THE Water Research FOUNDATION®

Wastewater Surveillance of the COVID-19 Genetic Signal in Sewersheds

Recommendations from Global Experts

The Water Research Foundation convened the International Water Research Summit on Environmental Surveillance of COVID-19 Indicators in Sewersheds in response to the overwhelming need for information regarding the distribution and prevalence of COVID-19. The global water sector has mobilized to investigate the use of wastewater surveillance of the genetic signal of SARS CoV-2 as an indicator of the distribution of COVID-19 in communities. This paper presents recommendations of global experts who contributed to the Summit, including potential uses of wastewater surveillance for tracking COVID-19, sampling design, analytical tools, and communication of results to public health decision makers, the public, and other key stakeholders.

The Water Research Foundation (WRF) is a 501(c)(3) nonprofit organization that provides a unified source for One Water research and a strong presence in relationships with partner organizations, government and regulatory agencies, and Congress. WRF conducts research in all areas of drinking water, wastewater, stormwater, and water reuse. The Water Research Foundation's research portfolio is valued at over $700 million.

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For more information, contact:
The Water Research Foundation
1199 North Fairfax Street, Suite 900
Alexandria, VA 22314-1445
P 571.384.2100
www.waterrf.org

6666 West Quincy Avenue
Denver, CO 80235-3098
P 303.347.6100
info@waterrf.org

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Working Group 1:

Co-chairs: Chuck Gerba, University of Arizona; Jim Pletl, Hampton Roads Sanitation District; Daniel Gerrity, Southern Nevada Water Authority; Alice Fulmer, WRF

Participants: Mark Sobsey, University of North Carolina at Chapel Hill; Amy Pickering, Tufts University; Mark Jones, UK Water Industry Research (UKWIR); Katrina Charles, Oxford University; Kelly Hill, Water Research Australia; Christoph Ort, Eawag – Swiss Federal Institute of Aquatic Science and Technology; Matt Burd, New York City Department of Environmental Protection; Kaylyn Patterson, Metropolitan Water Reclamation District of Greater Chicago; Scott Meschke, University of Washington; Amy Kirby, Centers for Disease Control and Prevention (CDC)

Working Group 2:

Co-chairs: Krista Wigginton, University of Michigan; Frederic Been, KWR Water Research Institute; Joan Rose, Michigan State University; Christobel Ferguson, WRF

Participants: Raul Gonzalez, Hampton Roads Sanitation District; Kellogg Schwab, Johns Hopkins University; Scott Meschke, University of Washington; Rosina Girones, University of Barcelona; Kaye Power, Sydney Water; Sudhi Payyappat, Sydney Water; Zia Bukhari, American Water; Farida Bishay, Metro Vancouver; Tiong Gim Aw, Tulane University

Working Group 3:

Co-chairs: Chuck Haas, Drexel University; Doug Yoder, Miami-Dade Water and Sewer; Gertjan Medema, KWR Water Research Institute; Vanessa Speight, University of Sheffield; John Albert, WRF

Participants: Mia Mattioli, CDC; Jay Garland, U.S. Environmental Protection Agency (EPA); Jeff Soller, Soller Environmental LLC; John Norton, Great Lakes Water Authority; Jeff Prevatt, Pima County Regional Wastewater Reclamation Department; Dimitri Katehis, New York City Department of Environmental Protection; Steve Rhode, Massachusetts Water Resources Authority; Ken Williamson, Clean Water Services; Paul Kadota, Metro Vancouver; Reynald Bonnard, Suez Environmental Research Center

Working Group 4:

Co-chairs: Jim McQuarrie, Metro Wastewater Reclamation District; Cathy Bernardino Bailey, Greater Cincinnati Water Works; Dan Deere, Water Futures; Lexie Vean, WRF

Participants: Jeff Oxenford, Rural Community Assistance Partnership; Vince Hill, CDC; Claire Waggoner, California State Water Resources Control Board; Karen Mogus, California State Water Resources Control Board; Chris Impellitteri, EPA; Diane Taniguchi-Dennis, Clean Water Services; Gabriella Rundblad, King's College London; Josef Klinger, Technologiezentrum Wasser (TZW); Bruno Tisserand, Veolia; Stephanie Rinck-Pfeiffer, Global Water Research Coalition; Yvonne Forrest, Houston Water

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Context

EARLY IN THE COVID-19 PANDEMIC, scientific studies demonstrated that the genetic material of the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2)—an enveloped RNA virus—could frequently be detected in the feces of infected individuals. This provided a strong indication that the genetic signal could potentially be detected in wastewater. The global water sector mobilized to investigate the use of wastewater surveillance of the genetic signal of SARS CoV-2 as an indicator of the distribution of COVID-19 in communities. The Water Research Foundation (WRF) noted that many similar efforts were occurring in parallel, and that there was an urgent need for greater coordination to align research to accelerate progress in this critical area. To address this need, WRF held the International Water Research Summit on Environmental Surveillance for the Genetic Signal of SARS-CoV-2 in Sewersheds.

Wastewater surveillance is not a new area of research. It has been critically important in detecting the presence of poliovirus to support the World Health Organization (WHO) program of eradication of poliomyelitis,[1] and has also been used to investigate opioid use in communities. What is new, however, is the exploration of its potential to provide an integrated, community-level indication of the presence of COVID-19. At present, the only effective tools to combat the virus are physical distancing and the use of personal protective equipment (PPE), and the best available indicator of disease prevalence and trends in most countries is confirmation of disease in patients who are sufficiently ill to get tested. However, in many countries, widespread clinical testing is not available, and clinical diagnosis is a lagging indicator of disease. On an exponential growth curve, this can have tragic consequences for communities.

> Wastewater surveillance (also called sewershed surveillance or environmental surveillance) is the monitoring of community health indicators of interest by periodically collecting and analyzing wastewater samples from the sewer network for the presence of chemical or microbiological targets.

Wastewater surveillance of COVID-19 is a rapidly evolving area of research that holds great promise as an early, cost-effective, unbiased community-level indicator of the presence of COVID-19. Our mission at The Water Research Foundation is to advance the science of water to improve the quality of life. In hosting this Summit, we sought to accelerate the development of wastewater surveillance for the detection of the genetic signal of SARS-CoV-2 to help inform the management of resources to combat COVID-19. Summit participants agreed that there is an urgent need to provide credible information to public health authorities and elected officials to help support risk mitigation decisions for the communities they serve.

Summit participants identified key principles and best practices for the collection, analysis, interpretation, and communication of wastewater surveillance results with key audiences, and identified priority research opportunities to rapidly advance wastewater surveillance of the genetic signal of SARS-CoV-2 in sewersheds.

[1] Deshpande J. M., S. J. Shetty, and Z. A. Siddiqui. "Environmental Surveillance System to Track Wild Poliovirus Transmission. Appl. Environ. Microbiol. 2003; 69(5): 2919–2927. doi:10.1128/aem.69.5.2919-2927.2003

Summit Structure

IN PREPARATION FOR THE SUMMIT, The Water Research Foundation invited over 50 leading water experts from utilities, academia, consulting, and government to join working groups focused on sample collection, sample analysis, data interpretation, and communication. In the two weeks preceding the Summit, the working groups assessed the current state of knowledge for wastewater surveillance in sewersheds and identified knowledge gaps and research needs. This paper summarizes the expert opinions provided throughout the course of the Summit, which arose through discussion of cumulative experience and knowledge of the existing scientific literature. Each working group had a specific task:

1. Develop best practices and standardized procedures for the collection and storage of wastewater samples
2. Develop best practices for the use of tools to identify the genetic signal of SARS-CoV-2 in wastewater samples
3. Develop recommended approaches for the use of data on the genetic signal of SARS-CoV-2 to inform trends and estimates of community prevalence
4. Develop strategies to communicate the implications of wastewater surveillance results with the public health community, elected officials, wastewater workers, and the public

The Opening Session, held on April 27, 2020, introduced the key issues in each of these areas and provided an opportunity for the working groups to share their initial progress with the public. Attendees were invited to submit questions that arose during the Opening Session. Over the following two days, the working groups continued their work and considered these questions as they refined their recommendations. During the April 30 Closing Session, the working groups presented guiding principles, recommended best practices, and near-term research opportunities in each of the four areas.

Recommended Use Cases for Environmental Surveillance of the Genetic Signal of SARS-CoV-2 in Sewersheds

Introduction

A CRITICAL FIRST STEP IN the design and implementation of any research study is to outline the intended use of the data being collected. Summit participants developed recommended uses of the data generated from wastewater surveillance of the genetic signal of SARS-CoV-2.

How Can SARS-CoV-2 Wastewater Surveillance Data Be Used?

SUMMIT PARTICIPANTS AGREED THAT WASTEWATER surveillance in sewersheds is a rapidly developing area of research that has the potential to inform public health policy decisions in the context of the current pandemic. Although this area of research is relatively new, a number of groups have demonstrated the proof of concept for COVID-19. Table 1 outlines the recommendations for how wastewater surveillance can be applied, along with the current feasibility of those applications. Additional details, including explanations of the feasibility designations, are provided in the following section.

