into characterized real sample matrix. Include multiple samples to check a range of samples.
13. Precise molecular standards
14. Quantitated sample exchange between labs for validation.

Methods:

1. Sample prep for both quantification and for sequencing
2. Consensus method
3. Analytical procedure for liquid and solids analysis
4. Sampling, RNA Concentration, Extraction and quantification
5. I think development of a 'best practice' method for concentrating/extracting/quantifying the target of choice (i.e., viral RNA for SARS-CoV-2) would be extremely helpful.
6. Testing protocol.
7. standardized test method

Documents:

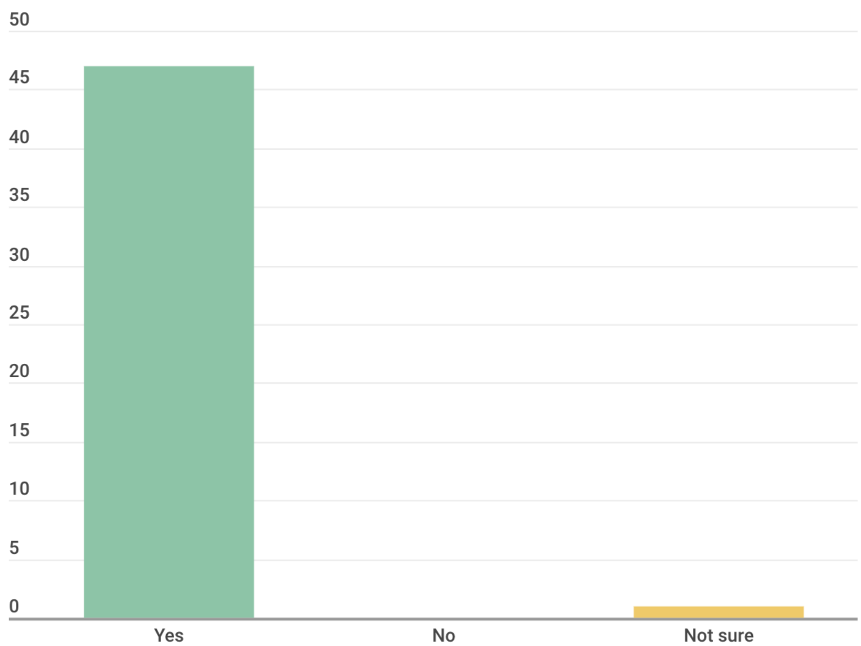
8. Guidance for analysis and reporting would be extremely beneficial
9. standardized way of reporting the data generated.

Other:

10. Testing sites that are close and readily available to all people doing work.
11. testing
12. different variant tracking

Poll questions asked during the workshop

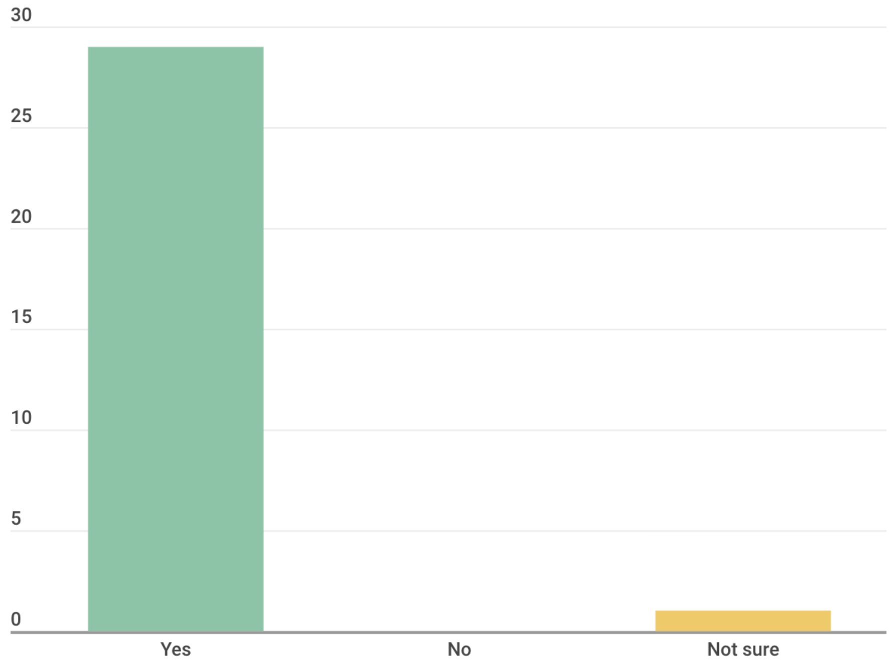
Monday Q1: Are standards needed for wastewater surveillance sampling (anything that happens before the sample is handed over to the lab)? (48 respondents)



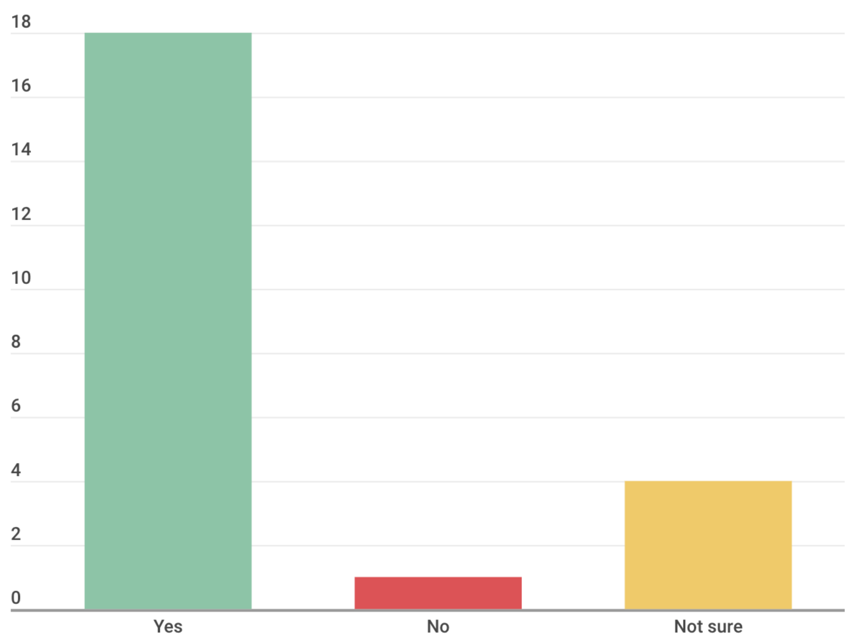
Monday Q2: Do you have any suggestions for specific standards needs related to sampling? (9 respondents, open text)

- Researchers need to understand well the nature of the sampling location, esp what types of waters are going into the location. E.g. Is it at the main outlet of a building and thus capturing water from many sources inside a building, or is it closer to the sewage treatment plant, where it would be capturing water from even more sources (including stormwater in combined systems)? Even though I don't think we know enough about what the quantities detected mean to worry too much about dilution right now, it is still important to characterize the sources especially if anyone is starting to make public health practice decisions based on these data.
- What about lab certification and/or accreditation?
- QA and QC recommendations for the entire process
- Trip control and field blanks at a minimum.
- Field replicates (splits off of the same composite) will help assess the variability of the lab process.
- Metadata on how the sample was collected
- No doubt there is need to standardize sampling if data were meant to inform trends and comparability. I think one or more of the panelists mistook this for a prescription for sampling methods.
- Metadata required for samples (and how that data is to be used), controls at the various points in the testing system (matrix, processing, extraction, detection, etc.) and how used
- Controls! Make sure positive and negative controls are explicitly standardized for sampling locations. Should a spike be added into a sample in every sampling event?

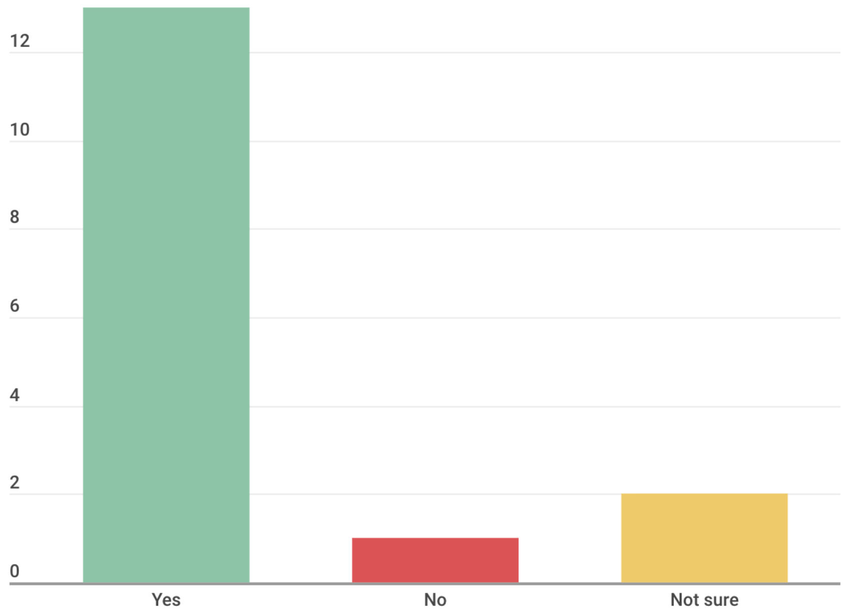
Tuesday Q1: Are standards needed to support laboratory testing for wastewater surveillance? This covers the steps from when the sample is received to when the data is collected. (30 respondents)



Tuesday Q2: Are standards needed to support data reporting and analysis for wastewater surveillance? This covers the steps from when the data is received to when it is passed to the decision makers. (23 respondents)



Tuesday Q3: Are standards needed to support decision-making based on wastewater surveillance results? This covers the steps from when the results are received to when decision makers decide how to respond. (16 respondents)

