FREQUENTLY ASKED QUESTIONS ABOUT PUBLIC HEALTH WASTEWATER MONITORING

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THE BASICS

What is public health wastewater monitoring and why is it useful for COVID-19 and other public health targets?

Public health wastewater monitoring involves systematic sample collection, processing, and analysis followed by interpretation of wastewater data to inform public health practice. Wastewater and other forms of environmental surveillance (e.g., monitoring of air, soil, or high-touch surfaces) can be valuable complements to more traditional public health tools such as syndromic surveillance and clinical testing.

Before 2020, public health wastewater monitoring had already been in practice for decades (e.g., to facilitate the eradication of polio), but the COVID-19 pandemic further magnified its value. People infected by SARS-CoV-2 shed viral material in their bodily fluids, including feces, which allows for the detection of SARS-CoV-2 RNA in wastewater. Similarly, detection of other RNA or DNA targets (e.g., Mpox virus DNA) is possible due to shedding by infected individuals. People who are asymptomatic or only mildly symptomatic may not know they are infected or seek clinical testing by a healthcare provider. Also, widespread access to at-home rapid antigen testing for COVID-19 eventually led to reduced clinical testing and reporting of COVID-19 cases, thereby creating a public health surveillance 'blind spot'. Because public health wastewater monitoring does not require people to seek testing from a healthcare provider and can be representative of large portions of a community, it gives public health officials a way to detect and characterize infection trends in a non-invasive, timely, and cost-effective way. In North America, community-level public health wastewater monitoring can provide coverage for the approximately 80% of U.S. households (<u>US EPA, 2008</u>) and 90% of Canadian households served by municipal wastewater collection systems (<u>Statistics Canada, 2020</u>).

What exactly is detected in the wastewater for public health wastewater monitoring programs?

For COVID-19, public health wastewater monitoring programs target genetic material, specifically the RNA of <u>SARS-CoV-2</u>, which is the virus that infects people and causes COVID-19. In many cases, public health wastewater monitoring programs search for the same fragments of the SARS-CoV-2 genome targeted by clinical tests that rely on <u>polymerase chain</u> reaction (PCR). By targeting specific fragments, or genes, PCR-based methods can be highly specific to the target of interest (e.g., SARS-CoV-2) and quantitative, thereby allowing for the determination of wastewater concentrations. Raw data are often reported as "gene copies" (or "gc") per volume of wastewater analyzed (gc/L). It is relatively easy to adapt PCR-based methods to other targets of public health interest, including Mpox virus DNA and antibiotic resistance (AR) genes. In fact, many public health wastewater monitoring programs

simultaneously monitor for the RNA of pepper mild mottle virus (PMMoV), which is an indicator of human fecal contamination. See the answer to the FAQ on <u>What is normalization and how</u> <u>is it used by NWSS</u>? for more information on the use of PMMoV to normalize wastewater data. The use of wastewater concentrations to estimate infection totals is still an evolving science, but trends in wastewater concentrations have successfully indicated when community transmission is increasing, decreasing, or plateauing.

Early in the COVID-19 pandemic, public health wastewater monitoring programs typically focused only on detecting and quantifying SARS-CoV-2 RNA. As the pandemic progressed and the virus mutated, it became increasingly important to also characterize the variants circulating in the community. In response, some public health wastewater monitoring programs targeted different fragments of the SARS-CoV-2 genome that could distinguish <u>variants of interest or concern</u>. Some also implemented a more flexible genome sequencing approach to identify important changes in the SARS-CoV-2 RNA present in wastewater samples. See the answer to the FAQ on <u>What is next generation sequencing and how does it work?</u> for more information on genome sequencing.

It's important to keep in mind that detection of nucleic acid fragments (DNA or RNA) in wastewater doesn't necessarily mean the wastewater contains infective forms of those targets. For example, as explained in the answer to the FAQ on *What is the latest PPE guidance for handling wastewater during an outbreak?* and described in detail in <u>Sobsey (2021)</u>, researchers have not detected infective SARS-CoV-2 in untreated wastewater, despite detecting relatively high concentrations of SARS-CoV-2 RNA. However, influent wastewater does contain infective forms of other pathogens so it is important to take proper precautions when implementing public health wastewater monitoring. Public health wastewater monitoring programs may eventually incorporate culture-based methods for detection of some targets of public health interest, including *Candida auris* and other antibiotic resistant pathogens. In contrast with PCR-based methods, culture-based methods can detect and quantify viable and potentially infective forms of the pathogens of interest.

Where are wastewater samples taken?