Use Cases

THE FEASIBILITY OF USE CASES identified in Table 1 represents the potential application of wastewater surveillance data for the SARS-CoV-2 genetic signal based on the information known at this time. Trend analysis, including initial occurrence and changes over time, requires accurate, repeatable analysis of the presence and strength of the SARS-CoV-2 genetic signal. Use of wastewater surveillance to assess increasing trends in occurrence of COVID-19, especially in early detection, has been successfully demonstrated. While the use of wastewater surveillance to identify a decrease in disease occurrence is also technically feasible, it may be complicated by the persistence of fecal shedding of SARS-CoV-2 RNA long after individuals have recovered from infection. In other words, wastewater surveillance is an effective leading indicator of COVID-19 emergence but may be a lagging indicator of subsidence, at least relative to clinical data. This use will benefit from additional research regarding the correlation of the RNA signal to reductions of COVID-19 in the community.

Although the assessment of the prevalence of community infection was identified as the highest priority use case, Summit participants found that there are additional variables that need to be evaluated for wastewater surveillance to be effectively used in such a manner. Two of the most important data gaps discussed included (1) the need for accurate clinical data to quantify the viral shedding rates (RNA copies/g feces) and their dynamics over the course of the infection in symptomatic and asymptomatic individuals, and (2) the difficulty of obtaining accurate clinical and system data to

Table 1. Use Cases for SARS-CoV-2 Wastewater Surveillance Data

General Use Cases	Can Inform	Current Feasibility
Trends/Changes in Occurrence	Early detection of occurrence/reemergence. Tracking the impact of medical and social interventions: A) curves increasing B) curves decreasing	A) ++ B) +
Assessment of Community Prevalence	Tracking disease prevalence in the community. Identification of areas of concern, as well as areas that are not impacted by the virus. Estimation of the level of infection in a community.	+/-
Risk Assessment	Risk to utility workers and those exposed to raw sewage	+/-
Viral Evolution	Source tracking of the virus (emergence of genetic variants and their locations)	-
Key–Current Feasibility: ++ very feasible, + somewhat feasible, +/- may or may not be feasible, - not feasible at this time		

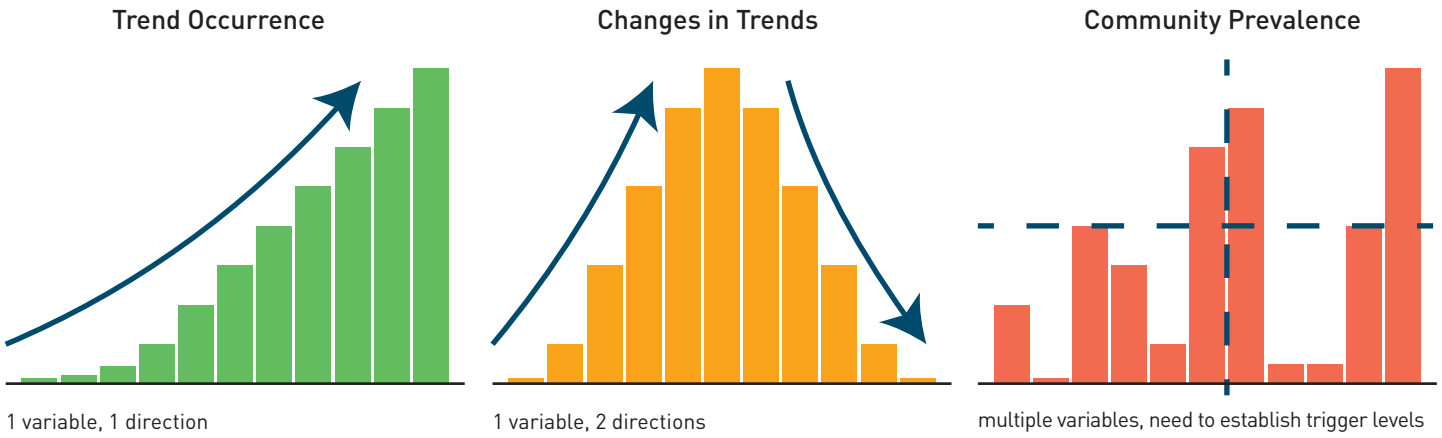


Figure 1. Increasing complexity for wastewater surveillance applications

be able to calculate community prevalence from the viral RNA gene copy number. In many cases, an assessment of community infection may also require a more comprehensive understanding of sewershed characteristics to estimate dilution in the sewer collection system and more accurately relate signal strength back to the population in the community. Still, Summit participants agreed that wastewater surveillance could offer valuable information to public health decision makers on the potential prevalence of community disease. This includes the identification of "hot spots" within a community, provided that an appropriate spatial sampling approach is implemented, as well as identifying areas that are not currently impacted by the virus. Summit participants also identified that intensive spatial sampling of wastewater could be used to provide information on the potential occurrence of COVID-19 in specific confined sub-communities such as correctional facilities, aged care facilities, or schools.

Figure 1 illustrates the increasing complexity of data interpretation required for the application of use cases for wastewater surveillance of COVID-19.

With respect to risk assessment, Summit participants emphasized the importance of occupational health and safety, specifically the protection of in-sewer operators and laboratory staff at wastewater treatment utilities. The participants agreed that general risk assessments for worker safety could be conducted now using information from other well-characterized viruses and exposure routes. However, it was clear that additional information was needed to perform a refined occupational risk assessment for SARS-CoV-2. In particular, critical information needed to quantify potential exposures includes the infectivity of SARS-CoV-2 in waste water matrices and potential differences in exposure to the virus in solids, liquids, or aerosols.

The viral evolution use case aims to characterize and track changes in the viral genome across time, and can also be used to identify spatial components (e.g., the origin of the viral signal) in a particular community.[2] This type of analysis requires sequencing of longer gene sequences than are currently being targeted for wastewater surveillance. Hence, this use application will necessitate collaboration with laboratories with special analytical capability to amplify sufficiently long regions of viral RNA to determine single nucleotide polymorphisms and point mutations that accumulate over time. The majority of laboratories proposing to implement wastewater surveillance for the genetic signal of SARS-CoV-2 are currently using either quantitative polymerase chain reaction (qPCR) or digital droplet PCR, both of which target relatively short sequences of RNA and would be unlikely to include sufficient material to track whole genome changes of the virus. However, as sampling and molecular methods advance, it may be possible for additional laboratories to use wastewater surveillance to help assess the evolution of infectious microorganisms within a community.

What Else Can Wastewater Surveillance Be Used For?

SUMMIT PARTICIPANTS INDICATED THAT ENVIRONMENTAL surveillance can also be conducted for other purposes if different sample types are collected and analyzed. For example, monitoring at different stages of the wastewater treatment process can provide information on the effectiveness of specific steps of the wastewater treatment process in removing or damaging the viral genetic material.

[2] For example, see Nextstrain. 2020. "Genomic Epidemiology of Novel Coronavirus—Global Subsampling." https://nextstrain.org/ncov/global

Communication of Wastewater Surveillance Results with the Public Health Community, Elected Officials, Wastewater Workers, and the Public

THE PUBLIC HEALTH CRISIS ASSOCIATED with the pandemic has created tremendous public and media interest in wastewater surveillance and other effective tools to track the progression of COVID-19 in communities. A focus on effective communication strategies with the public health community, elected officials, wastewater professionals, the media, and the public is a critical component of efforts to utilize wastewater surveillance for tracking trends in disease advance.

Guiding Principles for Effective Communication

WATER PROFESSIONALS HAVE EXTENSIVE EXPERIENCE with communicating complex issues to a variety of audiences. Successful approaches to communicating about wastewater surveillance of the genetic signal of SARS-CoV-2 rely on the same key guiding principles that have been utilized for other critical public health and environmental concerns:

Understand Your Audience

WHO IS YOUR AUDIENCE? WHAT do they care about and what motivates them? The more you understand about the needs and interests of your audience, the better you can tailor your message to reach them.

Build Trust

DO YOU HAVE AN EXISTING relationship with your audience? Have you communicated with them before? Your audience members are more likely to believe what you say if they trust you. Look for opportunities to build trust and form relationships, and do what you say you are going to do.

Define Roles and Present Unified Messaging

CONSIDER WHO ELSE IS COMMUNICATING about the issue at hand; your organization might not be the only entity that plays a role in the issue you need to communicate about. What other organizations and entities in your community are involved in the issue? Consider what they may need to communicate, and how that might differ from what you need to communicate. If possible, coordinate with them in advance of your communication to the public and try to unify your messaging, where applicable. For example, when communicating about wastewater surveillance, wastewater utilities should work with their public health agencies to make sure they are telling the same story. Although their roles in the community and the exact details of their messages might be different, they can present a united front.

Communicate in an Iterative and Evolving Manner

WHEN COMMUNICATING ABOUT EMERGING ISSUES or emergencies, situations tend to evolve quickly. How and what you communicate needs to evolve to match the situation.

Draw from Past Experiences

THE WATER SECTOR, AND UTILITIES in particular, has significant experience communicating during natural disasters and other emergencies, and about constituents of emerging concern. Some recent examples include per- and polyfluoroalkyl substances and lead in drinking water. The water sector can draw on these past experiences to inform how to communicate during the current pandemic.

For example, during the current pandemic, there has been much concern in the press about whether water resource recovery facility workers are safe when dealing with sewage on the job. By leveraging existing knowledge from recent studies on similar viruses, we can be reasonably sure that standard operating procedures and PPE recommendations already in place will keep these workers safe.

Be Transparent

WHEN COMMUNICATING ABOUT ANY ISSUE, be open about what questions you do and do not have answers to. There is a lot known about wastewater surveillance and its ability to show disease prevalence in a community. Even though COVID-19 is a new disease that we need to learn more about, we already have expertise that can help us along the way—we aren't starting at square one.