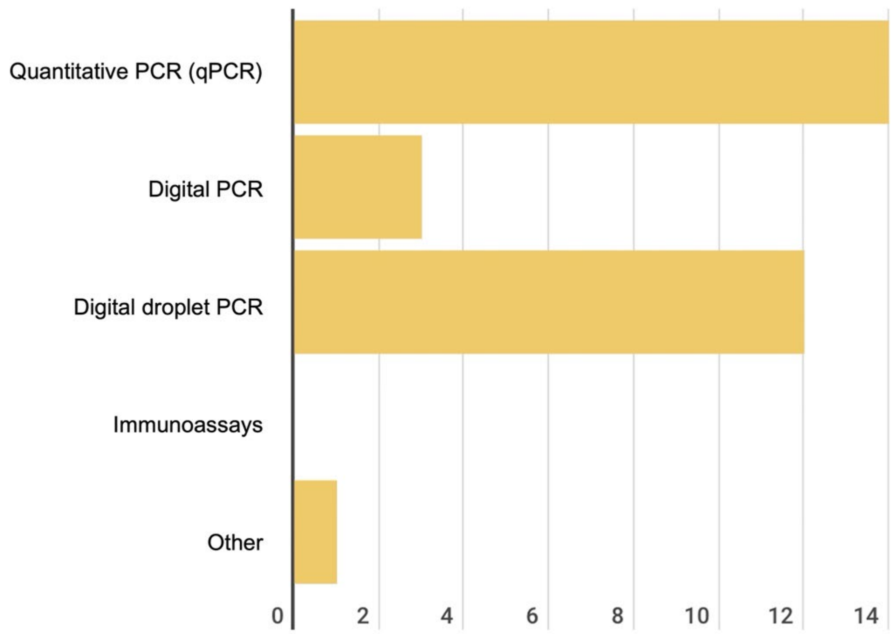


Appendix F: Methods – Focused Feedback Poll Results

1. What type of organization do you represent? (32 responses)

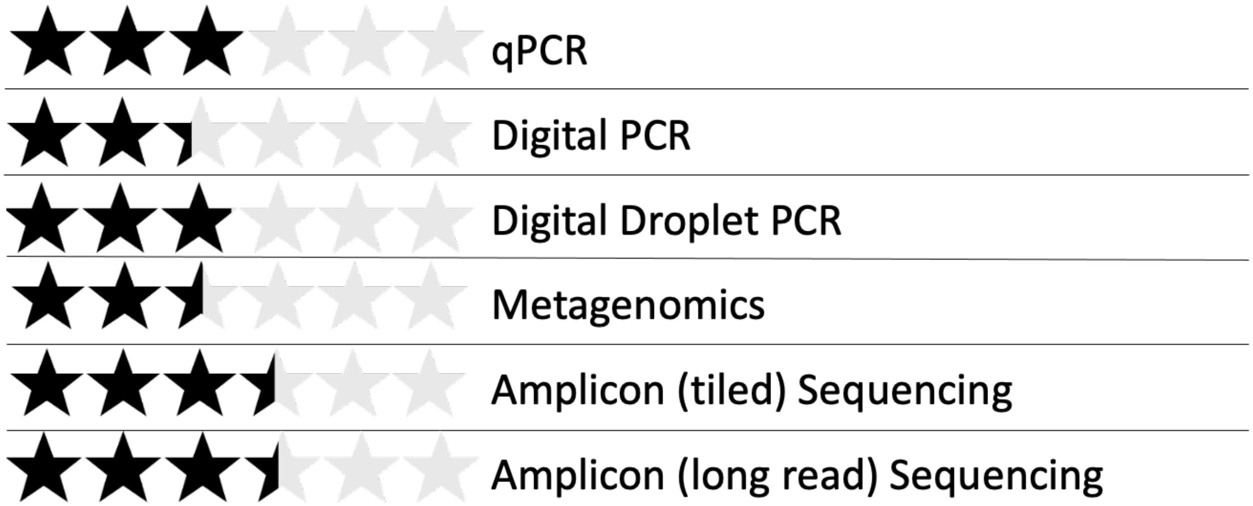
Other: Manufacturer; Not given

2. For SARS-CoV-2 quantitation, what is the preferred, state-of-the-art analytical technology? (30 responses)

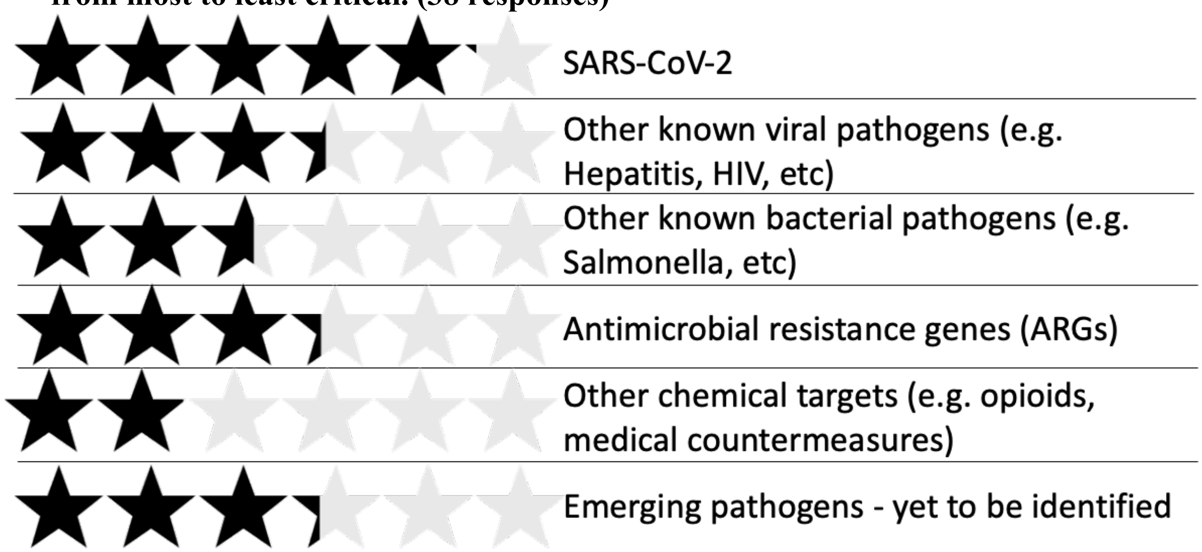


Other: all PCR methods listed will work, in my experience

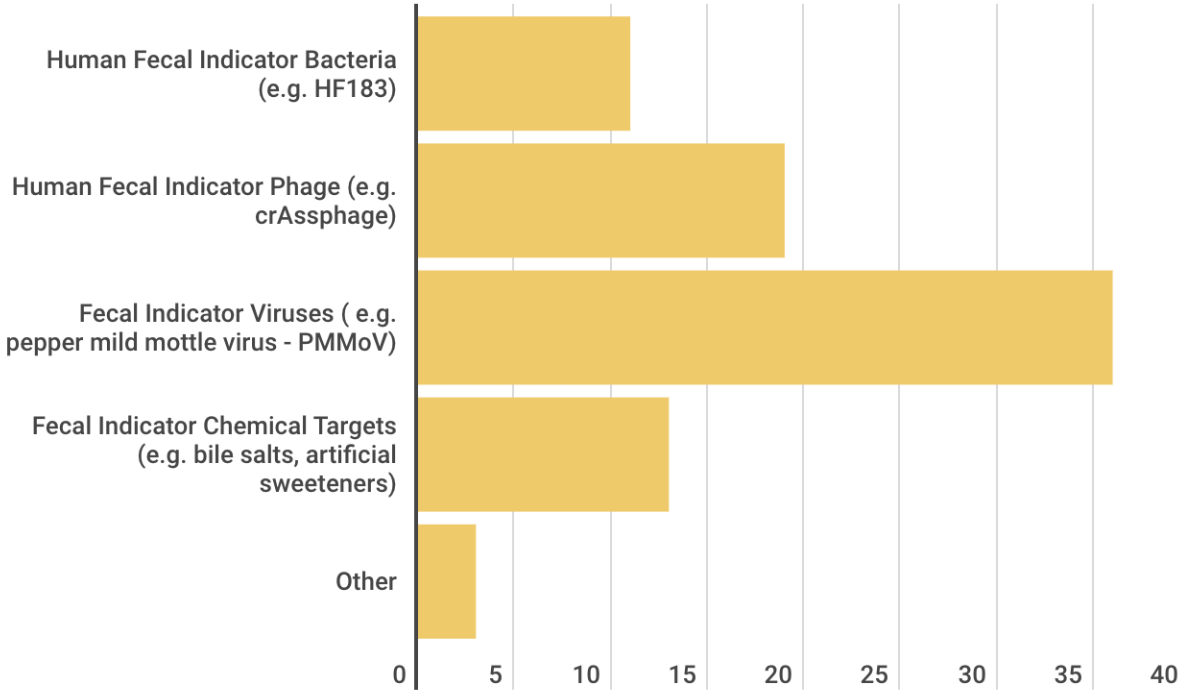
3. **For SARS-CoV-2 variant tracking, rank the state-of-the-art analytical methods from most to least impactful (29 responses)**



4. **What analytes/targets associated with wastewater surveillance are most critical? Rank from most to least critical. (38 responses)**

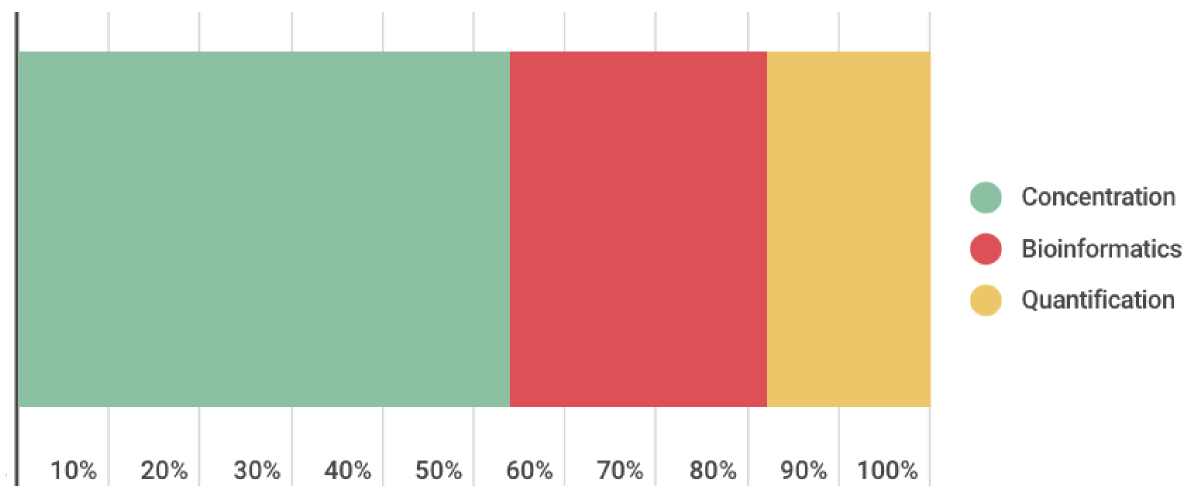


5. What normalization targets should be prioritized? (39 responses)



Other: Depends on what is being tracked. For bacteria, bacterial normalization control; viruses: crAssphage, PMMoV; I don't think we have enough information to know which of these microbial targets will perform best; If the current detection technology is nucleic acid-based, the indicator should also be for feasibility of scale up, which eliminates the chemical markers for the immediate term; This depends on target of interest, SARS-CoV2 or other virus, we need viral norm target. Bacteria target should have bacterial norm target.