Wastewater samples for surveillance efforts can be taken from many different types of locations, such as the influent to a water resource recovery facility (WRRF), a pumping station or manhole within the WRRF collection system, a manhole or building cleanout that captures sanitary flows from a specific facility (such as a university dormitory, prison, nursing home, or homeless shelter), or even community pit latrines. The U.S. Environmental Protection Agency provides examples of COVID-19 public health wastewater monitoring at many of these types of locations in their <u>Compendium of US Wastewater Surveillance to Support COVID-19 Public Health</u> <u>Response</u>. In most cases, wastewater utilities play a role in sampling program design, if not also in sample collection, as described in the answer to the FAQ on <u>What is the wastewater</u> <u>utility's role in NWSS and public health wastewater monitoring generally?</u>.

Who should participate in public health wastewater monitoring?

Everyone! The more utilities—whether small, medium, or large—participate in NWSS, the more useful the wastewater information will be for protecting public health. For more information on getting started, please see:

- How do I start working with my health department?
- What are ELC and NWSS Wastewater Coordinators and how do I get in touch with them?
- How can I obtain ELC funding through my health department?
- How much does it cost to participate in a public health wastewater monitoring program?

What do public health officials do with wastewater data?

Communities—whether cities, neighborhoods, or facilities—that participate in public health wastewater monitoring use the data in several ways, and ultimately this decision is up to the relevant public health entity. These public health entities would not have access to this critically important information were it not for the participation of wastewater utilities in sampling design and sample collection efforts.

Examples of how communities have used wastewater SARS-CoV-2 trends to track COVID-19 include:

- Confirm other public health surveillance data— Early in the pandemic, wastewater data were used to complement clinical testing data. As the pandemic progressed and the frequency of clinical testing decreased while the use of at-home testing increased, wastewater became one of the primary sources of public health surveillance data for COVID-19. Today, wastewater data provide a more timely indication of changes in disease community transmission than other available public health surveillance datasets, such as COVID-19 hospitalizations and emergency department visits.
- Alert healthcare facilities and the public Wastewater data can be used to provide an early warning to both healthcare providers and the public about the potential for an increase in COVID-19 transmission in a community. This early warning enables informed decisions at the facility (such as about infection control procedures and treatment therapies) and individual (about masking and other behaviors) levels.
- Indicate presence of variants of concern Wastewater may be the first indication that a <u>variant of concern</u> is present in a community. For example, <u>wastewater in the</u> <u>California cities of Merced and Sacramento tested positive for Omicron</u> before it showed up in nasal swabs in those counties.
- Direct resources where they are needed Health departments allocate a wide variety financial and non-financial resources to address public health needs in their jurisdictions. (See <u>this infographic</u> for more information on health departments). These resources can include communication and messaging efforts, testing supplies, vaccines, and others—all of which can be informed by wastewater data. Wastewater data can be especially helpful for informing communication to local health departments and the public.

Health departments have started using wastewater data for other pathogen targets in ways similar to those outlined above for COVID-19.

Please see the following dashboards for examples of how health departments and their partners are using wastewater to answer these questions:

- <u>California Department of Public Health</u>
- City of Tempe, Arizona
- <u>Colorado Department of Public Health and Environment</u>
- Florida Department of Health
- Houston Health Department
- Illinois Department of Public Health
- Indiana Department of Health
- Maryland Department of the Environment
- Massachusetts Department of Public Health
- Michigan Department of Environment, Great Lakes and Energy with Michigan
 Department of Health and Human Services
- <u>Missouri Department of Health and Senior Services</u>
- Nevada Empower Program
- New York State Department of Health
- North Carolina Department of Health and Human Services
- Ohio Department of Health
- Oregon Health Authority
- <u>Utah Department of Environmental Quality</u>
- Virginia Department of Health

What should I tell my elected officials, utility leadership, and/or the public about public health wastewater monitoring?

Here are five key points about public health wastewater monitoring:

- Health departments want wastewater data. Public health wastewater monitoring is a cost-effective and efficient method for assessing the health status of a community, regardless of access to healthcare or testing resources. It serves as a valuable tool for health departments, helping them validate other public health surveillance data derived from clinical tests, hospitalizations, emergency department visits, and mortality records. This information aids in resource allocation, enhances public awareness, and enables timely alerts to healthcare facilities. For further insights into how public health officials leverage wastewater data, refer to <u>"What do public health officials do with wastewater data?"</u>
- Public health wastewater monitoring is here to stay. Testing sewage for bacterial and viral pathogens has a long history, dating to the 1930s in the U.S., although the use of this tool saw rapid expansion during the COVID-19 pandemic. There is substantial support for continued use of public health wastewater monitoring, as evidenced by the conclusions of the 2023 Consensus Study Report on Wastewater-based Disease Surveillance for Public Health Action from the National Academies of Sciences, Engineering, and Medicine and the rapid growth of the <u>CDC's National Wastewater</u> Surveillance System (NWSS) since its establishment in September 2020. NWSS now covers 120 million people in the U.S. through sampling at more than 1,200 sites.
- **Public health wastewater monitoring is not just about COVID-19.** Many health departments in the U.S., with guidance from the CDC, are already testing for influenza, respiratory syncytial virus, and other pathogens in wastewater. The CDC NWSS team has announced their plans for expansion of the <u>National Wastewater Surveillance</u> <u>System core pathogen panel</u>. And many other pathogen targets are already being analyzed by <u>WastewaterSCAN</u> and other programs, as described in this <u>infographic</u>.
- Public health wastewater monitoring supports a wastewater utility's mission to

protect public health and can complement pretreatment and water reuse programs. While providing samples for public health surveillance programs doesn't necessarily help with permit compliance, using wastewater to track community health is an incredibly popular topic with the public. This <u>bill stuffer</u> may be helpful for informing rate payers about how a utility's participation in public health wastewater monitoring is just another way they work to protect their community's health. Public health wastewater monitoring can also more directly complement wastewater operations in two ways. First, tracking pathogen targets in untreated influent can be important for understanding inputs into a water reuse treatment process, if applicable. Second, taking samples from the collection system can support efforts to identify unknown discharges from industrial users.

Health departments strive to minimize the burden on wastewater utility staff. A wastewater utility participating in a public health wastewater monitoring program is generally expected to help health departments identify sampling locations, collect and package samples for transport, and provide sample-related metadata (see this infographic for more information). These activities require staff time, but should be limited to about 20 to 40 minutes per sampling event (as described in this infographic). Standard practice is for health department partners to provide sample kits, sample shipment or courier services, and sample analysis at no cost to the utility. In addition, some health departments offer stipends to their utility partners, as described in this memo, and will work with the utility to minimize staff burden as much as possible.

Find more resources on our <u>Utility Roadmap</u> page or in our <u>Infographic Library</u>.

What is the National Wastewater Surveillance System (NWSS)?

The <u>National Wastewater Surveillance System (NWSS</u>) seeks to understand the spread of infectious disease in a community, specifically through strategic monitoring of wastewater throughout the U.S. NWSS was initiated by the U.S. Centers for Disease Control and Prevention (CDC) and the U.S. Department of Health and Human Services (HHS), in collaboration with <u>multiple federal agencies</u>. While the initial focus was on COVID-19, NWSS was designed to be a long-term addition to CDC's overall public health surveillance infrastructure and has already been expanded to include new targets (e.g., Mpox virus) analytical capabilities (e.g., <u>next generation sequencing</u> to characterize circulating variants). NWSS is a flexible system that can be adapted to changing public health priorities, such as tracking <u>antimicrobial resistance</u>, guiding emergency response, responding to emerging infections, and preparing for bioterrorism and future pandemics.

The CDC maintains a <u>NWSS</u> website, which includes guidance on <u>sampling strategy</u>, <u>testing</u> <u>methods</u>, <u>data reporting and analytics</u>, and <u>public health interpretation and use of public health</u> <u>wastewater monitoring data</u>.

What is the wastewater utility's role in NWSS and public health wastewater monitoring generally?

The wastewater utility plays four important potential roles in any NWSS public health wastewater monitoring program: (1) identification and collaboration with appropriate program partners, ideally including local, regional, and/or state health department partners; (2) development of a wastewater sampling plan in coordination with these partners; (3) ongoing collection of wastewater samples, including sample packaging for transport to the analytical laboratory as well as administrative tasks related to the completion of the sample chain of

custody; and (4) documentation of sample-related data per the sampling plan, which can include and sewershed service area, number of people served by the utility, treatment processes, types of samples collected (hourly or flow-weighted composites), and wastewater flow rates during sample collection. For some public health wastewater monitoring programs, utilities play a larger role by performing the analysis of the wastewater samples or paying for these analyses to be performed by an outside, commercial laboratory. This is not typical, however, and is not expected for NWSS efforts.

See this infographic for more information.

DEFINITIONS

How is "public health wastewater monitoring" different than "wastewater-based epidemiology"?

In practice, public health wastewater monitoring and wastewater-based epidemiology are often used interchangeably. However, epidemiology specifically refers to "<u>the study of</u> <u>the distribution and determinants of health-related states or events in specified populations, and the application of this study to the control of health problems</u>". Public health wastewater monitoring, specifically the monitoring of targets of interest in wastewater, can help identify where a health problem is occurring (the distribution) but does not explain why (the determinants) or how to control the problem. As such, it is only one component of epidemiology, albeit a critically important component.