In terms of wastewater surveillance data, your communications might be framed this way: "We have some wastewater surveillance data, and even though we know a lot about that data, we are

still discovering things about them. We are working hard to determine how best to interpret the data so that our community can derive the largest benefit from this information."

Roles and Responsibilities

AS MENTIONED ABOVE, EACH ORGANIZATION should remember that it has a specific role to play, and that role can shift as a situation evolves. One helpful responsibility matrix is known as a RACI chart, with the letters in the acronym standing for responsible, accountable, consulted, and informed.

In the current pandemic, several parties might be interested in utilizing wastewater surveillance to track disease occurrence, and a RACI chart might show the following roles. The wastewater utility might be responsible and accountable for gathering wastewater samples and sending them to an independent laboratory. The laboratory could be responsible and accountable for analyzing the samples and informing the utility and the local public health agency of those results. The public health agency would then be responsible and accountable for analyzing the results and deciding on a course of action. During all of these activities, the above parties might need to consult with others, such as scientists at universities. And finally, even more parties need to be informed throughout the entire process: government officials and policymakers, the media, and the general public.

Communication Tools

COMMUNICATION PROFESSIONALS HAVE A VARIETY of communication tools at their disposal, but some tools work better for certain audiences. Table 2 shows several options for communication tools along with the audience(s) they might be best suited to.

Table 2. Tailoring Communication Tools Based on Complexity and Audience

AUDIENCE (From General to Specialized)	INFORMATION (From Basic to Complex)			
	Explanatory Graphics	FAQs/Glossary of Terms	Fact Sheets	Scientific/Technical Research Reports
General Public	●	●		
Wastewater Workers	●	●		
Decision Makers/ Elected Officials	●	●	●	
Public Health Agencies	●	●	●	●

Greater Cincinnati Water Works and Lead Service Line Replacement

SEVERAL YEARS AGO, GREATER CINCINNATI Water Works (GCWW) undertook a lead service line replacement program. It was a two-tiered approach: Tier One focused on education and communication, and allowed us to build trust with the public, show them value, and tell the story of what we planned to do. Tier Two focused on creating the actual program to help people replace their lead service lines.

During Tier One, GCWW formed a Speaker's Bureau to attend community council meetings. During those meetings, we framed the issue the community was facing using statements such as: "Yes, we have lead service lines in all 52 neighborhoods of our community," "Yes, we believe we know where the lead service lines are because we have good GIS data," and "Yes, we need to educate you on this issue as well as put together a program to help you remove the lead service lines."

Cathy Bernardino Bailey, Executive Director, Greater Cincinnati Water Works

Once GCWW was confident that the community understood the issue, we told them about new tools we were developing for them: a new website, a dedicated phone line, a service line look-up map, and free water testing.

We also took special care to communicate with the local school systems: "Yes, we know some schools have lead service lines," "Yes, we believe that if we collect samples from your school, we will detect lead," and "Once we detect it, we will help you determine next steps."

From there, we worked with the health department to figure out how we could tackle this issue in the schools and put together a robust plan to share information. After locating all of the lead service lines and testing the samples they collected, we sent a letter to each school that shared the sample results and provided a roadmap of practices they could put in place to mitigate the risk of lead exposure in the schools.

This program's success has largely been due to GCWW keeping communications best practices and guiding principles in mind—understanding our role and our audiences, building trust, and being as transparent as possible.

Sample Plan Design, Sample Collection, and Preservation of Wastewater Samples

IN ORDER FOR ENVIRONMENTAL SURVEILLANCE of the SARS-CoV-2 signal in sewersheds to be useful and informative, the data generated must be comparable across a community or region, and this requires a consistent approach and thorough documentation. Therefore, Summit participants developed the following guiding principles and best practices for the collection of wastewater samples. It is not possible to address every possible scenario that might arise; this is intended only as a starting point for developing a sampling plan for wastewater surveillance of the SARS-CoV-2 signal.

Guiding Principles

RECOMMENDATIONS FOR SAMPLE PLAN DESIGN, sample collection, and sample preservation were developed with the following guiding principles and assumptions in mind:

- Worker safety is a prime consideration in all sampling and sample preparation guidance.
- Data comparability requires consistency in practices and documentation/metadata.
- The proposed guidance assumes collection of a liquid stream from a centralized water resource recovery facility, recognizing that alternative procedures may be needed for special situations (e.g., pit latrines, septic tanks, settled solids).
- The recommendations provided can be adapted/modified to best meet needs for a specific situation.
- The intentions are to not inhibit utility operations during a pandemic and to balance study goals with practical considerations in terms of resources, staff capabilities, storage space, and budget.
- Some best practices for sample collection apply to all use cases, whereas others are use-case-specific.
- The guidance provided can currently be considered a proof of concept sufficient to support practice and research, with the potential to support meta-analysis and retrospective learning in the future.

Wastewater Surveillance Sample Plan Design: General Considerations

RESEARCHERS DEVELOPING SAMPLING PLANS FOR wastewater surveillance of the SARS-CoV-2 signal (or any other chemical or microbiological target) need to consider several factors. First and foremost, the goals of the monitoring program must be clearly established. Summit participants identified four general use cases (Table 1). Each of these uses will drive different decisions in developing wastewater sampling plans. For this reason, it is critical to partner with stakeholders in advance to identify how data will ultimately be used (particularly wastewater utilities, academic researchers, and public health professionals). These initial discussions will help determine how limited resources can be allocated in the most efficient manner possible. In addition to study goals, key considerations include:

- Worker safety
- Wastewater system characteristics
- Sample type, timing, and location
- Sample frequency and duration
- Sample collection, transport, preservation, and storage
- Consistency in sampling methods
- Availability and collection of metadata

Worker Safety

IN ACCORDANCE WITH GUIDANCE FROM the CDC, standard practices associated with water resource recovery facility operations should be sufficient to protect worker safety. Such practices include the use of the personal protective equipment (PPE) normally required when handling untreated wastewater, such as safety gloves and glasses, masks, or face shields. Safety recommendations may vary between handling wastewater and processing samples. Ensuring worker safety may involve consultations with environmental health and safety representatives or biosafety committees at the local institution/agency to develop an acceptable plan for the research effort.

Wastewater System Characteristics

THE COMPLEXITY OF WASTEWATER INFRASTRUCTURE varies widely around the world: from latrines to single-family septic systems, from community systems to conventional wastewater treatment systems with hundreds of miles of pipes, lift stations, and multiple facilities serving millions of people. When determining sites for sample collection for more complex analyses, it is important to coordinate with wastewater utility personnel and local public health officials who can provide context on preferred sampling points. This is particularly true when attempting to determine community prevalence or to identify specific community locations with changing trends in infection. In most cases, sampling of the influent to the water resource recovery facility can provide an

overall integrated estimate of signal strength for the portion of the community served by that facility. In urban areas where large populations are served by complex sewer networks, it may be possible to sample separate sewer trunk mains to investigate and identify locations where a high proportion of COVID-19 cases is suspected.

Sample Type, Timing, and Location

FOR MOST USE CASES, OBTAINING a representative sample is critically important, otherwise artifacts of sample design could skew results and final conclusions. Therefore, in terms of sample type, composite samples are generally preferred over grab samples, though grab samples may be acceptable if composite sampling is not practical. For example, the use of grab samples may make it easier to monitor multiple locations within a service area, including pump stations or hospitals that could act as possible sentinels for infection. Grab sampling may also be the only practical approach to sample smaller and more rural communities, or communities that are not served by central water resource recovery facilities. Additional research will help to inform the degree to which the SARS-CoV-2 genetic signal varies with a grab sample and the sensitivity of the detection methods. It is also presently unclear how significantly the SARS-CoV-2 signal in sewage varies throughout the day. Viruses exhibit different behaviors in response to chemicals and can demonstrate significant retardation in the sewershed, enabling them to be detected over several days.

Longer composite durations (e.g., 24-hour) will presumably give the most representative signal, though alternative durations (e.g., 2-, 4-, or 8-hour composites) may also be adequate. If collecting grab samples, consideration may need to be given to the time of day sampled. It is also important to understand travel time in the sewer and hydraulic retention times within the water resource recovery facility (e.g., primary clarifier) to understand what time of day is reflected by a particular sample. It may be beneficial to try to capture the morning flush, though there is some debate as to whether this would capture the strongest signal or result in a more diluted signal. The impact of daily commuting or tourism activities will need to be considered in transient service areas when attempting to align the SARS-CoV-2 signal with local public health data. More complex systems serving larger communities with high baseline flows may not see significant differences in signal over the course of a day or week. Close collaboration with wastewater utility partners is critical to identify and characterize these issues.

Similarly, it is not yet clear whether the type of composite sample (i.e., time-proportional vs. flow-proportional) impacts the results for wastewater surveillance of the SARS-CoV-2 signal. Flow-proportional composites are generally preferred in most applications, and are often required for compliance monitoring of other constituents. Therefore, flow-proportional composite samples might be readily available at most centralized water resource recovery facilities. Regardless, composite samplers should purge following the collection of each discrete sample to avoid accumulation of solids and sample carryover. Composite samples should also be refrigerated during collection, if possible.