6. Which area has the greatest need for advances in technology for wastewater methods? (39 responses)



7. For SARS-CoV-2 and other biological targets, list the state-of-the-art technologies for sample concentration? (24 responses, open text)

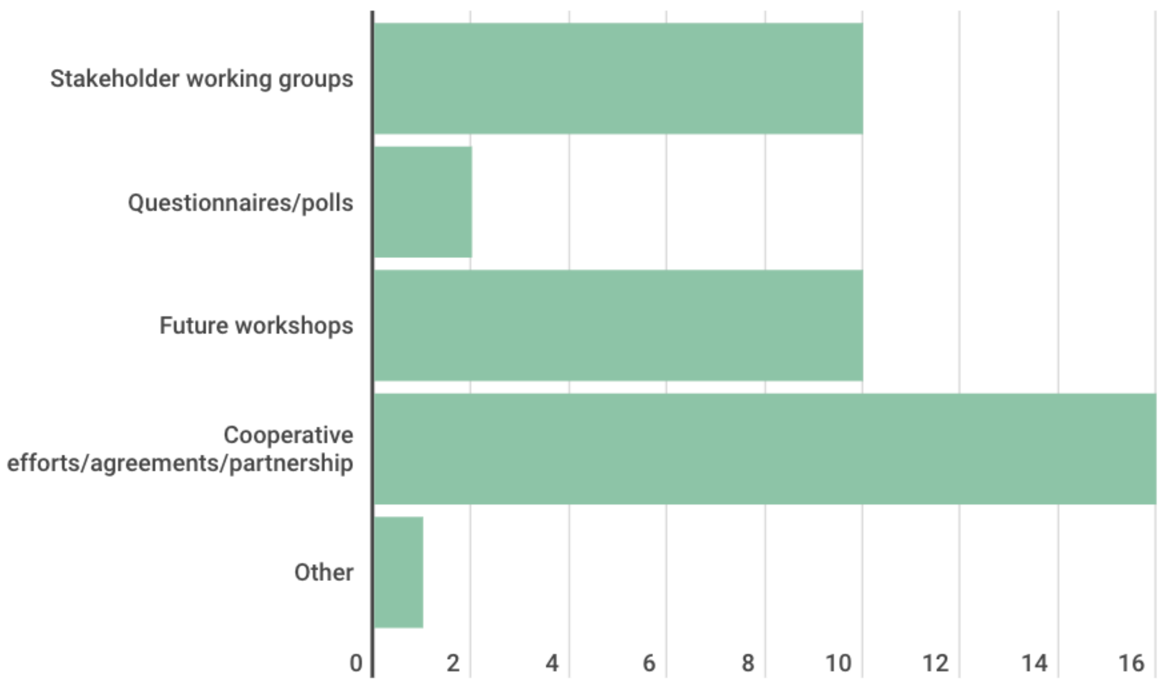
Other suggestions: (1) Best to have no concentration which is possible if you use solids which have extremely high concentrations of virus rather than liquids where concentration is needed. Concentration steps are expensive, difficult to scale, and add variability in the measurement.
(2) The presence of SARS-CoV-2 in wastewater is measured by determining the virus gene concentration in the liquid portion of the wastewater sample. The gene concentration is more accurate and representative if all virus attached on the solid surface can be effectively detached from the solid to the solution by a physical or chemical detaching method during the sample preparation process before the qpcr process. I am not aware of any standardized sample preparation process or state-of-art technologies to detach virus from solid to solution. I hope NIST can develop a standardized and effective process for this purpose. Thanks.

8. Please provide suggestions on a universal data output for comparing data from different regions. (16 responses, open text)

- Common platform like CDC
- gene copies per person per day (use flow or fecal virus to account for flow)
- For PCR: Ct Values or Copies/Liter
- normalized to non-biological persistent analyte
- Need MIQE like guidelines for comparison... concentration method, quantification method, gene target, normalization target (e.g. PMMoV), flow rate (if known), population of watershed (if known).
- I do not know
- I think Gene copy / L is still the most common approach, but it would be best to try and normalize that to sewage strength. But this is more difficult to execute.

- Ratio of COVID to PMMoV
- A universal data output is different depending on what is being tracked. Concentrations normalized to PMMoV appear to work well for quantitative tracking. For sequencing in sewage, a more structured way to bin the mutations is needed to categorize the SARS-CoV-2 variants present in the mixed sample is still sorely needed.
- I would suggest a different approach based on a site and temporal comparison (trending over time).
- relative analyte level = target genome unit per liter / fecal indicator genome unit per liter
- Synthetic sequencing standards can be used as both quantitative normalization controls and to distinguish false negatives from failed reactions.
- Concentration normalized to original sample input volume (pre-concentration) with matched data on appropriate markers, rich metadata on flow etc.
- Different geographic region? volume concentrated, method of concentration. extraction method. %tested. testing method. quant/qual method. sewershed population
- Number of target molecules detected per 100ml sample volume extracted. Greater than 100mL is to much to quickly filter or process effectively.
- An Excel spreadsheet

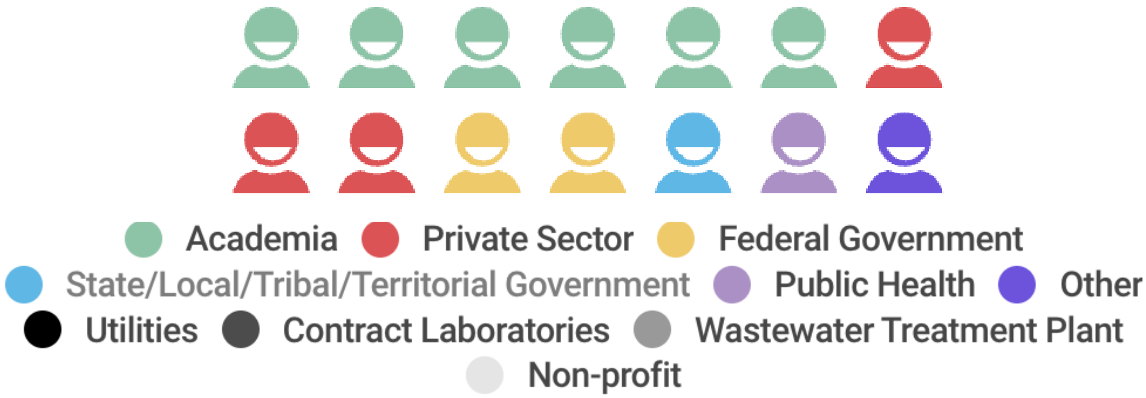
9. In your opinion, what is the best forum or mechanism to ensure stakeholder feedback in the development of tools to support/improve methods for wastewater surveillance? (39 responses)



Other: Combination of all of it. Make sure all have a voice and not only the "charismatic ones"; Discuss how to validate and share methods for wider and faster enablement

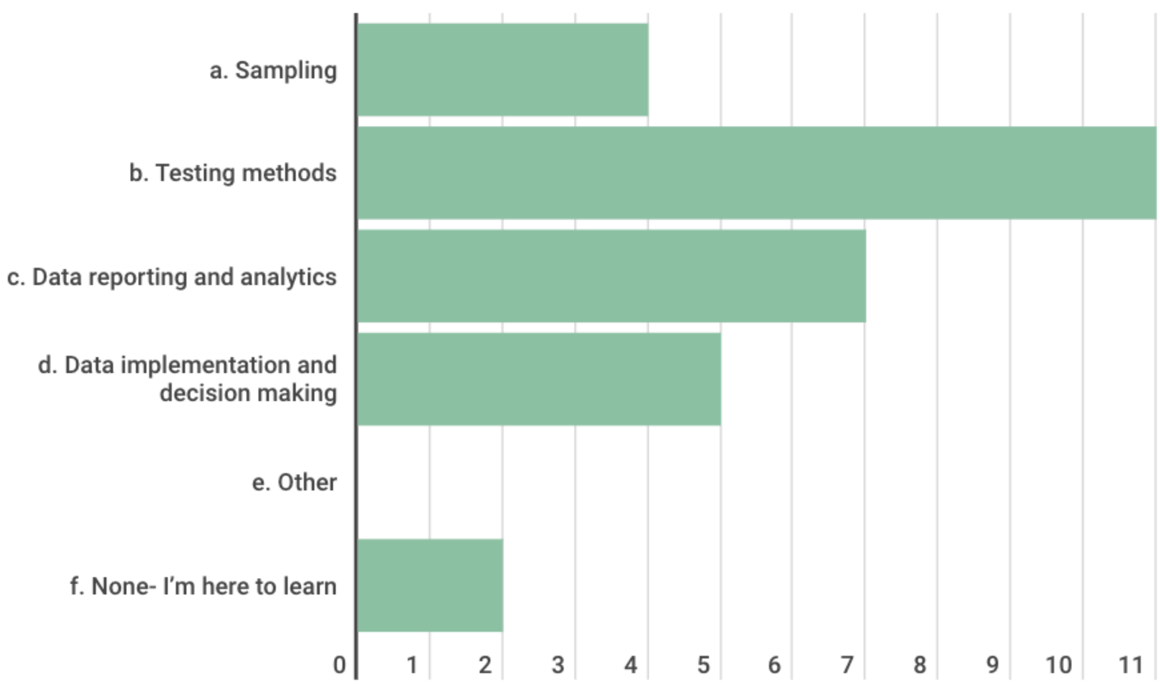
Appendix G: Reference Materials – Focused Feedback Poll Results

Warm-up: Select your stakeholder group and/or interest sector (14 respondents)

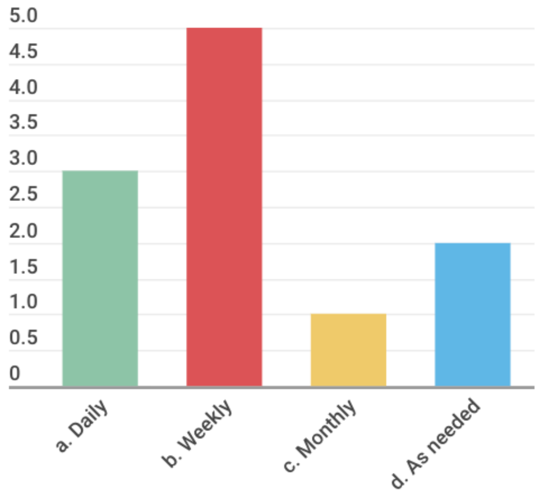


Other: Molecular Biology kit manufacturer, (Promega Corp)

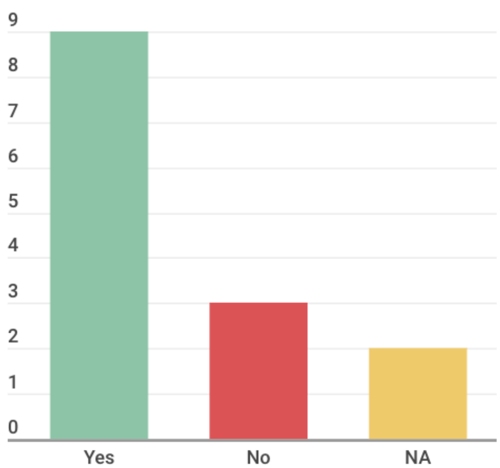
Q1. Which step(s) of the wastewater surveillance process do you work in and/or contribute to? (14 respondents)



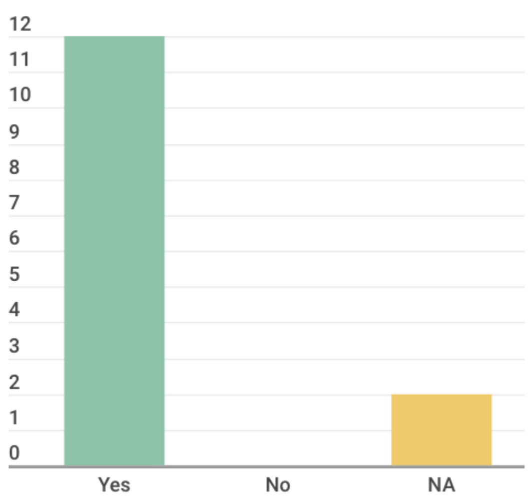
Q2. How often are you testing wastewater? (11 respondents)



Q3. Do you currently use a reference material (RM) for a wastewater surveillance application? (14 respondents)



Q4. In your process, do you include quality control materials in every run? (14 respondents)