Why is the word "surveillance" sometimes used?

The use of the term "surveillance" is consistent with the fact that wastewater surveillance is another example of "public health surveillance", or the "<u>ongoing, systematic collection, analysis,</u> <u>and interpretation of health-related data essential to planning, implementation, and evaluation of</u> <u>public health practice</u>". Moreover, surveillance is integrated into the name of the "National Wastewater Surveillance System" and is helpful to distinguish wastewater testing for tracking community health from wastewater testing for routine monitoring and compliance. If surveillance is associated with an overly negative connotation, the following alternative terms could be considered:

- Community wastewater health assessment/monitoring
- Public health wastewater monitoring
- Wastewater indicators of community health (WICH)
- Wastewater indicators of public health (WIPH)
- Wastewater intelligence
- Wastewater monitoring for infectious diseases
- Wastewater public health monitoring
- Wastewater-based infectious disease surveillance

THE FUTURE

What will NWSS be expanding to after COVID?

NWSS was designed to inform diverse disease surveillance strategies, including:

- **Core** surveillance for endemic or common diseases, such as flu or antibiotic resistance, to provide regular and consistent updates at minimal cost
- **Emergency** surveillance of sporadic but expected diseases (e.g., shigellosis or polio) for rapid response during outbreaks (e.g., Mpox virus), emergencies, and natural disasters to provide acute, timely updates
- **Pandemic preparedness** surveillance to evaluate the potential for rare, unexpected diseases such as typhoid or Ebola

Currently, NWSS shares data on its dashboard for influenza A, mpox virus, respiratory syncytial virus, and SARS-CoV-2. In partnership with the <u>NWSS Centers of Excellence</u>, the CDC will continue to assess priority targets to expand the list of core targets. Examples of priority targets may include:

- Pathogen targets
 - Respiratory
 - SARS-CoV-2
 - Influenza A and B
 - Respiratory syncytial virus
 - Enteric
 - Adenovirus 40/41
 - Norovirus GI and GII
 - Shiga-toxin-producing coli
 - Campylobacter
 - Emerging pathogens
 - Candida auris
 - Mpox (non-variola orthopox)
 - Antibiotic resistance genes
 - Carbapenemases
 - Extended-spectrum beta-lactamases (ESBL)
 - Colistin resistance
 - Vancomycin resistance
 - Normalization and process controls
 - Pepper mild mottle virus (PMMoV)
 - CrAssphage
 - Bovine coronavirus

Some of these pathogen targets are already being analyzed as part of state and local programs. And the <u>national testing contract</u> includes testing for influenza A and B and respiratory syncytial virus, in addition to SARS-CoV-2 and Mpox virus. Health departments will communicate with their partners which of these targets or others they intend to include in their own programs.

COLLABORATIONS

What types of settings can be monitored using public health wastewater monitoring?

During the pandemic, more than 4,000 COVID-19 public health wastewater monitoring programs have cropped up around the globe, as illustrated on <u>this map from the University of</u> <u>California Merced</u>. These include monitoring efforts in many different settings, such as:

- Cities and regions (<u>Gerrity et al. 2020</u>; <u>Gonzalez et al. 2020</u>; <u>Medema et al.</u> <u>2020</u>; <u>Hopkins et al. 2023</u>; <u>Brighton et al. 2024</u>)
- Neighborhoods (Spurbeck et al. 2021; Li et al. 2024; Starke et al. 2024)
- Schools, including university/college campuses and pre-K through 12 schools (<u>Bivins</u> and Bibby 2021; <u>Harris-Lovett et al. 2021</u>; Wolken et al. 2023)
- Correctional facilities (Kennedy et al. 2023; Klevens et al. 2023)
- Airplanes, airports, and cruise ships (Ahmed et al. 2020; Morfino et al. 2023)
- Shelters and unhoused encampments (Harrington et al. 2024)
- Healthcare facilities, including hospitals, long-term care facilities, nursing homes and others (Acosta et al., 2021; Keck et al. 2023)

Another good summary of public health wastewater monitoring programs is available via the <u>W-SPHERE data repository</u>, which aims to serve as a global data center and provide public health wastewater monitoring data in reusable formats.

How does CDC work with health departments for NWSS?