Each use case is likely to dictate the most appropriate sampling location. For example, hot spot monitoring would likely require sampling upstream in the sewershed, while sampling at the water resource recovery facility might be more appropriate for trend analysis or assessment of overall community prevalence. Influent wastewater (post-headworks) is likely to be most representative of the community served by the water resource recovery facility, although primary effluent may also be suitable if influent wastewater is unavailable. Given the propensity for enveloped viruses to partition to solids, sampling downstream of primary effluent is not recommended unless there is a need to evaluate treatment efficacy or effluent water quality.

Assuming a representative liquid stream is the target matrix, samples should be collected from continuously flowing, well-mixed locations to avoid solids accumulation. In the absence of an optimized protocol for a particular location and use case, many laboratories are currently testing 1 L samples, primarily for logistical reasons. The optimum volume of sample for analysis may also depend on the analytical approach being utilized. A 1 L sample can be split into 4 aliquots of 250 mL in order to allow for immediate analysis, archiving for future or retrospective analysis, and quality control purposes (e.g., replication, method development, recovery spikes). If samples are intended for freezing, bottles should not be completely filled to allow room for expansion upon freezing.

Sample Frequency and Duration

SAMPLE PLAN DESIGN MAY DEPEND on the timing of events affecting a local community, and this is particularly true for COVID-19 and other disease outbreaks. For trend analysis, sampling should ideally capture critical points along the epidemiological curve (e.g., emergence, peak, waning). This may require adjustments to sample frequency and duration over time, particularly when considering resource availability and funding limitations.

Assuming a representative composite sample, the SARS-CoV-2 genetic signal is unlikely to vary considerably from day to day, particularly since fecal shedding of SARS-CoV-2 by an infected individual can persist for weeks. Therefore, it may

not be necessary to sample daily if sufficient resources are unavailable. A possible exception might be in the early stages of an outbreak when the case load may increase by orders of magnitude (i.e., 1 case to 10 cases to 100 cases) over a short period of time. Another possible exception would be hot spot monitoring using grab samples. The critical wastewater "pulses" for this use case may be more difficult to capture without increasing the frequency of grab samples. Also, if a critical pulse is captured, concentrations might be higher than with a composite sample collected at a centralized water resource recovery facility due to lower dilution ratios.

In most cases, sampling multiple times per week could be justified, but one sample per week may be sufficient to adequately monitor a local outbreak, at least until a significant change in the signal is detected. As the disease subsides within the community, it may be beneficial to establish a long-term monitoring program to quickly identify, and potentially respond to, secondary waves. Such a program could be extended over longer durations by reducing sample frequency (e.g., every two weeks or even monthly).

Sample Collection, Transport, Preservation, and Storage

PRIOR TO COLLECTION, EQUIPMENT AND bottles will need to be cleaned and prepared. Sample bottles should be new if possible. The next-best alternative would be to use autoclaved bottles or, at a minimum, bottles cleaned with bleach and thoroughly rinsed to avoid the effects of residual bleach on the SARS-CoV-2 genetic signal. If autoclaving, the materials should be verified for autoclave compatibility. No specific preservative is recommended at this stage. It is critically important that all bottles be properly and clearly labeled, particularly when planning for long-term storage.

After collection, samples should be refrigerated during transport, or kept cool with ice if refrigeration is not available. Once at the laboratory, sample temperature should be recorded along with other information as documented in Appendix 1 and in the following section. Samples for analysis should be stored at 4°C and analyzed as soon as possible (up to a maximum of two weeks). If this is not possible samples should immediately be frozen at -80°C, -40°C, or -20°C (in decreasing order of preference). The impact of freezing on the strength of the PCR signal has not been quantified; however, it may be significant. Storage at -80°C and avoidance of freezer defrost cycles will likely reduce the loss of signal compared to storage at -40°C or -20°C. However, practical considerations may dictate the storage temperature, so these are provided as guidelines and not absolute requirements.

To reduce storage space requirements and potentially improve SARS-CoV-2 signal preservation, it may be preferable to store filtered and/or concentrated samples rather than raw sewage samples; potential sample processing options are noted later in this document. Another step that may be required by some institutions is pasteurization of the wastewater to ensure worker safety. If pasteurization is used, it should be noted that the SARS-CoV-2 genetic signal could be adversely impacted, so it is important that this be accounted for during final data analysis.

Consistency in Sampling Methods

A SAMPLING PLAN SHOULD UTILIZE a documented procedure and follow it consistently. Users should ensure that the procedure can be implemented in all cases prior to beginning the study, and staff taking samples should be properly trained. Quality assurance elements such as audits and data traceability reviews should be included in a monitoring program. Consistent and thorough documentation of the sampling protocol and metadata will enable more universal use of the data. Also, when deviating from the sampling plan, changes should be clearly documented.

Critical Metadata

FOR ALL USE CASES, IT is important to compile metadata that can ultimately be used to provide context for sample characterization and to assist data interpretation. This is particularly important in this early phase of rapidly developing knowledge about the source, fate, and persistence of SARS-CoV-2 and its genetic signal. For example, ambient air temperature, sample water temperature, and temperature of the sample as received at the laboratory and during subsequent storage provide indications of the potential for a sample result to be impacted by this parameter. Until the specific survival characteristics of both SARS-CoV-2 and its genetic signal are known, it will not be possible to define an optimized process for sample collection, transport, storage, and analysis for the genetic signal of SARS-CoV-2.

Other water quality parameters, such as wastewater flow, pH, total suspended solids, and ammonia, can provide information about the operational status of the water resource recovery facility. This can be useful in identifying the occurrence of events that may impact the strength of the genetic signal (e.g., dilution due to ingress of stormwater runoff). More comprehensive characterization of flows (e.g., diurnal variability) or composition might be warranted depending on the specific use case targeted by the wastewater surveillance effort.

A field sample collection form is provided in Appendix 1 to facilitate this documentation. In addition to the water quality parameters indicated above, critical information to document during collection includes:

- Date and time the sample is collected
- Type of sample (e.g., grab versus composite)
- If composite sample, type of composite (e.g., time-proportional or flow-proportional) and composite duration
- Name of individual who collected the sample
- Location of sample collection (including streets, locality, and/or landmarks, as appropriate)
- Sample identification number, barcode, or other identifying information consistent with container labeling
- Collection volume and number of aliquots
- Wastewater flow rate at time of collection
- Weather information, particularly whether it rained the previous day or the day of collection
- Type of sewer (separate, combined sewer, or other)
- Additional water quality parameters such as specific UV absorbance that might provide indications of potential matrix inhibition for molecular analyses
- Sample transportation or shipping notes

Once samples are transported or shipped to the lab, additional information to document includes:

- Date and time the sample is received
- Temperature of the sample upon receipt
- Storage temperature
- Any sample processing prior to storage
- Preservation agent (if applicable)

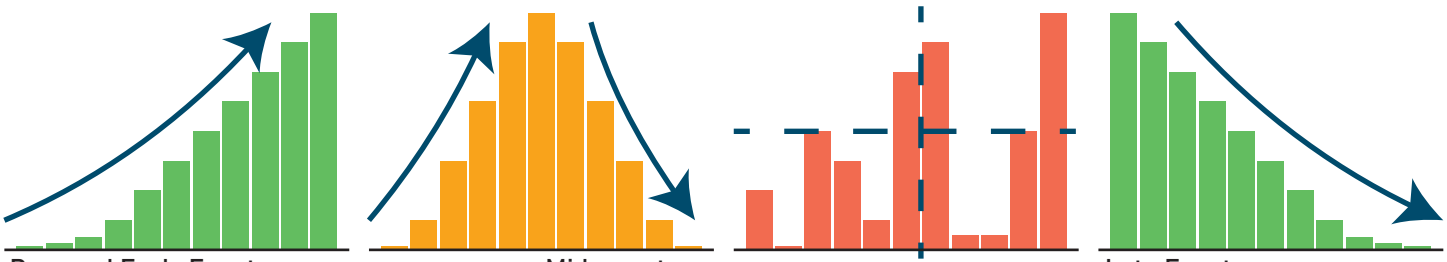
For the use case of community prevalence, it will also be important to know information about community characteristics in the service area where the sample was collected, such as the population served and the presence of commercial, industrial, or residential land uses within the area, in order to correlate results with relevant public health data. For example, nursing homes or hospitals in the service area are likely to have higher numbers of infected individuals, and may represent important point sources of a positive genetic signal. Water use patterns and composition may also change during the day depending on commuting patterns and the presence of commercial or industrial operations, so it will be important to know the residential population, the presence of commercial and/or industrial operations, and daily commuting patterns for the area of interest. Wastewater utilities with existing source control programs may already compile much of this information.

General Use Case: Trends/Changes in Occurrence

WHEN DESIGNING A SAMPLING PLAN to identify trends or changes in occurrence during an outbreak, it is helpful to think of the event in three phases: (1) pre- and early event, (2) mid-event, and (3) late event (Figure 2).

In the pre-event or early event stage, detection of a signal is most important, rather than quantitation. Monitoring of centralized water resource recovery facilities will be most efficient. However, in rural communities where this may not be feasible, sentinel sites like hospitals or aged care facilities could be targeted to detect the first indications of possible infection. Other alternatives, including health clinics and large sporting, concert, and festival events, may provide convenient opportunities for hot spot detection.

For the mid-event phase, quantitation may be more important, and composite samples might be preferred. Major conveyance junctures or plant influent samples are desirable. Downward trends in signal strength can provide confirmation of the effectiveness of management interventions, while new peaks can provide early warning of a re-emergence of infection hot spots.