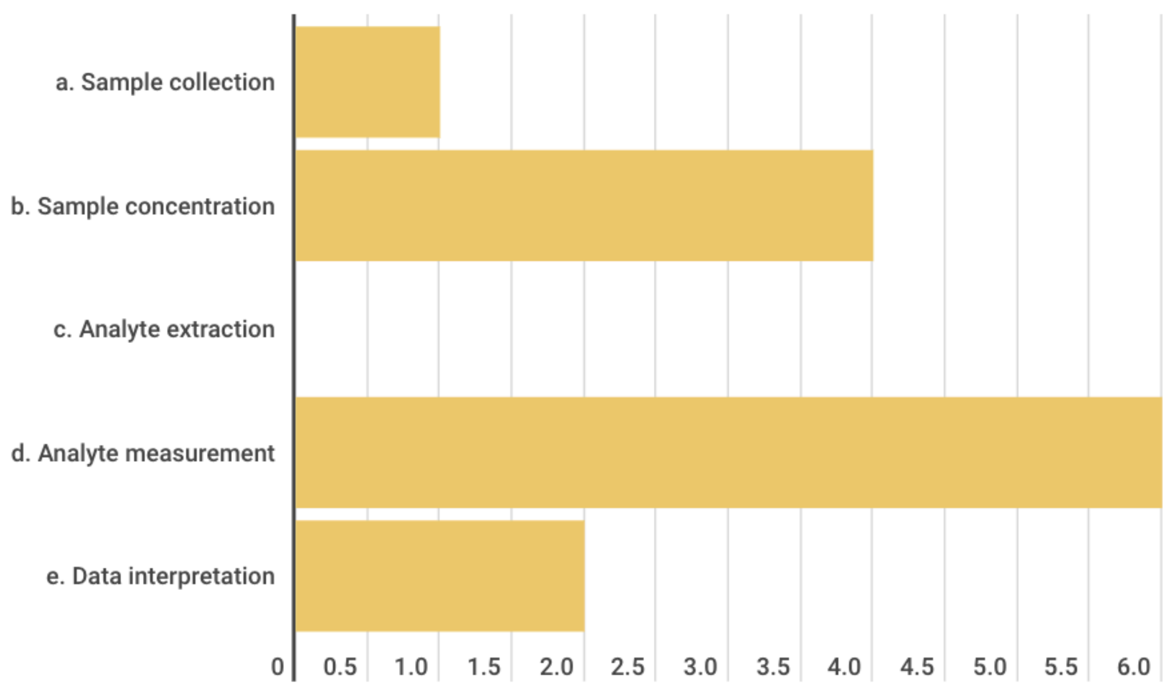


Q5. If so, what analytes are you currently measuring? Please provide up to three targets (ie. SARS-CoV-2, antibiotic resistance genes, fecal indicators, pharmaceuticals, etc). (10 respondents)

1. SARS-CoV-2 CDC N1; PMMoV; bovine coronavirus
2. SARS-CoV-2, mtDNA
3. Fecal indicators, Bovine coronavirus process control
4. SARS-CoV-2
5. SARS-CoV-2
6. SARS-CoV-2
7. SARS-CoV-2 (N1, N2, E), PMMoV (fecal indicators)
8. PMMoV fecal indicator virus
9. SARS-CoV-2, fecal indicators
10. Illicit drugs, pharmaceuticals

Sudden acute respiratory distress syndrome-Coronavirus-2, SARS-CoV-2; SARS-CoV-2 Nucleocapsid gene region N1, N1; Pepper Mild Mottle Virus, PMMoV; SARS-CoV-2 Nucleocapsid gene region N2, N2; SARS-CoV-2 Envelope gene, E; mitochondrial DNA, mtDNA

Q6. In your opinion, which step of wastewater surveillance laboratory testing has the greatest need for a RM? (13 respondents)

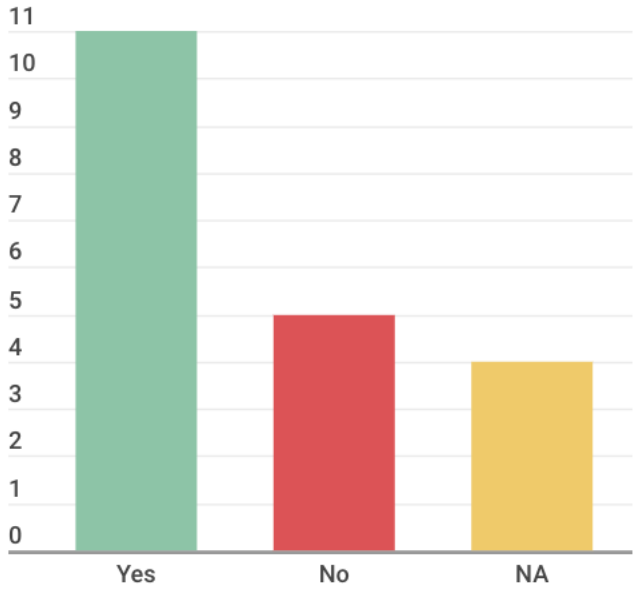


Q7. For SARS-CoV-2 wastewater surveillance applications, please rank matrix-based RMs from most to least useful for future development? (18 respondents)

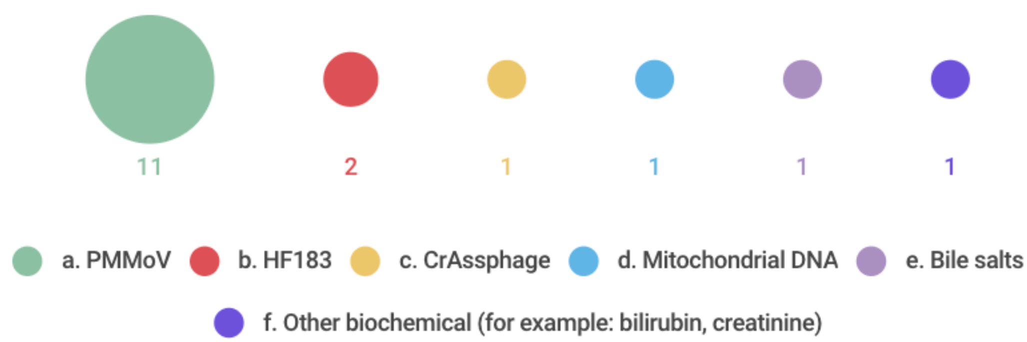
Other: Synthetic wastewater including defined amount of defined inhibitors

Q8. For SARS-CoV-2 wastewater surveillance applications, please rank calibration RMs from most to least useful for future development (*defined mixture)? (18 respondents)

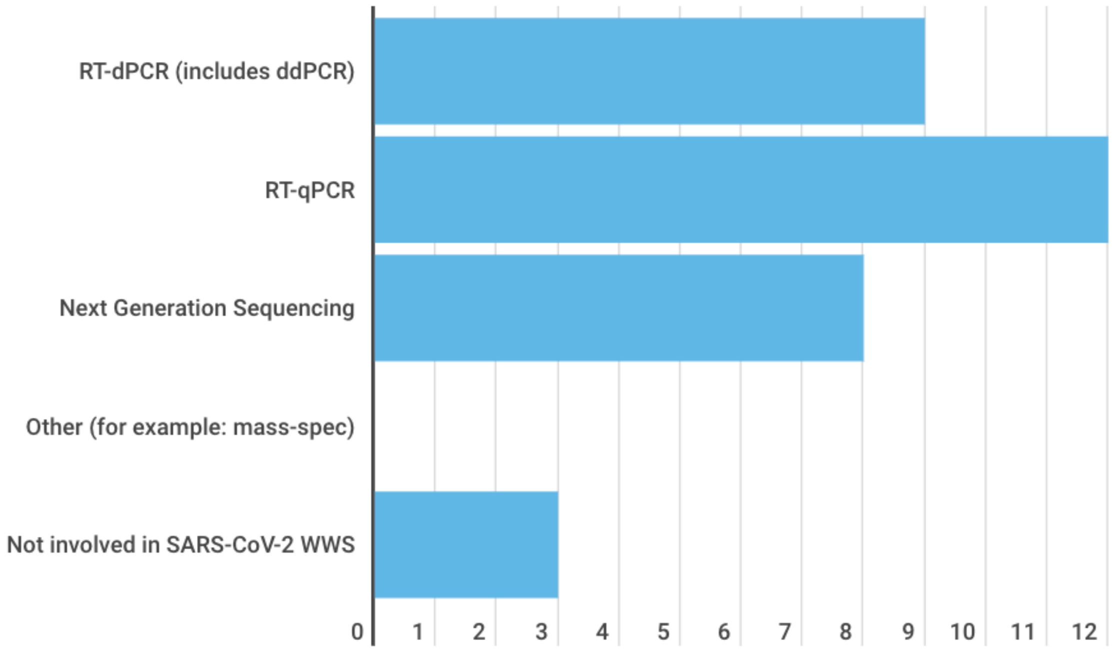
Q9. For interpretation of SARS-CoV-2 measurements in sewage, are you normalizing to a human fecal waste-associated target such as PMMoV, HF183, CrAssphage, and/or biochemical? (20 respondents)



Q10. If normalizing SARS-CoV-2 measurements to a human fecal waste-associated target, which one are you currently using? (select all that apply) (14 respondents)

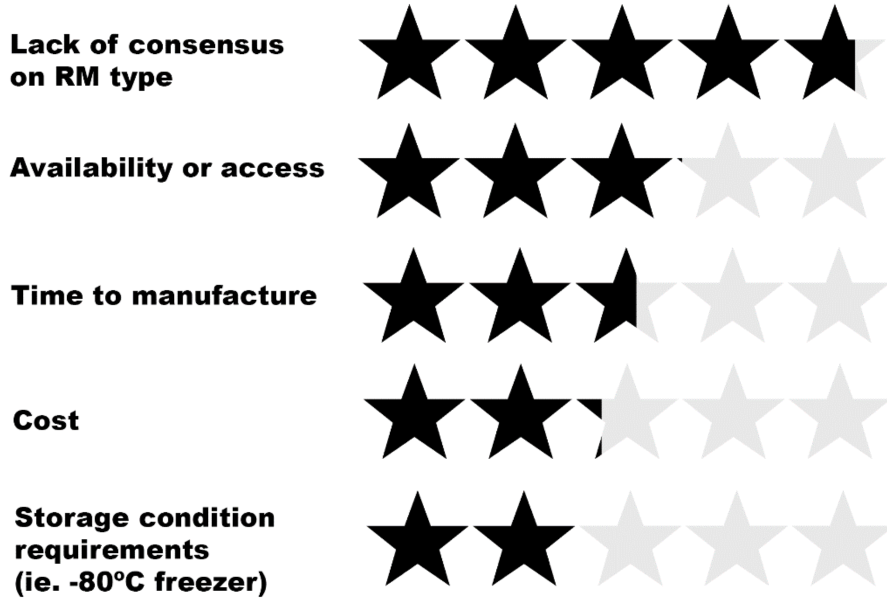


Q11. What surveillance technology platform are you currently using to characterize SARS-CoV-2 analytes in wastewater? (select all that apply) (19 respondents)