CDC works directly with health departments to implement NWSS. In this case, a "health department" can be a state health agency, a city health department, a tribal health entity, or a territorial health organization. CDC funds these health departments for NWSS activities through the Epidemiology and Laboratory Capacity for Prevention and Control of Emerging Infectious Diseases Cooperative Agreement (ELC) program (this program is described more in the answer to the *How does ELC funding work?* FAQ). CDC is also currently funding NWSS activities through a long-term commercial wastewater analysis contract to support communities that want to participate in NWSS but do not have access to analytical services. See more information about the commercial testing contract in the answer to *What is the NWSS commercial testing contract?* FAQ. Similar to the Utilities Community of Practice (CoP), there is also a Health Department CoP in which the CDC works collaboratively with public health partners to share best practices and advancements in the use of public health wastewater monitoring data.

As of early 2024, 57 jurisdictions (49 states [all but ND], five cities [Chicago, Houston, Philadelphia, New York, and Washington DC], one county (Los Angeles) and two territories [Guam and Puerto Rico]) have received funding from CDC for NWSS-related public health wastewater monitoring.

How do I start working with my health department?

Start by reaching out to the NWSS Wastewater Coordinator (see <u>What are ELC and NWSS</u> <u>Wastewater Coordinators and how do I get in touch with them?</u>) in your relevant jurisdiction and expressing your interest. Most of these coordinators are in state health departments. If your jurisdiction is not already funded for NWSS activities, reach out instead to the ELC coordinator or even the <u>CDC NWSS team</u>. More information is provided in the answer to <u>How does ELC</u> <u>funding work?</u>

FUNDING

What is the NWSS national testing contract and who is the current contractor?

Since 2020, the U.S. Health and Human Services (HHS), including the CDC, has contracted with commercial laboratories to provide wastewater testing for SARS-CoV-2 and, more recently, Mpox virus. Because they enable utilities to participate in NWSS without having to pay for sample kits or analyses, these contracts are designed to complement the efforts of state and local health departments by filling gaps in wastewater testing coverage. All contracts awarded so far, as well as any amendments to those contracts, are shown in the table at this link. The most recent commercial testing contract is between the CDC and Verily Life Sciences LLC (Verily) that provides twice-weekly testing for:

- SARS-CoV-2 (quantitative levels and sequencing)
- Mpox (non-clade-specific and clade 1 quantitative levels and sequencing)
- Flu A (quantitative levels)
- Flu B (quantitative levels)
- RSV (quantitative levels)

Note that WastewaterSCAN, for which Verily is a partner, is entirely separate from the NWSS national testing contract. WastewaterSCAN is not funded by the CDC, although they do share their data with CDC via upload to DCIPHER. Also, any free testing for pathogen targets being offered by Biobot (such as through the "Biobot Network") is also separate from the NWSS national testing contract. Biobot does not share their data with CDC via upload to DCIPHER.

How does ELC funding work?

To facilitate participation in NWSS, CDC provides funding to health departments (HDs) for public health wastewater monitoring activities through the Epidemiology and Laboratory Capacity for Prevention and Control of Emerging Infectious Diseases Cooperative Agreement (ELC). The ELC "provides financial support and technical assistance to the nation's HDs to support their efforts to detect, prevent, and respond to emerging infectious diseases". ELC funding is available to HDs in <u>64 US jurisdictions</u>, including:

- All 50 states
- Five large cities: Chicago, Houston, New York City, Philadelphia, and Washington DC
- One county: Los Angeles County
- Eight territories: American Samoa, Federated States of Micronesia, Guam, Marshall Islands, Northern Mariana Islands, Palau, Puerto Rico, and US Virgin Islands

In support of the COVID-19 response, the ELC has awarded more t\$100 million to support the implementation of NWSS programs through the ELC.

ELC funds can be used to pay for autosamplers and other items that support utility participation in public health wastewater monitoring programs. Utilities should coordinate directly with their relevant health department to find out more. If you're not sure who to reach out to at your health department, please <u>email us</u> at the Water Environment Federation and we'll put you in touch with your Wastewater Coordinator, or reach out to the appropriate contact shown <u>on this map</u>.

What are the NWSS Centers of Excellence?

In August 2022, <u>NWSS</u> established Centers of Excellence (CoEs) to support the continued development of public health wastewater monitoring. Since then, NWSS has funded six CoEs to serve as leaders in public health wastewater monitoring implementation and coordination:

- California, led by the California Department of Public Health
- Colorado, led by the <u>Colorado Department of Public Health and Environment</u> in partnership with the University of Denver
- <u>Houston</u>, led by the Houston Health Department in partnership with Houston Public Works and Rice University
- New York, led by the New York State Department of Health in partnership with Syracuse University
- North Carolina, led by the North Carolina Department of Health and Human Services in partnership with the University of North Carolina-Chapel Hill
- Wisconsin, led by the Wisconsin State Laboratory of Hygiene

More information about the NWSS CoE partners, regions, and activities can be found on <u>CDC's</u><u>NWSS website</u>.