Pre- and Early Event
- Detection most important
- Sentinel site

Mid-event
- Quantitation important
- Major conveyance junctures or plant influent desirable

Late Event
- Detection again becomes important
- Sentinel sites serve, again, as a metric of occurrence
- Monitor well past last incident of detection

Figure 2. How sampling approach can vary during phases of an infection event

General Use Case: Assessment of Community Prevalence

WHEN USING WASTEWATER SURVEILLANCE TO assess community prevalence, quantitation of the genetic signal of SARS-CoV-2 becomes more important. In this situation, flow-based composite samples could support calculating the percentage of the population shedding genetic material. Sampling throughout a service area will be helpful to pinpoint areas contributing the strongest signal. Increased spatial sampling will require a balance between grab samples and composite samples.

General Use Case: Risk Assessment for Wastewater Exposure

THE RISK ASSESSMENT USE CASE requires information on the presence of infectious virus in fecal material or wastewater. In the absence of this data, this use case relies on the quantification of a genetic signal, rather than simply reporting its presence or absence. This will require characterization of the fate and transport of the genetic signal from the fecal source to the water resource recovery facility, including capture of the exposure from aerosols. In the absence of infectivity data, digital droplet PCR could potentially be used with appropriate assumptions in place. Samples should be collected, stored, and prepared in such a way that they support potential future infectivity studies, noting the potential for pathogen die-off.

General Use Case: Viral Evolution

FOR VIRAL EVOLUTION, COMPOSITE OR grab samples (preferably from a centralized treatment facility) could be used to represent a pooled sample. The analytical approach will be different from routine wastewater surveillance in order to capture longer gene sequences. These data will also need to be interpreted in conjunction with reported case data from public health agencies.

Tools to Quantify the SARS-CoV-2 Genetic Signal in Wastewater Samples

Introduction

ACROSS THE WATER SECTOR, MANY groups have rapidly mobilized to conduct wastewater surveillance for the genetic signal of SARS-CoV-2. In April 2020, The Water Research Foundation conducted a survey of these activities, which collected data from 127 respondents in the water sector. The survey showed that 54% of respondents were already testing for the genetic signal of SARS-CoV-2, and the remaining 46% were developing methods with the intention to commence testing.[3] The majority of respondents were utilizing virus concentration and genetic extraction methods previously intended for the isolation and detection of enteric viruses.

Table 3. Common Methods for the Analysis of Wastewater for the Genetic Signal of SARS-CoV-2

Method/Phase	Method
Primary Concentration	Membrane Filtration
	Centrifugal Ultrafiltration
	Cartridge Filter
	Hollow Fiber Ultrafiltration
	Ultracentrifugation
	Polyethylene Glycol (PEG) Precipitation
	Skim Milk/Filtration
Secondary Concentration	PEG Precipitation
	Skim Milk Flocculation
	Centrifugal Ultrafilter
	Membrane Filter
Genetic Extraction and Assays	Real-time (RT) qPCR SYBR
	RT-qPCR Taqman
	RT-qPCR Other
	Digital Droplet qPCR

Analysis Guiding Principles

ANALYTICAL TOOLS NEED TO BE appropriate for the intended use of the data—some approaches provide presence/absence information and others provide more quantitative results. While the detection of target nucleic acids (RNA for SARS-CoV-2) is a powerful tool, it can be fraught with challenges and potential misinterpretation. Ultimately, molecular methods need to provide reproducible, reliable, and preferably quantitative information.

It is important to carefully consider the complexity of the wastewater matrix when planning analytical approaches for measuring the genetic signal of SARS-CoV-2. Wastewater is frequently a combination of municipal and industrial effluents (which differ significantly from clinical samples). In addition, analysis of wastewater samples requires the careful consideration of potential biosafety hazards associated handling raw wastewater samples in advance of nucleic acid extraction.

Summit participants emphasized the importance of careful evaluation and validation of COVID-19 methods at this early stage of development, particularly given the analysis of a new, enveloped virus. Given the urgency for available information from wastewater surveillance of the SARS-CoV-2 signal, the Summit participants opted against development of a standard method of analysis. The participants recommended parallel investigation of a wide variety of approaches for the concentration of wastewater and the extraction of genetic material in order to rapidly provide information on the effectiveness of different methods for the recovery of the viral RNA signal from wastewater samples.[4]

The methods of analysis typically include sample concentration followed by extraction of the genetic material using molecular techniques to amplify, detect, and quantify the presence of one or more target gene sequences (Table 3).There have been some limited evaluations of the genetic assays available for the detection of SARS-CoV-2, the majority of which have been utilized for clinical specimens that are very different from the wastewater matrix. A comparison by

[3] A paper presenting the full results of the survey is being developed and will be published in the near future.

[4] In June 2020, WRF released a Request for Qualifications (RFQ) to identify a research team to lead a project evaluating existing methods and testing reliability for the genetic signal for SARS-CoV-2 in untreated wastewater.

Table 4. Common Gene Targets for the Detection of SARS-CoV-2

Primer/Probe	Sequence (5' to 3')	Target	# of SARS-CoV-2 Sequences with Mismatches in Primer/ Probe Sites* (n=180)
RdRP_SARSr-Forward	GTGARATGGTCATGTGTGGCGG	RdRp (Corman)	
RdRFP_SARSr-Reverse	CARATGTTAAASACACTATTAGCATA		
RdRP_SARSr-Probe	***FAM***-CAGGTGGAACCTCATCAGGAGATGC-***BHQ1***		
N_Sarbeco_Forward	CACATTGGCACCCGCAATC	N-gene (Corman)	1
N_Sarbeco_Reverse	GAGGAACGAGAAGAGGCTTG		
N_Sarbeco_Probe	***FAM***-ACTTCCTCAAGGAACAACATTGCCA-***BHQ1***		
E_Sarbeco_Forward	ACAGGTACGTTAATAGTTAATAGCGT	E-gene (Corman)	
E_Sarbeco_Reverse	ATATTGCAGCAGTACGCACACA		1
E_Sarbeco_Probe	***FAM***-ACACTAGCCATCCTTACTGCGCTTCG-***BHQ1***		1
nCoV_2019 Forward	CAAATTCTATGGTGGTTGGCACA	RdRp (UW)	
nCoV_2019 Reverse	GGCATGGCTCTATCACATTTAGG		
CoV_Probe	***FAM***-ATAATCCCAACCCATRAG-***MGB***		
CDC N1 Forward	GACCCCAAAATCAGCGAAAT	N-gene (CDC)	1
CDC N1 Reverse	TCTGGTTACTGCCAGTTGAATCTG		
CDC N1 Probe	***FAM***-ACCCCGCATTACGTTTGGTGGACC-***BHQ1***		1
CDC N2 Forward	TTACAAACATTGGCCGCAAA		
CDC N2 Reverse	GCGCGACATTCCGAAGAA		
CDC N2 Probe	***FAM***-ACAATTTGCCCCCAGCGCTTCAG-***BHQ1***		1
CDC N3 Forward	GGGAGCCTTGAATACACCAAAA		8
CDC N3 Reverse	TGTAGCACGATTGCAGCATTG		
CDC N3 Probe	***FAM***-AYCACATTGGCACCCGCAATCCTG-***BHQ1***		1
RNAseP Forward	AGATTTGGACCTGCGAGCG	RNAseP (CDC Internal Control)	N/A
RNAseP Reverse	GAGCGGCTGTCTCCACAAGT		N/A
RNAseP Probe	***FAM***-TTCTGACCTGAAGGCTCTGCGCG-***BHQ1***		N/A

A total of 180 SARS-CoV2 sequences (GISAID) were compared to identify polymorphisms in primer/probe sequences.
*Total number of sequences with SNP in primer/probe region. Blank cells represent 100% homology.
N/A: Not applicable. RNAseP have no homology with SARS-CoV2 sequences.
FAM: 6-carboxyfluorescein
BHQ1: Black Hole Quencher-1
MGB: Minor Grove Binder

Source: Adapted from Nalla et al 2008[5]

5 Nalla, A. K., A. M. Casto, M. -L. W. Huang, G. A. Perchetti, R. Sampoleo, L. Shrestha, Y. Wei, H. Zhu, K. R. Jerome, and A. L. Greninger. 2020. "Comparative Performance of SARS-CoV-2 Detection Assays Using Seven Different Primer-Probe Sets and One Assay Kit." *Journal of Clinical Microbiology*, 58 (6): e00557-20; DOI: 10.1128/JCM.00557-20.

Nalla et al. (2020)[6] evaluated seven different primer/probe sets and one assay kit (Table 4), and determined that the most sensitive assays were those that used the E-gene primer/probe set described by Corman et al. (2020)[7] and the N2 set developed by the CDC.[8] However, all assays tested were found to be highly specific for SARS-CoV-2, with no cross-reactivity with other respiratory viruses regardless of the primer/probe set or kit used. Overall, Nalla et al. (2020) "found assays using the CDC N2 and Corman E-gene primer/probe sets to be particularly sensitive."

However, Summit participants indicated that there is insufficient information to support selection of a single set of primer/probe sets at this stage, or even to nominate a specific gene target. Further information on the sensitivity of the primers using wastewater matrices is essential to discern comparative performance of the assays. There is some evidence to suggest that the N1 primer provides early detection and that the combination of N1 and N2 primers is particularly effective with the E-gene primer sets of Corman et al. (2020). However, significantly more comparative data need to be available showing the effectiveness of the various methods for the analysis of wastewater samples before methods can be assessed for accuracy and precision.