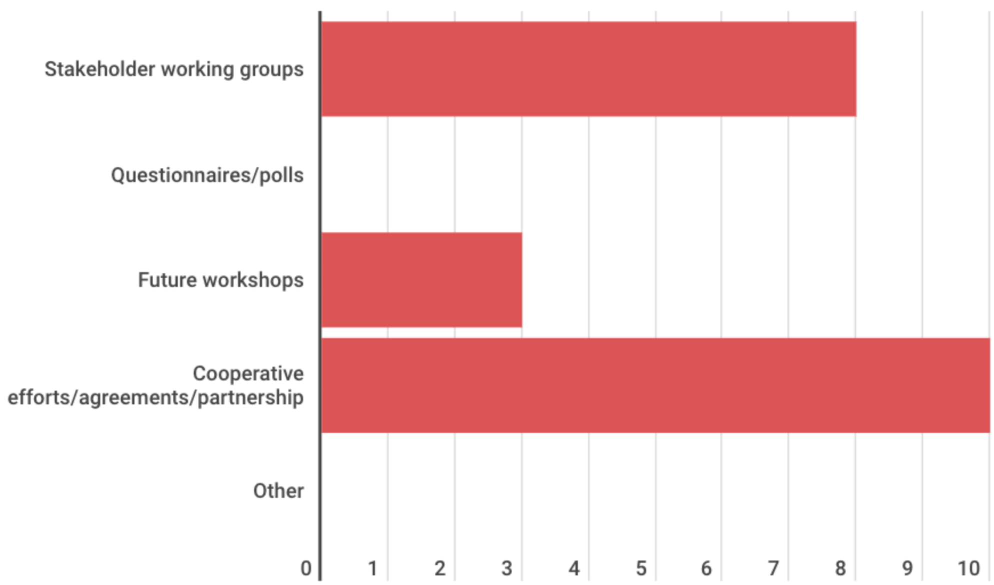


Q12. Rank the following from most to least important towards establishing confidence in a sewage surveillance monitoring program. (21 respondents)

Q13. Rank the following potential barriers (from most to least impactful) for development and implementation of a RM. (20 respondents)



Q14. In your opinion, what is the best forum or mechanism to ensure stakeholder feedback in the development of a RM? (21 respondents)



Other Suggestions:

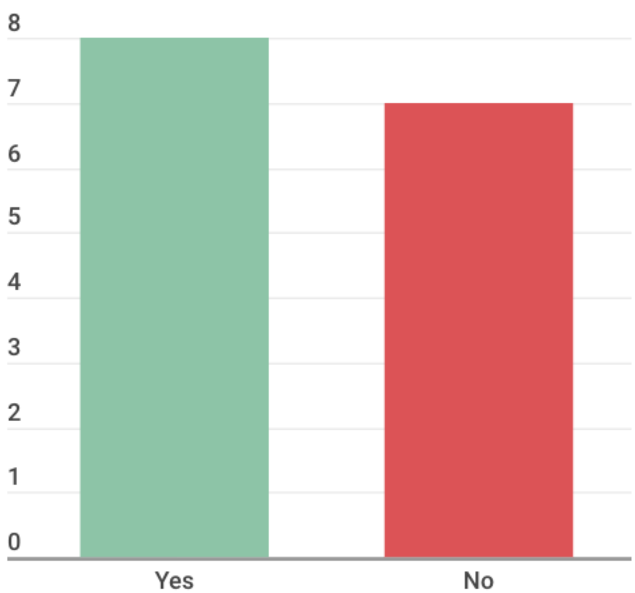
- Seminar and specific working group in worldwide conferences like IWA, WEF, etc.
- A combination of all above as a consultant to a stakeholder working group

Appendix H: Documentary Standards/Guidance Documents – Focused Feedback Poll Results

1. What type of organization do you represent? (15 respondents)

2. If you answered "Other" to question 1, please elaborate. (no response)

3. Are you currently using any documentary standards and/or guidance documents related to WWS? (15 respondents)



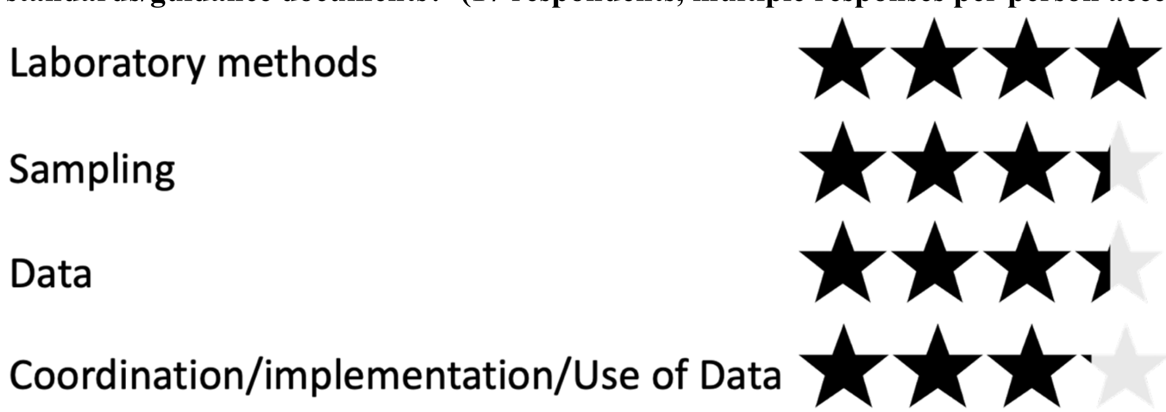
4. If you answered yes to Question 3, please elaborate. What documents do you use? (7 respondents)

- CDC-China protocol for sewage sampling
- US Geological Survey National field sampling and safety manuals.
- NWSS Standards
- We currently conform to the requirements and recommendations of CDC's NWSS, particularly as it relates to data submission and what information is collected.
- Reagent manufacturers' instructions
- wbe.stanford.edu
- Field sample collection procedure. Site location selection procedure. Laboratory analysis procedure.

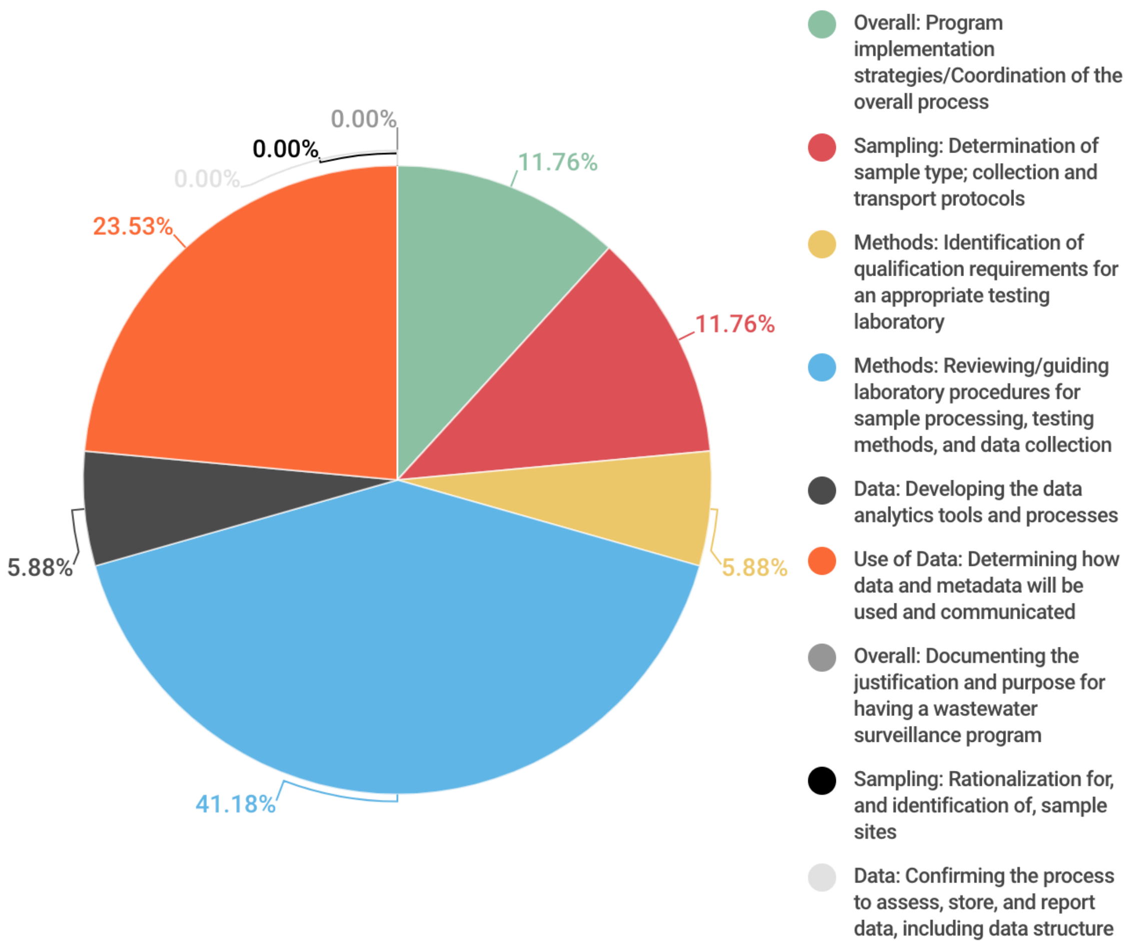
5. Are you aware of any documentary standards/guidance documents that are available or under development? Please describe. (8 respondents)

- CDC-China protocol for sewage sampling
- Yes USGS is updating guidance manual to include influent and effluent wastewater sample collection and handling.
- I believe that both COSeS and the NSF RCN on Wastewater Surveillance have generated and are in the process of publishing guidance documents.
- I believe that CDC is working on refining their existing guidance and making it more comprehensive.
- NA
- No
- Spain has a documentary standard/guidance document for WWS that was made available early on during the COVID-19 pandemic.
- I understand NWSS is developing these but they are not yet available. We are developing our own at UCSD to share with other institutions.

6. How would you prioritize these areas, in terms of their need for documentary standards/guidance documents? (17 respondents, multiple responses per person accepted)



7. More specifically, what step in the wastewater surveillance process could benefit MOST from documentary standards? (choose one) (17 respondents)



8. Why did you prioritize this area? What are the primary challenges in this area that could be addressed with standard/guidance documents? What specific challenges could be addressed immediately? (13 respondents)

- Standard protocol
- There is really no established uniform SOP from sample collection, concentration, testing and data analyses and reporting. In every scenario, the determining factor is the CONOPS for a given organization.
- Having communicated results to customers, it is clear that there is a lack of understanding, and evolving understanding, in how to apply wastewater testing results to pandemic management and public health.
- Relying on existing compliance monitoring standards is inadequate. The need to have nationally consistent and statistically rigorous Quality Assurance/ Quality Control

(QA/QC), sample collection, design, handling/shipping, lab handling and processing, analysis, data storage, retrieval and interpretation all coordinated is key to the integrity and credibility of this enterprise. This includes various scenarios such as surges, variants, new pathogens, etc that require specific attention and potentially unique protocols.

- Need more translational research to help health departments understand how to use the data, and when it's robust enough to guide decisions.
- This area is most challenging because it straddles both the wastewater and microbiological community and the public health community. More needs to be done to bring those two groups together to understand what data should be collected that is most useful for public health outcomes.
- There are currently many lab methods that can (and are) be used, but they do not all perform equally well. As the (current) main use of wastewater data is observing trends over time, comparability of the data is of the utmost importance. As such, identifying an appropriate, well performing lab method early in the implementation of a wastewater surveillance program is key. While having stronger standards or guidance docs here wouldn't eliminate the need for validation of a method at individual labs, it would at least be extremely helpful in providing a strong foundation for starting the process (and potentially get us further on the road of being able to realistically compare data between programs). I would like to mention that a very close second for me would be standards/guidance docs for data analytics and processes. There is absolutely a strong need for work in this area (I'm not sure that any programs are analyzing their data in precisely the same way). However, having a more unified set of standards/recommendations for lab protocols can also be important here and is an earlier step in the process.
- The results are only as good as the sample provided. With sample standards, sample storage and transport temperature, and everyone knows you are dealing with a quality sample. A person will not have to convince an administrator or utility employee they need to refrigerate wastewater samples.
- The diversity in test methods could be normalized when standards and guidance documents are available. This will help data reporting and epidemiological analysis.
- It seems that this is one of the areas in which there is the least amount of guidance and consistency across labs.
- Metadata is least standardized but has massive impact on interpretation of the results.
- Need standardization of testing to compare results between areas
- Sample collection has generally not been well documented by current WWS, and as the point of sample generation getting a good sample is a critical and often hidden part of the WW surveillance chain.