The NWSS CoEs are distinct from the 16 <u>Centers of Excellence for Wastewater Epidemiology</u> <u>established by Ceres Nanosciences</u>, which were supported by an \$8.2 million award from the National Institutes of Health Rapid Acceleration of Diagnostics (RADx) Initiative.

What are ELC and NWSS Wastewater Coordinators and how do I get in touch with them?

Each state, city, and territorial health department eligible for ELC funding has an ELC Coordinator who manages applications for annual ELC funding for programs including public health wastewater monitoring and other aspects of HD operation. Moreover, any state, city, or territorial health department that has already received ELC funding for NWSS activities either has hired, or is in the process of hiring, a NWSS Wastewater Coordinator (who may also be known as a program lead or program manager or another title) who coordinates with wastewater utilities for public health wastewater monitoring activities. Please use <u>this map</u> to identify the contact information for your health department partner, or <u>email</u> or <u>call</u> WEF for help getting connected.

Are wastewater utilities eligible for ELC funding?

Although utilities are not eligible to receive ELC funds directly, you are encouraged to work with your health department to determine whether their ELC funds can be used to support the purchase of autosamplers or other items that support utility participation in public health wastewater monitoring programs.

If you did not have a chance to coordinate with your health department yet, it is worth reaching out to the appropriate coordinator in your home jurisdiction to express your interest in participating in NWSS sampling. Please <u>email</u> or <u>call</u> WEF for the name of your ELC Coordinator or Wastewater Coordinator, as applicable, if you're not sure who to reach out to. It may be helpful for you to develop a budgetary estimate of costs associated with labor, supplies, and even equipment – such as autosamplers or flow metering equipment – needed to effectively implement public health wastewater monitoring sampling for the expected duration of

the public health wastewater monitoring program. Expand the answer to the FAQ below for more guidance on estimating costs.

How much does it cost to participate in a public health wastewater monitoring program?

The scope of your program will dictate which costs you'll incur when participating in a public health wastewater monitoring program as a utility. Below, we've provided some detailed information on different cost categories, as well as links to specific budgeting resources. Have you participated in, or led, a wastewater program? If so, please share your costs anonymously through our feedback form so we can improve the information provided here.

Cost Categories

It's helpful to break out costs into different categories, not all of which will necessarily apply to every public health wastewater monitoring program, as follows: labor, equipment, supplies, analytical, and miscellaneous. Considerations for each of these categories are offered below. Note that the discussion below is organized around the assumption that your wastewater utility is *not* responsible for running the SARS-CoV-2 analysis in your laboratory.

Labor

Labor costs include all personnel time related to sample collection and project administration. For sample collection, personnel may be needed to: walk or drive to sampling locations; retrieve samples from autosamplers, manholes etc.; reset/manage/program autosamplers; package samples and fill out paperwork (the "chain of custody") for transport/shipment to the analytical laboratory; and/or courier/drop off samples for shipment/transport. Project administration labor may include time spent on data management and project management (*e.g.*, procurement, team coordination).

For a utility collecting wastewater samples from one water resource recovery facility influent sampling point where an autosampler is already set up and programmed to collect a daily 24-hour composite sample, the labor costs will be minimal: one staff member will need to spend about 15 to 45 minutes per sample collected. For a utility collecting samples from locations within the collection system (*e.g.*, manholes), a team of two staff members will need to spend about an hour per site for sample collection and autosampler management, not including travel time.

For budgeting purposes, sampling labor costs should be developed by estimating the total number of hours required for sample collection and using total, burdened labor rates. Project administration costs can be estimated as a percentage of total project costs (*e.g.*, 10 to 15%).

Equipment

One-time equipment purchases required to start up a public health wastewater monitoring program may include: autosamplers (if used for composite samples); peristaltic pumps (if used for grab samples); bucket dippers (if used for grab samples); flow meters to measure flow; meters (handheld or benchtop) to measure pH, temperature, and electrical conductivity; coolers to manage sample transport; certain personal protective equipment (PPE) items requiring a one-time purchase (*e.g.*, rubber boots); and/or any other items needed to equip the sampling team. In addition, it may be necessary to purchase a refrigerator to store samples before shipment.