Controls need to be included in each step during initial method validation so that the impact on subsequent steps is clearly understood. Once a method is developed, routine evaluation for sample analysis should be accompanied by controls that estimate overall recovery and detection per batch of samples, rather than for every individual sample, to increase analytical efficiency and reduce costs.

Summit participants emphasized the importance of carefully documenting metadata at each sampling or analytical step to ensure that the appropriate context can be given for subsequent interpretation of results. For example, it is important to report all of the factors in the study that could impact the result, such as heavy rain events that could impact flow and water quality and dilute the genetic signal.

The Summit participants indicated that a quality assurance and quality control (QA/QC) checklist is essential and that the limit of detection/quantification for any assay needs to be established for the sample matrix being analyzed. The sewage matrix is notoriously variable and complex. The presence of industrial and trade waste discharges, for example, can introduce chemicals and constituents that may complicate the extraction and detection of genetic targets. Preparation of sewage samples for detection of gene targets often results in a trade-off between larger volumes providing greater sensitivity of detection and at the same time concentration of inhibitors that may interfere with the detection of the genetic signal.

QA/QC Checklist

DURING METHOD DEVELOPMENT, THE VALIDATION of the assay should include:

- Initial precision and recovery controls
- Matrix spike (periodic assessment)
- Estimate of the limit of detection (lowest level at which a signal can be detected) and limit of quantification (the lowest level of signal that can be quantified with accuracy and precision)
- Reporting of the equivalent volume of sample analyzed

Once an assay has been developed and validated, the minimally acceptable QA/QC standards for every assay include:

- Detection assay controls (positive, negative, and inhibition)
- Ongoing precision recovery
- Reporting of the equivalent volume of sample analyzed
- Periodic matrix control spikes

The primary error in qPCR occurs when the standard curve is generated. Each standard curve should be checked for validity. For some that focus on the detection method only, a valid standard curve may be generated with plasmid DNA or RNA transcripts in purified lab water. This curve is good for trying to understand the performance of the PCR assay in an optimized setting, but will almost certainly underestimate the amount of virus present in an environmental sample. The matrix effect must be understood for any attempt of RT-qPCR to be quantitatively interpreted. This is one of the reasons that

[6] Nalla, A. K., A. M. Casto, M. -L. W. Huang, G. A. Perchetti, R. Sampoleo, L. Shrestha, Y. Wei, H. Zhu, K. R. Jerome, and A. L. Greninger. 2020. "Comparative Performance of SARS-CoV-2 Detection Assays Using Seven Different Primer-Probe Sets and One Assay Kit." *Journal of Clinical Microbiology*, 58 (6): e00557-20; DOI: 10.1128/JCM.00557-20.

[7] Corman, V. M., O. Landt, M. Kaiser, R. Molenkamp, A. Meijer, D. K. W. Chu, T. Bleicker, S. Brünink, J. Schneider, M. L. Schmidt, D. G. J. C. Mulders, B. L. Haagmans, B. van der Veer, S. van den Brink, L. Wijsman, G. Goderski, J. -L. Romette, J. Ellis, M. Zambon, M. Peiris, H. Goossens, C. Reusken, M. P. G. Koopmans, and C. Drosten. 2020. "Detection of 2019 Novel Coronavirus (2019-nCoV) by Real-Time RT-PCR." *Euro Surveill.*, 25 (3). https://doi.org/10.2807/1560-7917.ES.2020.25.3.2000045.

[8] Centers for Disease Control and Prevention. 2020. *2019-Novel Coronavirus (2019-nCoV) Real-Time rRT-PCR Panel Primers and Probes*. https://www.cdc.gov/coronavirus/2019-ncov/downloads/rt-pcr-panel-primer-probes.pdf.

digital droplet PCR approaches have considerable utility for monitoring in the environment, as they do not require the use of a standard curve.

Surrogate Organisms

SURROGATE ORGANISMS CAN BE USED as internal controls to estimate recovery efficiency and as alternatives for experimental evaluation of fate and persistence. The use of surrogate organisms for this latter purpose is particularly relevant when so many characteristics of an organism are unknown, as is the case for SARS-CoV-2. The use of surrogates enables laboratories without biosafety level 3 (BSL3) classification to conduct experiments with similar, less pathogenic organisms, and helps to accelerate the acquisition of new knowledge to inform risk assessments. The Water Research Foundation's survey results indicated that a wide variety of control organisms is currently being used as process controls, including F-specific phages (e.g., MS2), Hepatitis G, mouse hepatitis virus (MHV), murine norovirus, pepper mild mottle virus (PMMoV), and human coronaviruses 229E, OC43 and NL63.

Summit participants indicated that preferred surrogates to represent SARS-CoV-2 (in decreasing order of suitability) include:

- A non-human infectious coronavirus strain (e.g., MHV)
- An attenuated coronavirus (e.g., Bovine Coronavirus vaccine strain)
- A surrogate enveloped virus (e.g., Pseudomonas φ6)
- Armored RNA (expensive and not an enveloped virus)
- An indicator virus that is highly abundant in wastewater (e.g., PMMoV)

Summit participants recommended that molecular research studies conform to the guiding principles and establish that QA/QC checks are working to enable confidence in the sample results. The examination of sequential positive assay results and/or positives from multiple sites is an appropriate strategy to provide confidence that a trend reflects actual changes in the incidence of infection with SARS-CoV-2 in the community (including negative results to indicate downward trends and/or absence of community infection).

Summit participants highlighted the critical importance of engaging early and proactively with health officials regarding the types of analyses to be undertaken and how the results are going to be communicated to ensure successful collaboration, sharing of information, and support of complementary health outcomes.

Research Opportunities to Strengthen Wastewater Surveillance of the SARS-CoV-2 Genetic Signal in Sewersheds

Priorities for Near-Term Research

SUMMIT PARTICIPANTS IDENTIFIED FOUR KEY themes for near-term research opportunities to accelerate progress in this work, as shown in Table 5. These specific areas of research will help to accelerate progress on implementation of the specific use cases.

Methods

INTRA- AND INTERLABORATORY ASSESSMENTS OF the analytical assays are critical to understand the variability, reproducibility, and reliability of the molecular methods currently being used to conduct wastewater surveillance. There is no standard method currently available, and numerous research groups have developed, or are currently developing, sample concentration methods and molecular assays for the detection of the genetic signal of SARS-CoV-2 in wastewater. Many existing sample concentration and analysis methods have been developed for non-enveloped viruses, and hence may not be optimal for the detection and quantification of the SARS-CoV-2 genetic signal. How well do PEG precipitation, ultrafiltration, charged membranes, and skim milk flocculation methods recover the genetic signal of SARS-CoV-2?

An interlaboratory evaluation of methods is necessary to enable comparison of a wide variety of analytical approaches and simultaneously provide an opportunity to evaluate an individual laboratory's ability to produce replicable and robust data. This project would enable laboratories to evaluate their own performance by using their methods (complying with the guiding principles) to analyze "standard" samples and compare their results with other participants. If a sufficient number of laboratories are engaged in the project, then further analysis of the data could answer key research questions identified by the working groups, including:

- How do common sample concentration methods differ in terms of recovery of enveloped viruses?
- What is an appropriate volume and concentration factor of wastewater?
- How do the different molecular assays (e.g., N1 vs. N2) compare in their detection of SARS-CoV-2?
- What method controls are required to have confidence in analytical results?
- Is digital droplet PCR (ddPCR) more accurate and sensitive than qPCR?
- Do we need different sample concentration methods, sample processing methods, and controls for qPCR, ddPCR, culturing, and metagenomics?

This project would effectively accelerate method development and provide a benchmark for laboratories to assess the effectiveness of their methodologies. The outcomes of the project would complement any desktop literature review of potential methods for the detection of enveloped viruses, focusing on the concentration from the wastewater matrix, and would provide potential verification data for the experimental comparison and pros and cons of each approach. Identification and confirmation of the advantages and disadvantages of various method steps would support the development of minimum information for publication of quantitative real-time experiments (MIQE) guidelines. Although MIQE guidelines already exist for qPCR, they need to be adapted to the context of wastewater surveillance and should be expanded to encompass the concentration, purification, and extraction of genetic material and detection steps.

Summit participants also agreed that implementation of wastewater surveillance at a scale to meaningfully inform health practitioners and decision makers will require significantly increased capacity for analysis from utilities and researchers, some of whom will be new to wastewater surveillance. The commercial laboratory sector will be able to support these activities; however, regional centers of excellence will likely be required to mentor local analytical laboratories as they implement the new methods. Video recordings demonstrating methods and analyses were identified as effective means to provide additional support and training, and they have been used successfully in previous laboratory methods projects.[9] Key topics for video support materials could include:

[9] e.g., https://www.youtube.com/watch?v=9d3fUlyQTrA

- Sample collection
- Sample concentration
- Genetic extraction
- Molecular assays
- Data interpretation and communication of results

Shedding Rate and Genetic Signal

THE VIRAL SHEDDING RATE OF SARS-CoV-2 in fecal material (RNA copies/g) for both symptomatic and asymptomatic individuals determines the strength of the genetic signal that can be detected in wastewater. Shedding rates are partly determined by diet, and other demographic considerations that also need to be understood include age, sex, and immunocompetent status of the infected person. Zheng et al. 2020[10] found that there was no difference in shedding rate between mild and severe symptomatic patients; however, men shed for a significantly longer duration than women. These types of assessments are currently being undertaken by the clinical sector.