9. What type of standard/guidance document would help address these challenges? (Examples include: Data reporting template, metadata requirements, sampling guidance, guidance on controls, other - add your own) (12 respondents)

- Data reporting template and LOD
- All the above
- Data reporting template to simplify interpretation and comparison of results; Sampling guidance to improve assay robustness; Guidance on controls (and common sources) to improve data quality and speed methods development
- Soup-to-nuts protocols and design. From planning to collect samples based on specific needs to sample collection to analysis and interpretation of data including metadata and QA/QC data (field and lab). There can be individual chapters but also needs to show a continuum.
- Analytics around signal vs. noise, thresholds that matter, etc
- A document describing the kinds of data needed to address specific questions such as tracking outbreaks, tracking variants, and so on. Then additional guidance on how the data needs to be collected and used to be useful. Including details such as frequency of sampling.
- I think there are a number of things that could be helpful (all of the examples mentioned in the question could be useful). To my mind, some of the especially helpful standards/guidance documents would be guidance on choosing an appropriate set of laboratory methods (some comparison studies would likely be helpful, similar to the existing WRF study) and recommended methods for analyzing and interpreting the resulting data. Some other sets of standards, like metadata requirements and lab controls, would naturally follow from this work (at least in part).
- Sampling guidance. Sampling receiving and storage.
- data reporting template; guidance on controls; sampling guidance; metadata requirements; performance testing guidelines
- guidance on sampling procedures (comps v grabs v passive); guidance on inhibition controls and reducing inhibition from ww; Data reporting requirements (flow rate, environmental factors, controls)
- Metadata and data requirements and reporting template
- Sampling guidance for both composite (down to specifics of time or flow weighted settings) and grab samples (what type of cup on rope to use and how to disinfect between sample sites) for both wastewater utilities and when using students to collect samples with limited environmental sampling experience.

10. In the area of sampling, what guidance/standard document would have the greatest impact? (open text, 12 respondents)

- Sample types address from the treated and untreated sources.
- Sample collection and concentration
- Proportional sampling best practices and sample storage
- One that shows the big picture and specific data quality objectives for various scenarios
- SOPs for primary influent or sludge at treatment plants and at smaller sewer catchments including frequency of collection.
- I think that information on sample handling/transport/storage would likely be the most helpful. I' not sure there's much disagreement on the type of sample that's optimal (~24-hour composites).
- transport
- guidance on collection, storage, shipment
- Type of appropriate sample
- Where and how to sample
- Use of solids
- Sampling guidance for both composite (down to specifics of time or flow weighted settings) and grab samples (what type of cup on rope to use and how to disinfect between sample sites)

11. In the area of laboratory methods, what guidance/standard document would have the greatest impact? (open text, 11 respondents)

- Method standiratization including primers and probes.
- SOP
- Assessing sample quality (inline controls and control markers)
- A big picture showing national consistency in meeting data quality objectives for various scenarios (eg surges, standard surveillance etc)
- SOPs for quantitative tracking and sequencing including necessary processing, extraction, and inhibition controls.
- A document recommending a valid, well performing lab method (or set of methods), with recommended conditions and controls (including matrix recovery and human fecal content as options).
- controls
- extraction methods, QA/QC of qPCR, if and how to use % recovery data from a matrix spike, quantification standards, fecal indicator standards
- Inhibition controls
- Which methods are/aren't acceptable (many produce similar results, e.g. ddPCR vs qPCR)
- ddPCR

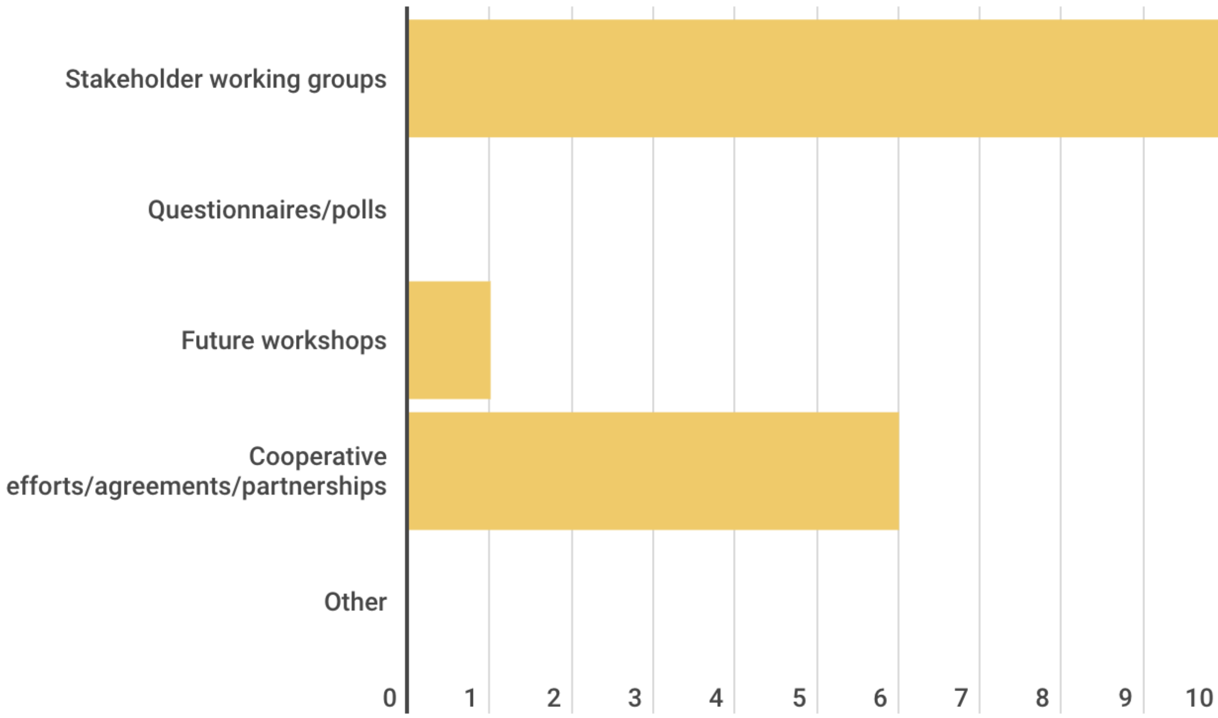
12. In the area of data, what guidance/standard document would have the greatest impact? (open text, 10 respondents)

- LOD
- Data standards
- Data standards for variant calling, updated variant classifications, versioning
- Metadata (including qa/qc field and lab) analysis and interpretation
- An SOP laying out what needs to be included in data reporting in order to be useful for translation to public health.
- We really need more work developing guidance on how best to analyze the data and detect trends/events of interest. It seems that everyone does something different, and I haven't seen one that's "best"
- Reporting
- what to include in reporting
- Standard template for reporting results
- What to include as meta data, including for quality assurance samples.

13. In the area of coordination/implementation/use of data, what guidance/standard document would have the greatest impact? (open text, 10 respondents)

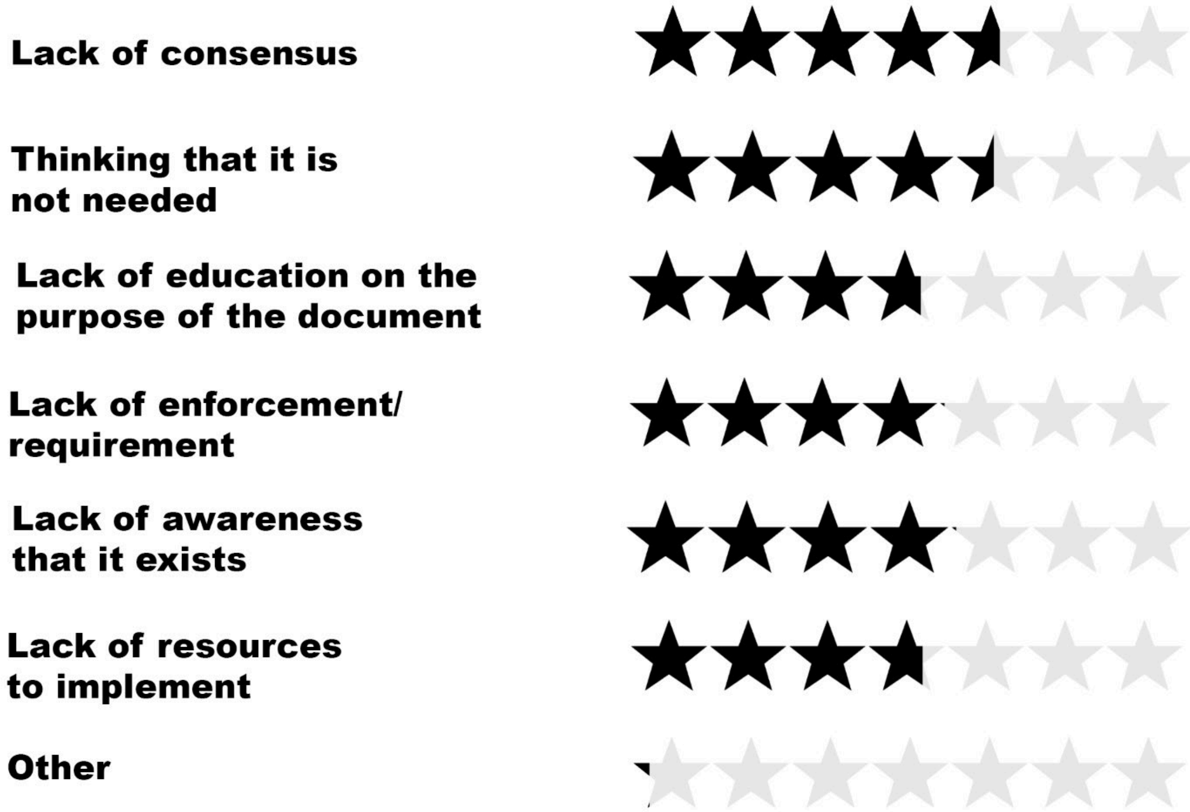
- Implementation
- Data analyses and reporting
- Reporting standards (mutation vs. viral strain)
- Same as above. In other words, 10-13 are a continuum that need to be coordinated via national guidance and protocols
- A document describing the kinds of data needed to address specific questions such as tracking outbreaks, tracking variants, and so on.
- Recommendations on how best to use the data and present it to partners would be helpful, ideally with examples/case studies demonstrating its utility in different situations.
- trends
- national database/dashboard
- Not sure
- The RCN is doing a good job currently of WWS coordination.