Although costs for each of these items will vary by location and equipment specifics, here are some typical values:

- Autosampler: about \$8,000 to \$9,500 for a compact, portable, battery-powered model including extra tubing, bottle arrays, strainers and an extra battery depending on the setting
- Peristaltic pump: about \$1,500 to \$1,750 with extra battery and tubing
- Bucket dipper: about \$200 to \$250 for 1-liter sampler
- Flow meter: will vary substantially, depending on installation specifics
- Handheld meter to measure sample pH, temperature, and electrical conductivity: about \$800 to \$1,000
- Cooler (reusable): about \$40 to \$60 for 48-quart with hinged lid
- Rubber boots: about \$120 to \$150 for a pair of steel-toe, knee-height rubber boots with oil-resistant soles
- Refrigerator: about \$4,000 for a laboratory refrigerator, although a \$300 model may be suitable as well

Supplies (or consumables)

Supplies will need to be purchased on an ongoing basis to support sampling activities. Depending on the scope of the public health wastewater monitoring program, these supplies may include:

- Disposable PPE, such as gloves, goggles, and coveralls (for safety during sample collection)
- Hand sanitizer (for use in the field after sample collection)
- Sample bottles (for sample transportation/shipment)
- Ice packs (for sample transportation/shipment)
- Boxes/coolers (for sample transportation/shipment)
- Bags of ice (if needed for portable autosamplers)
- Paper towels (for clean up during sampling activities)
- Garbage bags (for clean up during sampling activities)
- Traffic cones (for traffic management during sampling activities, g., at manhole in street)

Note that most commercial laboratories provide sample bottles, boxes (or small, disposable coolers), and ice packs at no additional cost (beyond the fee associated with the SARS-CoV-2 analysis. All sample supplies, sample shipment or courier services, and analytical costs are covered by the NWSS national testing contract.

Analytical

Although paying for laboratory analysis is often outside the scope of a wastewater utility's participation in public health wastewater monitoring activities, it is helpful to understand which analytical costs might be part of a public health wastewater monitoring program. These include:

- PCR for:
 - Target quantification (e.g., SARS-CoV-2, Mpox virus, influenza, etc.)
 - Quantification of <u>a fecal indicator</u> (such as pepper mild mottle virus [PMMoV], crAssphage, F+ prophage) that can be used to normalize data
 - Measurement of gene targets specific to variants of interest or concern
- Next generation sequencing for variant identification (less common and is more complex and expensive but can be extremely valuable for public health partners)

The PCR cost, including quantification of both SARS-CoV-2 and a fecal indicator (but not targets specific to variants of interest or concern) ranges from about \$300 to \$400 per sample, including sample bottles, ice packs, boxes/small coolers, shipping, and analysis. Each additional target (e.g., MPox virus, influenza, etc.) and approach (e.g., next generation sequencing) may incur additional costs.

Miscellaneous

In addition to labor, equipment, supplies, and analytical costs, there may be miscellaneous costs associated with public health wastewater monitoring programs, such as:

- Sample shipment, if not included in the analytical cost
- Vehicle mileage
- Police or traffic detail for sampling locations in the collection system in high-traffic areas

Note that the sample shipment costs, and the logistics involved in shipping samples, can be substantial. We recommend that you reach out to the shipping service early in the development of your public health wastewater monitoring program to plan ahead. We also recommend including a project contingency in your program budget to cover unforeseen costs.

Budget Development Resources

The following resources offer more specific guidance on how to develop a public health wastewater monitoring program budget for your utility:

- <u>Online calculator</u> (opens new window)
- <u>Downloadable spreadsheet template</u> (Utilities CoP membership is required to access this file: join today!)

What information should I be ready to share when I talk to my Wastewater Coordinator?

Your Wastewater Coordinator would be interested in knowing:

- The basics of your wastewater treatment system, such as population served, collection system area boundaries, number and size (i.e., flow rate) of your treatment facilities, and details of the samples that can be collected [e.g., location (collection system vs. influent vs. primary solids), type (grab vs. composite), frequency, and duration];
- Whether you have performed any public health wastewater monitoring activities in the past;
- Number of operators/staff on-site and whether your staff are able to support sample collection, administration, and shipping activities;
- Availability of autosamplers, if needed for program; and
- Whether you need any funding to facilitate your participation in NWSS sampling activities and, if so, what your funding request is;
- How involved you'd like to be in data use and reporting (e.g., receiving periodic data updates, participating in public health wastewater monitoring team meetings, etc.).

Ideally, this will start a discussion with the Wastewater Coordinator about how to best implement a public health wastewater monitoring program.

OCCUPATIONAL HEALTH

What is the latest PPE guidance for handling wastewater during an outbreak?