Table 5. Near-term Research Opportunities to Support Wastewater Surveillance of SARS-CoV-2

Priority	Theme	Specific Research Opportunity	Appropriate Resource Provider	Required for Use Case
High	Methods	Intra- and interlaboratory assessments on sampling regimes and molecular methods	Water Sector	Trends and Community Infection
High	Shedding Rate and Genetic Signal	Effect of wastewater pre-treatment on genetic signal	Water Sector	Trends and Community Infection
High	Shedding Rate and Genetic Signal	Dilution and persistence of the genetic signal in the sewer collection system—Targeted integrated study (in well characterized systems that have good hydraulic models)	Water Sector	Trends and Community Infection
High	Risk	Evaluation of potential for infectious virus in wastewater and generation of aerosols	Water and clinical sector	Risk Assessment
High	Interpretation of Results	Correlations to clinical data for the assessment of community prevalence—How can we leverage wastewater surveillance to provide useful data to the public health stakeholders?	Water and clinical sector	Community Infection
High	Interpretation of Results	Define partnership opportunities	Water and clinical sector with state and federal government	Trends and Community Infection
High	Shedding Rate and Genetic Signal	Viral shedding rate, duration, and demographics in symptomatic and asymptomatic infections (RNA copies per gram of feces)	Clinical sector	Community Infection
Medium	Methods	Impacts of sample collection method (grab vs. composite, duration of composite, time of day)	Water sector	Community infection
Medium	Methods	Distribution of virus (or RNA copies) in liquid and solid phase	Water sector	Community infection
Medium	Interpretation of Results	How to effectively translate COVID-19 research into pandemic preparedness and wastewater surveillance for future needs?	Water and clinical sector with state and federal government	Risk Assessment
Low	Methods	Which spike organism to use for QA/QC purposes?	Water sector	Trends and Community Infection
Low	Methods	Comparative methods review for enveloped viruses—focusing on the concentration from wastewater matrix	Water sector	Trends and Community Infection

[10] Zheng, S., J. Fan, F. Yu, B. Feng, B. Lou, Q. Zou, G. Xie, S. Lin, R. Wang, X. Yang, W. Chen, Q. Wang, D. Zhang, Y. Liu, R. Gong, Z. Ma, S. Lu, Y. Xiao, Y. Gu, J. Zhang, H. Yao, K. Xu, X. Lu, G. Wei, J. Zhou, Q. Fang, H. Cai, Y. Qiu, J. Sheng, Y. Chen, and T. Liang. 2020. "Viral Load Dynamics and Disease Severity in Patients Infected with SARS-CoV-2 in Zhejiang Province, China, January-March 2020: Retrospective Cohort Study." *BMJ*, 369. doi: https://doi.org/10.1136/bmj.m1443.

The strength of the genetic signal is also determined by the concentration of fecal material in sewage and the partitioning of the signal into both the liquid and solid phases. The duration and intensity of the genetic signal within the sewershed are related parameters that, though not well understood at this point, have significant implications for data interpretation. The water sector is well-equipped to undertake this type of study. A short-term, thorough, targeted, integrated assessment using well-characterized sewersheds with different characteristics that have good hydraulic models and geographic information system (GIS) data would improve understanding of the persistence of the genetic signal for wastewater surveillance. Simultaneous collection of appropriate metadata parameters would also enable assessment of site-specific variability. Comparing measurements to determine trends from the same sewershed using the same methods over time will reduce the impact of some of the analytical uncertainty. Comparing across sewersheds requires greater certainty about the replicability of the assays and the quality and consistency of the data.

Persistence of the genetic signal will be impacted by:

- Shedding rate, volume, and duration
- Virus partitioning between solid and liquid phases
- Degradation of the genetic signal in the sewer system
- Impact of sewage constituents, pH, ammonia, total suspended solids, presence of chemicals and industrial discharges, dilution, and saltwater ingress
- Sewage transport at ambient temperatures throughout the collection system, including loss and inactivation through leaks, seepage, sedimentation, biofilms, etc.
- Time taken for sample transport and storage
- Impact of sample pre-treatments, e.g., pasteurization
- Assessments of viral gene copy numbers and correlations to the rate of infection
- Size of the system and population of the community being sampled

Understanding the correlation between the clinical data and viral gene copy numbers identified within a sewershed is critical for making any assessments regarding community prevalence.

Interpretation of Results

DURING THIS RAPID DEVELOPMENT AND independent study phase, Summit participants noted that it is critically important to coordinate and share information to enable rapid assessment of approaches and to avoid duplication of effort. Ideally, a meta-analysis of wastewater surveillance data and projects would identify general approaches that show the greatest promise of supporting the identified use cases, and lead to more rapid refinement of methods.

Performing and reporting on an inter- and intralaboratory assessment of existing and developing methods for the detection of the genetic signal of SARS-CoV-2 will enable decision makers to have increased confidence in the quality of the data collected for wastewater surveillance. Standardization of the measurements to meet minimum standards for analytical quality and for the collection of metadata associated with the samples will facilitate comparison and analysis of the data between laboratories and across geographic areas.

For the detection of trends and the estimation of community prevalence, it will also be necessary to have a quantitative method that is sufficiently well-characterized to allow a determination of how large of an increase or decrease in the genetic signal can be considered a significant change. For the detection of subsequent increases in community rates of infection, it will be necessary to determine how the signal strength might change between initial onset, the increasing part of the curve, and the decreasing part of the curve. Does the genetic signal return to zero after an epidemic, or is there a long tail on the epidemic curve due to persistent shedding from people recovering from infections? If so, will this mask future increases?

To estimate community prevalence, it will be important to interpret the genetic signal in the context of the sample that was collected and analyzed, and the characteristics of the system where and when the sample was collected. Careful documentation of the metadata associated with each sample and the characteristics of the sewershed, including the resident population dynamics, will enable the identification of correlations to other sewage parameters and assist with the identification of trends and prevalence. Comparing measurements to determine trends from the same sewershed using the same validated methods over time will increase confidence in the effectiveness of the approach.

Identifying the intensity and duration of the genetic signal through wastewater surveillance will only be possible if there is a high-quality genetic signal in the community that is being sampled. Setting minimum data requirements will be important. There needs to be a focus on the link between the genetic signal and clinical information on infection prevalence within the community.

Key research needs related to reducing uncertainty in estimating community prevalence include identification of the fecal shedding rates of the virus over the course of the disease and after symptoms have resolved. A better understanding of how fecal shedding rates differ between individuals (based on gender, age, and/or pre-existing conditions) is also needed. Ideally, this would be paired with high-quality estimates of the number of people infected in a targeted, integrated study.

Risk

UNDERSTANDING THE GENE COPY TO infectious virus ratio is important to enable exposure assessments to be evaluated for risk assessment calculations. The relationship between genetic signal strength (copy number) and the presence of infectious viable virus will likely be highly dependent on the matrix environment.

As new information becomes available, it will be important to continually refine guidance related to safe practices associated with conducting wastewater surveillance, including use of PPE, and recommended procedures for the collection, processing, and analysis of samples.

Current studies have demonstrated little success in recovering infectious SARS-CoV-2 virus from stool samples. Therefore, although there is a genetic signal for the presence of the virus, there may not be infectious virions present in wastewater. A key research need is to understand the correlation between infectious virus concentrations with the genetic signal and good estimates of fecal shedding rates.

In order to fully address the potential risks to wastewater operators and technicians, it will be important to understand the potential pathways of exposure for the virus (droplets, contact, fomites), including the role of aerosols and the potential for fecal-oral transmission.

Conclusion

WASTEWATER SURVEILLANCE OF THE GENETIC signal of SARS-CoV-2 holds significant promise for helping to better understand and predict community spread of COVID-19. Research in this area is rapidly evolving. As the sector continues to refine approaches and strengthen the scientific credibility of the information gathered through wastewater surveillance of the genetic signal of SARS-CoV-2, it will be critical to ensure that effective communication occurs between water utilities, researchers, and public health decision makers.

The investment in research to advance wastewater surveillance to detect the genetic signal of SARS-CoV-2 could potentially strengthen the relationships between the water sector, clinical health practitioners, and policy and decision makers, building a platform of information- and knowledge-sharing that could proactively assess other potential threats to human health. As wastewater surveillance becomes more widespread, it could support the tracking of different strains of the virus across geographic areas, providing insight into the spread of COVID-19 and the potential effectiveness of various control measures and intervention strategies. In this context, many partnership opportunities exist.

The response to the current COVID-19 pandemic has been largely reactive. Current efforts have the potential to utilize wastewater surveillance data to develop a more proactive state of pandemic preparedness (Figure 3). The Water Research Foundation will continue to operate at the intersection of all types of water utilities and key stakeholder groups, including public health agencies, academic researchers, and innovators, to unlock opportunities to meet the greatest challenges facing the water sector.