14-15. In your opinion, what is the best forum or mechanism to ensure stakeholder feedback in the development of a standard/guidance document(s)? (17 respondents)



Other suggestions: Building more partnerships of utilities (small and large), academics and the private sector to allow greater range of lesson learned for both better data and better WWS project efficiency (don't reinvent the wheel, learn from friends)

16. What might potentially hinder the development and use/implementation of a documentary standard or guidance document related to wastewater surveillance? Rank from most to least likely to hinder. (20 respondents)



Appendix I: Chat and Q&A from Days 1-2

Appendix I provides a record of the questions received through the GoToWebinar QnA feature as well as the sli.do polling platform. Some questions received through GoToWebinar were answered using the chat feature; these answers are recorded below. GoToWebinar questions without a written answer below were either not answered during the webinar or were answered verbally (as indicated in some cases by "Answered in Q&A").

Monday June 14th: GoToWebinar QnA

Question	Answer
Does the Centers for Disease Control and Prevention (CDC) National Wastewater Surveillance System (NWSS) require a population normalizer to improve reporting of viral concentrations across the country?	
I was curious to know how to get links to the background literature you mentioned earlier.	
Address to Pat Phillips: kindly elaborate on the refence material, papers you've just referred to.	
is Coronavirus in feces more than urine If yes, how to extract virus from solid in wastewater into liquid sample for analysis?	SARS-CoV-2 is detected more frequently in feces than in urine. There are still questions around whether liquids, solids or both are more appropriate for wastewater surveillance and the best methods for each sample type. We will hear more about those issues in the testing session tomorrow morning.
70 gal/person/day wastewater flow was used in our study to determine the viral load. Is this a representative unit flow number to use? If not, please recommend a number. Thanks.	
Are you concerned about the ability of sewage surveillance to detect SARS-CoV-2 as COVID-19 incidence decreases?	
Are you concerned about the sensitivity of sewage surveillance to detect SARS-CoV-2 as COVID-19 incidence decreases?	Yes. We expect to lose the wastewater signal in a community as cases decreases. It is not clear what the overall limit of detection will be for this surveillance approach and it likely varies between communities and sampling locations. We are evaluating approaches to increase the sensitivity, such as sub-sewershed sampling or primary sludge testing, but it isn't clear how feasible those will be at scale.
@Kate Griffiths - When was this inter-lab study done? Is your report available online?	The study was run in February this year. I have written the report and literally have a day of work to do to finish it. It will then be "branded" and go onto the WaterRA website for the members to access, but this isn't publicly available. However, the plan is to publish the full report in the scientific

	literature asap. The calibrant is also available for sale.
I realize this is CDC and "disease". The NWSS seems to be only focused on disease markers, genes, ribonucleic acid (RNA), etc... Is there interest in small molecule detection such as stress or health markers?	
can we be reminded of what the DCIPHER acronym represents? I don't see it in the documents you sent	Data Collation and Integration for Public Health Event Response (DCIPHER) platform
LMIC?	Low and middle income countries-got it
This may be the time to ask about the ethical public health concerns regarding confidentiality and similar concerns - how small is too small for a sewershed regarding public health ethics?	
do you know anything about the (Randazzo group) co-precipitaton with AlOH3 instead of PEG@dr. griffiths	No I'm not, though at the national metrology institute (NMI) we don't run wastewater surveillance as our focus is on the calibrants and proficiency. For this study the participants had independently developed their methods, so were asked to use these. I had no influence on the methods they used.
How does you project with BioBot Analytics lab fit in	
What do you hope to gain from project newly announced by BioBot Analytics lab?	There are two goals of the Health and Human Services (HHS) contract awarded to BioBot. First, it will complement existing wastewater surveillance capacity, particularly in jurisdictions that do not have state-led programs. Second, it will provide wastewater sequence data that CDC can use to evaluate variant tracking in wastewater.
DHHS has apparently contracted with one private lab to recruit 320 WWT systems to provide samples twice a week for 9 weeks which that lab will analyze. Impact on NWSS?	Sorry David. I missed your question. Let's bring this up during the panel discussion this afternoon
Does anyone of you actually sample septic systems?	
Do EPA or others offer general guidance for sampling septic systems?	
What is the status and approach for funding wastewater surveillance systems over the long term?	CDC funds wastewater surveillance through the Epidemiology and Laboratory Capacity (ELC) cooperative agreement, which is the primary way that we fund health department activities. In 2021, we are transitioning NWSS support from supplemental, one-time COVID awards to the annual ELC award. This transition will allow us to provide routine funding to health departments on

	an expected schedule that will support better programmatic planning and development.
How do you insure there is no inhibition within your wastewater sample? Does inhibition testing need standardization?	
Do you know if there is any different amount of shading between the different variants?	There are some indications that shedding may vary between variants, mostly inferred from differences in respiratory shedding. We are hoping to get more information on this soon.
What other taxa/pathogens/diseases will NWSS be used for beyond COVID?	
what is the role of commercial labs in NWSS?	
How are standards/protocols currently shared across wastewater management facilities?	
did you consider comparing raw wastewater samples and sludge samples?	The feedback I got from the participants during the planning indicated that the raw wastewater represented the majority of the testing. It's always a balance in a voluntary study to capture as much data as possible without burdening the participants with too much work. However, there is interest in running another round so we'd be keen to use different samples next time.
Recommendations on getting started with NWSS?	
Will the surveillance system also support surveillance of chemical substances as well? Meaning, will the infectious diseases surveillance be integrated with other surveillance?	CDC is evaluating the potential to measure chemicals as part of NWSS. It is unlikely to be included in the first expansion of NWSS activities, but that does not exclude future expansion as methods and epidemiologic need allow.
What about data normalization using artificial sweeteners?	
Is the NWSS/DCIPHER data available outside of the CDC for research purposes?	No, the NWSS DCIPHER data is currently only available to health departments but we are working to share the data on COVID Data Tracker in the next few months.
What if any specific plans are there for using NWSS to monitor other pathogens and/or detect emerging pathogens?	We are currently evaluating additional pathogens for inclusion in NWSS. We are early in the process so we do not yet have a list of specific targets. We hope to roll out the expanded NWSS panel within the next 2 years.
Are you considering using metagenomic sequencing as a method for pathogen-agnostic monitoring?	We are watching the development of metagenomic technologies and methods closely. While it does have the advantages of detecting a wide variety of pathogens, including those we don't even know about yet, metagenomics are not very good for quantitative measurements which are critical for

	NWSS. We think metagenomics is promising, but the technology isn't ready yet for this application. Hopefully, in 5-10 years, the methods will have improved enough to warrant incorporation into NWSS.
Is the DCIPHER dashboard public available?	https://www.cdc.gov/cpr/readiness/orr.html
Any thoughts on using this process in cruise ships?	
-How can private labs get involved?	
For quantification purposes, digital PCR has shown to be more reliable than RT-PCR? Is there any kind of homogeneity among labs?	We will hear more about digital Polymerase Chain Reaction (dPCR) versus quantitative PCR qPCR tomorrow. I will defer to those presenters.
Is there any recommendation for the untreated wastewater surveillance in the community absent of wastewater treatment plant (WWTP), especially in low and middle income countries (LMICs)? Thanks!	
Amy, can you elaborate more on the role of commercial laboratories.	
Besides microbes, are there any other agents bei8ng tracked? antibiotics, drugs of abuse or toxins?	
How did your team develop a workplan to sample from prisons or long-term care facilities to avoid the noise you were talking about earlier?	
This is for Amy Kirby, but perhaps too late now: Is CDC looking at the potential for incorporating sampling of primary (and possibly secondary) solids sampling for a standardized surveillance program, particularly based upon its potentially higher sensitivity to capturing prevalence when case counts are present but low. Advantages appear to be ability to use grab-sample of the solids rather than needing 24-hour composite sample.	Yes! We already have some locations that are testing primary sludge. The DCIPHER system can accept data from primary sludge. There are still some questions around when primary sludge performs better than wastewater. Hopefully, the discussions in this workshop can inform guidance on which type of sample is best in each setting.
Is grab sampling typically sufficient for sampling primary sludge?	
There hasn't been a lot of discussion about the importance of integrating flow monitoring with composite sampling, and the various ways of getting this data, relative to where the sample is collected. Comments?.	
in some countries there are discussions on mobile labs for wastewater based epidemiology	

(WBE) sample collection and analysis. Are such concept discussed in the US	
When cdc assays are used, do you notice difference in ct between n1 and n2	Yes we saw a lower Ct value from N1 compared to N2. It is assumed to be due to differences in secondary structure between the two assays impacting on the efficiency of the reverse transcription step. This variability is mostly cancelled out when using the calibrant.
Hi Dr Kirby - now that many academic labs have scaled out to test wastewater for COVID - including personnel - how do you envision the long-term role of these labs? Will that capacity have to be moved to state testing labs in order to continue contributing NWSS?	While academic labs can carry out rigorous wastewater testing, functioning as a surveillance lab doesn't benefit academic researchers in the long run. We would like to transition the routine testing to public health and environmental health labs. This will free up the academic labs to focus on the many research questions around wastewater surveillance, many of which have already been touched on today. CDC intends to stay engaged with the academic community to serve as a liaison between public health need and academic research, highlighting the research questions of greatest need and ensuring that research results are used to improve NWSS.
Who was the "opening remarks" speaker before Phillips and after the break? No name is listed on the agenda. Thanks	Here is the speaker info for the first talk in the Sampling Session: Utilities Perspectives and Needs, Mr. Claudio Ternieden, Senior Director of Government Affairs at Water Environment Federation. Please see the "handouts" in GoToMeeting for the latest version of the agenda, program book (bios/talk abstracts), and poster abstract book.
WILL you consider the in-sewer decay of viruses?	We have looked at decay and the inclusion of wastewater residence times in the data analysis. To date, it hasn't seemed to impact the results significantly. Because residence times are not readily available for all wastewater systems and they don't seem to have significant impact on the results, we have not included it in the NWSS analysis.

Tuesday June 15th GoToWebinar QnA

Question	Answer
How do you propose accounting for recovery rate of method? What is range based on enrichment?	
@Nichole - How do you define "variants of concern" and "variants of interest"?	They're defined by public health officials. The CDC has a good description of the classifications https://www.cdc.gov/coronavirus/2019-ncov/variants/variant-info.html.

What type of samples are you collecting from manholes?	Answered in Q&A
What sample volume do you test?	Answered in Q&A by Mariana.
@Sandra - sometimes public health agencies tell us "How will having this data change what we are already doing?" Do you have advice on how to answer that question?	
don't you spin down and discard the big solids prior to membrane filtration?	@David - some people do. others do not.
is that CSV sharing page open for us to view? Do we need an NWSS account to access the pages/resources	You can access general information on the CDC NWSS webpage, but only health department staff can access the NWSS DCIPHER data platform. We expect to post the NWSS metadata on the public CDC NWSS webpage soon.
is ppmov really known as a human biomarker?	Addressed by Aparna in Q&A
Is anyone working on potential standards pertinent to septic systems?	
Dr Wiley: Is NWSS receiving or open to receiving wastewater solids data?	(answered in the Q&A) - Yes.
Has Houston used clarifier solids at all? Please describe.	answered in Q&A
Aparna: Has CDC sought Mathematica insights for revising or improving NWSS?	Answered in Q&A: Not yet.
Mariana: How will performance of BioBot Analytics' new contract with DHHS differ from previous BioBot work on Coronavirus?	Addressed in the Q&A
How can one present WBE information for geographic areas served by septic systems (rather than WWTPs) — assuming collection and analysis for such information from at least some septic systems?	for data panel
Dr.Jennings: Could NWSS split "national" WBE information it provides into 2 (or more) presentations?	
Many WW utilities can provide sewershed info.	for the data panel
Mariana: How does BioBot handle issues with inhibition from wastewater?	Answered in Q&A
Do you have a sense of the influence of different solids settling methods on the ability to compare between locations?	One of our plants uses settling from influent and their results are similar to other plants that use primary clarifier solids. the data fall on the same line.