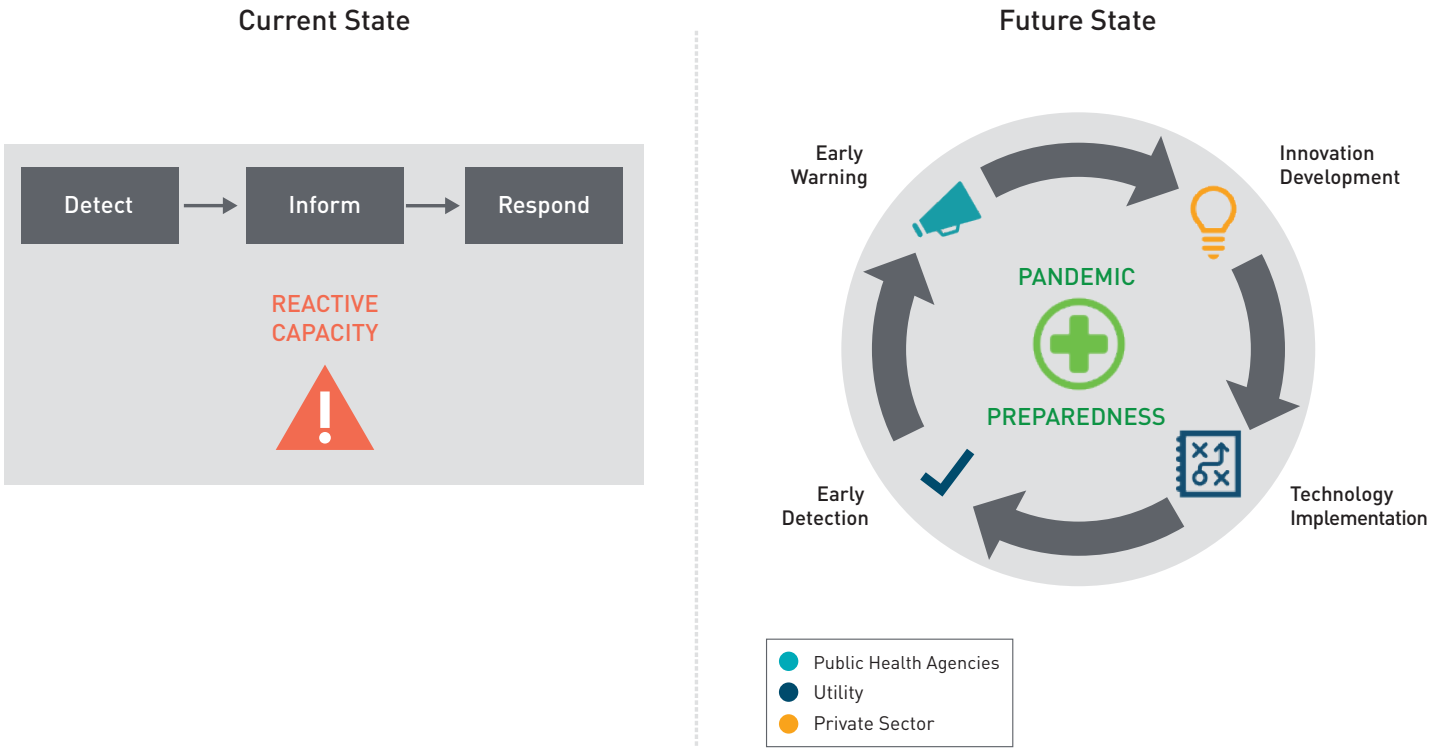


Figure 3. Current state of pandemic response (reactive) vs. potential future state of pandemic preparedness (proactive)

Appendix 1: Best Practices for Collection and Storage of Wastewater Samples to Support Wastewater Surveillance of the COVID-19 Signal in Sewersheds

THIS APPENDIX IS INTENDED TO provide guidance and consistency in the collection of wastewater samples to support wastewater surveillance of the genetic signal of SARS-CoV-2 in sewersheds. Appropriate sample type, location(s), frequency, duration, etc. will depend on study goals and resources.

Safety

IN ACCORDANCE WITH GUIDANCE FROM the CDC, standard practices associated with water resource recovery facility operations should be followed, including the PPE normally required when handling untreated wastewater, such as safety gloves and glasses, masks or face shields. Safety recommendations may vary between handling wastewater and processing samples.

Equipment and Preliminary Activities

CLEAN AND PREPARE ALL SAMPLE equipment, including composite sampler (if applicable) or sample bucket, 4 x 250 mL polycarbonate leak-proof bottles, cooler with ice, documentation/labels, tubing, and other equipment typically used for measurement of water quality and characteristics.

Sample bottles should be new if possible. The next best alternative is autoclaved bottles, or at minimum, bottles cleaned with bleach and thoroughly rinsed.

Sample Collection

ENSURE SAMPLING DRAWS FROM A well-mixed part of the stream. Sampling near the bottom of a stream may introduce more solids into the sample than what is representative of the entire stream. Collect a sample volume of at least 1 L. Samples may be split in 4 aliquots of 250 mL in order to allow enough sample for analysis, quality control, and archival for future. Do not fill bottles completely, in order to prevent issues upon freezing/storage.

Composite samples are preferred over grab samples, though either is acceptable. Composite samplers need to purge following the collection of each discrete sample to preclude accumulation of solids in sampler tubing. Composite samples should be refrigerated during collection and storage.

If possible, measure additional sample characteristics including air temperature, water temperature, wastewater flow rate, pH, total suspended solids, and chlorine residual.

Documentation

LABEL THE CONTAINERS WITH SAMPLE site, date, time, sampler initials, and sample identification number if applicable. Complete the documentation form on the reverse side.

Sample Transport, Storage, and Preservation

SAMPLES SHOULD BE REFRIGERATED DURING transport, or cooled with ice or cold packs if refrigeration is not available. Once at the laboratory, sample temperature should be recorded, along with other information as documented on the reverse side. Samples should be stored at 4°C and processed as soon as possible (up to a maximum of two weeks). If this is not possible, then samples should immediately be stored at -80°C, or alternatively at -40°C or -20°C (in decreasing order of preference). Avoid freezer defrost cycles.

If possible, it is preferable to store filtered samples rather than raw sewage samples. Methods used for solids removal and concentration should be documented (i.e., filtration, centrifugation, precipitation, skim milk approach). Impacts of pasteurization on the strength of the genetic signal are not known at this time.

Field Sample Collection Form

1. Date field sample collected: _____ Time: _____

2. Type of sample (check one): ___ Grab ___ Composite
   If composite, composite type: ___ Flow ___ Time Composite duration: _____

3. Collected by: _____

4. Location (include street, locality, and/or landmarks, as appropriate):
   _____

5. Sample ID number/container labeling: _____

6. Collection volume (in mL): _____ Number of aliquots/bottles: _____

7. Wastewater flow rate: _____

8. Did it rain yesterday? ___ Yes ___ No Did it rain today? ___ Yes ___ No

9. Type of sewer system (check one): ___ Separate ___ Combined

10. Air temperature (in degrees Celsius): _____

11. Sample water characteristics: pH: _____ Temperature (in degrees Celsius): _____
    Total suspended solids: _____ Chlorine residual: _____

12. Sample transportation/shipping notes:
    _____

Other Important Information (If Known)

13. Population served: _____

14. Service area notes: _____

15. Public health data: _____

Storage/Laboratory Information

16. Date sample received: _____ Time: _____

17. Temperature of sample upon receipt (in degrees Celsius): _____

18. Storage temperature (in degrees Celsius): _____

19. Sample processing: _____

20. Preservation agent: _____

Appendix 2: Glossary

BSL3: Biosafety Level 3. A BSL3 laboratory is designed so that operators can safely handle microorganisms that can cause serious or potentially lethal diseases.

Composite Sample: A combined sample consisting of a collection of numerous individual discrete samples taken at regular intervals over a period of time, usually 24 hours.

COVID-19: The infectious disease caused by the newly discovered coronavirus SARS-CoV-2.

Enveloped Virus: A type of virus that has a lipid envelope that fuses with the host cell membrane to allow the virus entry.

Environmental Surveillance: Involves the periodic analysis of wastewater samples for the presence of target material to infer information about the population served by that system—also called sewershed surveillance.

Fecal Shedding: The amount of a particular organism or substance that is excreted in fecal waste, usually reported per gram – also called viral shedding.

Genetic Signal: The strength of the genetic signal reflects the amount of RNA copies found in a test sample.

Grab Sample: A sample collected at one location at one point in time.

Infectious: An organism that is both viable and able to infect a host.

Inhibition: The presence of substances that impede the PCR reaction and can prevent detection of the target, causing a false negative result.

Negative Control: A test that is run with a batch of samples that is known not to include the target, and which therefore must produce a negative result as an assurance that there has been no cross-contamination.

Non-enveloped Virus: A type of virus that lacks an outer "envelope" layer of protection.

Nucleic Acid Extraction: The process of extracting an organism's nuclear material to examine the genetic code, frequently used to identify and detect microorganisms in the environment.

qPCR: Quantitative polymerase chain reaction, a method that multiplies target sequences of genetic material allowing small amounts to be detected

PEG: Polyethylene glycol (as in the precipitation method commonly used to concentrate viruses)

Positive Control: A test that is run with a batch of samples that includes the target that you are looking for, and which must therefore produce a positive result as an assurance that the test has been appropriately conducted.

PPE: Personal protective equipment such as safety gloves and glasses, masks, or face shields

RNA: Ribonucleic acid (SARS-CoV-2 is a positive sense, single-stranded RNA virus).

SARS-CoV-2: Severe Acute Respiratory Syndrome Coronavirus 2; the name of the virus causing the disease COVID-19.

Sewershed: The area served by sewer pipelines collecting wastewater that is delivered to a single endpoint.

Sewershed Monitoring: Taking samples and conducting analyses at different locations in wastewater systems to detect the presence of target organisms and/or compounds of interest.