@ Nathan LaCross, what was the priority rank of the wastewater surveillance system compared to other public health measures search covid-19 testing, treatment, etc? Any comment about how budgetary restriction /priorities at local state level	
lnfluenced setting up a wastewater surveillance system	
Q@Ali - How Solid samples were collected? What Equipment were used?	POTWs have access to their solids line from the primary clarifier that they use for standard sampling and maintenance. they use a bottle to collect the samples. Solids can be settled from influent using a standard method using Imhof cones which are readily used in POTW labs for compliance monitoring
are the different sequencing protocols listed somewhere?	
@Ali - How do results compare from Chemagic instrument versus Qiagen kit?	Chemagic recoveries are close to 100% whereas Qiagen is about 1-10%. Also the chemagic is set up for high throughput 96 well plates (which i know qiagen can do too though)
Could you review your assessment of normalization technique performance relative to case counts in the rural NC county comparative study?	When comparing different approaches for standardization to case counts in rural NC, we found that the correlations between the wastewater data and case counts did vary greatly across methods of standardization, but that the lead time suggested in the data varied greatly, by up to 6 days, across different normalization approaches.
From slido: With the small sample volume in BioFire arrays, what is the limit of detection in copies per liter of water or gram of solids?	
from slido: @Ellen Beasley How are relative abundances of mutation read copies impacted by primer specificity and sensitivity?	There is some reproducible performance variation between the amplicons across the S gene. However, the relative abundance of variants associated with particular variant are consistent between amplicons for a particular sample.
from slido: @Ellen Beasley - Is there any place where we can explore the details of MiSeq sequencing and bioinformatics analyses for variant detection	
from slido: This question for Dr. Boehm, I apologize if covered in the presentation and I missed it. Do solids had more PCR inhibitors compared to other samples types?	Sewage has a lot of inhibitors in general. You need to make sure there is no inhibition and yes - we use digital PCR to help with inhibition.
from slido for methods panel: can you discuss your experience with performance of matrix controls	
from slido: for Ohio speakers: Can you speak to the sensitivity of the ddPCR mutation assays	currently, we're using duplex assays that contain probes for both mutation and reference sequences

and cross-reaction with the wildtype that could impact the trend and abundance evaluation?	and can view potential cross-reactivity in each droplet. We find concordance among the quantities measured by N2 and duplex mutation assays.
from slido for methods panel: While matrix spikes and indicators of fecal strength are widely used they do not seem to be being used to adjust the results? What is the panel's view on this?	
from slido: @Houston Have private businesses such as nursing homes had any concerns regarding HIPAA or the impact of testing on business for site specific WW testing?	
Is there any place where we can find the protocol you have followed for miseq sequencing and bioinformatics analysis	We have not yet published our results, so we do not have a protocol that we are sharing. We designed the amplicon primers to avoid existing variants that have already been observed in SARS-CoV-2. We use shorter amplicons, 140-300 bp because we have found that the performance is more robust. We target 10,000+ reads/locus to have reasonable relative quantification.
What is the sampling distance between measurements to establish the trend and alert analysis	The trend analysis is based on 5 consecutive measurements; the temporal distance between those measurements is typically twice weekly, which yields a trend determination over 15 days of data. But the cadence of data collection can vary quite a bit, so it's challenging to define a trend metric spanning a specified timespan. (There is also a short-term trend determination made on 3 consecutive measurements.) Alerts are based on 5 measurements previous to the most recent measurement; a prediction interval is then generated for the most recent measurement and compared to that measurement.
what are you using for testing your recovery rate?	
Is the NWSS data dictionary publicly available?	for the data panel
'm surprised that wastewater treatment facilities don't already have these sewersheds mapped out. How come that data wasn't needed previously for their work?	for the data panel
Besides human fecal source indicator, is there any other significances of measuring crAssphage and PMMoV	
Hello everyone. Don't we need to know more about the biology of SARS-CoV-2 in the gut and drivers of fecal shedding as well?	
Besides SARS COV2 is there a plan to expand it other pathogens?	ODH is discussing this option with our state public heath lab, epidemiologists and our research

	partners. We will be considering monitoring of other viruses or microbiological agents.
Rebecca: Do you think your excellent use of wastewater surveillance has helped stimulate vaccination rate in Ohio?	ODH does not have sufficient information to know if communicating wastewater data has helped with vaccination rates. We have just built the ability to extract vaccination numbers by sewershed so perhaps further analysis will reveal some trends.
Ali: Did you do any work with secondary solids? Some WWTP have no primary settling but do have clarifiers and secondary solids. Is the RNA signal still there and useful to measure?	If a plant doesn't have a primary clarifier, then they should settle solids from the influent samples they already collect for their normal compliance monitoring. We don't recommend secondary solids because those have been subjected to treatment
Jordan: Have you used your methods to sample from portable toilets, such as a farm worker camp?	Thanks for the question - not specifically from portable toilets, but we have from black water systems on cruise ships which should behave similarly (vacuum system completely segregated from grey water). Happy to discuss further: jordan.schmidt@luminultra.com
Ali: Isn't a 24-hour composite influent sample analyzed by filtering the sample and analyzing essentially the solids on the filter? I'm missing something here, I believe. Thanks!	The goal is to get a lot of solids! Even if your "influent method" does not discard the solids, it only captures a small amount of solids. The goal of analyzing solids is to get as much solids as possible into your work flow. The usual TSS in influent is like 100-300 mg/L. So if you filer 100 ml you would collect just 10-30 mg and then after you extract RNA and aliquot into reactions, much much less. We analyze a virtual 225 mg in our PCRs
Thanks Ali for influent sampling response. I'll have to ask our WWTP folks if and how they are taking influent settleable solids samples (quantity). I think the great advantage you mentioned with primary solids about being able to grab-sampled and not needing to use a composite sampler breaks down when you depend on single settleable solids influent sample, due to expected during-the-day likely variability.	Yes but most plants collect 24 h composite influent sample.
Speaking of metadata, I've heard little discussion about the impacts of the wastewater temperature on the signal decay, which was addressed by some researchers early on, but haven't heard much about this recently. How much of our lower detections occurring currently impacted by this?	
for a state lab running these tests what is the expectation in throughput as we move ahead and COVID cases have gone down	Since Ohio is using this data as an early warning system, we work with our utility and lab partners to ensure same day pick up or shipping of the sample, analysis and reporting within a 4-5 day time period.

https://covid19.ncdhhs.gov/dashboard/wastewater-monitoring	
What is your recommendations for collecting samples in septic systems?	ODH has decided to not monitor septic systems due to a variety of challenges including privacy issues, variability in waste strength and flow, when and how to obtain samples, the affect of household chemicals on RNA extraction among others.

Sli.do Questions

June 14th 2021

Amy Kirby presenting

1. Do we have any sense of the fecal shedding rates of vaccinated individuals?
2. Any thoughts on using this process in WW from cruise ships?
3. Are there other agents besides microbe that are monitored: antibiotics and drugs of abuse?
4. I think you said that about 50% of infected individuals shed SARS-CoV-2 in their stools. Can you provide more detail on how is that sufficient?
5. Standardization efforts ahead of fully developed measurement science is referred to harmonization...still quite valuable to help in developing future standards.
6. Has there been any success monitoring homeless shelters that utilize port-o-potties?
7. Do you have thoughts as to which WWS processes are in highest need of standards?
8. What about rural areas where septic tanks are more prevalent? Will these communities be un-represented?

Kate Griffiths presenting

9. @Kate G – When the calibrant material was quantified using dPCR prior to the study, was the quantification performed before or after gamma irradiation?
10. What is the extent of wastewater surveillance across Australia? Is there a national coordinating system?
11. What is the variation in concentrations estimated across the labs (a quantification of the bar plot you showed)?
12. You mentioned that viral capsids may be lysing during irradiation. How much capsid lysing happens in actual wastewater before treatment?
13. How accessible is the standard from NMIA for other labs in other countries?
14. Is this report published?

Beverley Stinson presenting

15. Is this not the national strategy that NWSS is currently coordinating and doing with public health agencies?
16. What metric is being used to normalize dilution events like storm water runoff?
17. What are the different roles for these efforts between the various sectors, such as private industry and federal agencies?

Sampling Session and Panel Discussion

18. For sampling small systems, have you compared passive samplers to composite sampling options?
19. What is the timeline for USGS' sampling document?
20. Is there concern about using non-fecal associated endogenous normalization markers since the primary shedding measured in wastewater is fecal associated?
21. More recent EPA document: https://www.epa.gov/sites/production/files/2017-07/documents/wastewater_sampling306_af.r4.pdf

22. It appears that heat inactivation impacts are inconsistent. Could this be due to method differences compared to the WRF study that found the opposite?
23. When is piggy-backing disease surveillance samples on samples already collected for regulatory monitoring (in primary influent) possible and when not?
24. can the panel comment on the pros/cons of swab-based sampling? Are these reliable enough for use in cost/resource constrained situations?
25. How long wastewater samples can be stored in the lab prior analysis and at which temperature?
26. This seems like testing controls. What about sampling controls?
27. Couldn't weather (dilution) data be accounted for with endogenous controls?
28. how many copies/L is relevant, is there any understanding on this on a broader sense.

June 15th 2021

Testing Methods

29. Is the SARS-CoV-2 target generally found in the liquid phase of wastewater samples, or is it more associated with the solid phase?
30. How do you propose accounting for recovery rate of method? What is range based on enrichment?
31. Can you speak to the sensitivity of the ddPCR mutation assays and cross-reaction with the wildtype that could impact the trend and abundance evaluation?
32. Please describe how sequencing can provide info on relative abundance of VOC cases when fecal shedding titers are not well known and highly variable.
33. How are relative abundances of mutation read copies impacted by primer specificity and sensitivity?
34. With the small sample volume in BioFire arrays, what is the limit of detection in copies per liter of water or gram of solids?
35. Is there any place where we can explore the details of MiSeq sequencing and bioinformatics analyses for variant detection
36. for Dr. Boehm, I apologize if covered in the presentation and I missed it. Do solids had more PCR inhibitors compared to other samples types?
37. can you discuss your experience with performance of matrix controls
38. While matrix spikes and indicators of fecal strength are widely used they do not seem to be being used to adjust the results ? What is the panel's view on this?

Reporting and Analytics

39. Does CDC anticipate releasing open RFPs this year related to data analysis and reporting?
40. Are any of the python/R/java analysis scripts mentioned for NWSS analytics available in public software repositories?
41. @Wiley Is comparison via trends vs absolutes sufficient for your purposes? Or would you like to be able to compare absolutes? What would you need to do so?
42. Have private businesses such as nursing homes had any concerns regarding HIPAA or the impact of testing on business for site specific WW testing?
43. What's the main goal that the HHS contract is trying to achieve outside of NWSS?
44. I'm surprised that wastewater treatment facilities don't already have these sewersheds mapped out. How come that data wasn't needed previously for their work?
45. Is the NWSS data dictionary publicly available?

Role of Standards in Supporting the Use of WWS Data

46. As the outside temperatures get hotter, what are ways to protect the equipment (autosamplers, batteries) from malfunctioning?
47. @Sandra - sometimes public health agencies tell us "How will having this data change what we are already doing?" How do you answer that question?