Because wastewater contains a broad range of pathogens, handling wastewater carries inherent risk of exposure to biological hazards that can result in illness and disease. Therefore, protecting wastewater workers from occupational infections through engineering and administrative controls (e.g., task-specific job safety assessments), hygiene precautions, training, and personal protective equipment (PPE) is necessary to mitigate risks of pathogen exposure on a routine basis. During outbreaks, the concentrations of certain pathogens in wastewater may increase, but regular practices to protect workers from pathogen exposure would still be protective. <u>A Blue-Ribbon Panel</u> convened by <u>WEF</u> in April 2020 describes best practices in protecting wastewater workers from biological hazards.

In the case of SARS-CoV-2, the U.S. EPA recommends workers follow routine practices normally used when handling untreated wastewater and "[n]o additional COVID-19–specific protections are recommended for employees involved in wastewater management operations, including those at wastewater treatment facilities". These routine practices include engineering and administrative controls, hygiene precautions, specific safe work practices, and the use of PPE. According to the CDC, this PPE should include the following:

Goggles

- Protective face mask or splash-proof face shield
- Liquid-repellent coveralls
- Waterproof gloves
- Rubber boots

Workers should also follow basic hygiene practices and wash hands with soap and water *immediately after* removing PPE. <u>CDC also recommends</u> that wastewater workers be trained on disease prevention practices and consider vaccinations for tetanus, typhoid fever, polio, Hepatitis A, and Hepatitis B.

It is important to note that detecting SARS-CoV-2 RNA in wastewater is not the same as detecting infectious SARS-CoV-2. As described in <u>Ahmed et al. (2022)</u>, researchers have not been able to detect infectious SARS-CoV-2 in untreated wastewater, even though infectious SARS-CoV-2 can sometimes (but not always) be isolated from the feces of infected patients. Just because the SARS-CoV-2 RNA can be present at high concentrations does not mean that exposure to the wastewater can result in COVID-19 infections.

Additional guidance specific to other pathogens is available in this series of fact sheets.

SAMPLE COLLECTION AND SHIPMENT

What is the best way to collect samples?

There are many different approaches to sample collection and any sampling plan needs to establish where to sample [water resource recovery facility (WRRF) influent or out in the collection system], how often to sample, what to sample (untreated wastewater vs. primary solids), how to sample (grab vs. composite), and how to safely collect, store and ship samples. Each of these topics is addressed in detail on the <u>NWSS website</u>. Utility-specific information is important to consider when designing a sampling program. This includes information on the location of industrial dischargers in the collection system, whether the WRRF utilizes influent flow equalization, and the location of chemical (such as chlorine or ferric chloride) addition at the WRRF influent. It's important to find representative sampling locations that are practical and safe to access but are not confounded by chemical addition or other factors (e.g., dead zones).

It is also important to be consistent over time with the sampling approach, especially with respect to the "where", "what", and "how" of sample collection.

What is passive sampling?

Passive sampling of wastewater for COVID-19 is an alternative to traditional sampling techniques (composites and grabs) and involves deploying an absorbent material in the wastewater flow for a specified period of time to allow SARS-CoV-2 to associate with the material. At the end of the sample collection period, the material is transported to a laboratory for processing and analysis. Passive sampling can be done using cotton gauze, cotton cheesecloth, cellulose sponges, electronegative filters, or tampons (Lugali *et al.* 2016; Hayes *et al.* 2021; Bivins *et al.* 2021) which can be placed inside a housing (such as a 3D-printed cage [Rafiee *et al.* 2021] or a colander [Schang *et al.* 2021]) or hung from a string (*e.g.*, a Moore swab [Sikorski and Levine 2020]). Studies suggest that passive sampling can give comparable results to composite and grab samples, while being cheaper and easier to deploy (Rafiee *et al.* 2021; Schang *et al.* 2021). However, a key limitation of passive sampling is that it does not yield quantitative results (gene copies per unit volume of wastewater) like composite and grab samples can.

It should be noted that passive sampling is not used for NWSS programs.

How do I program my autosampler?

This will depend on the make and model of your autosampler, and the best thing to do is consult the user manual for your particular autosampler. Here are links to the user manuals for some commonly used autosamplers:

- Endress+Hauser Liquistation CSF48
- Hach AS950
- Teledyne/ISCO 5800
- Teledyne/ISCO GLS
- YSI Xylem WS Series

What is needed to package and ship samples?

Although the specific packaging needs will depend on the laboratory to which wastewater samples are being sent, packaging and shipping supplies may include:

- Chain of custody form (see <u>this example form</u> that can be used as a template; Utilities CoP membership is required to access this file: <u>join</u> today!)
- Sample bottle(s) (plastic, usually 50 to 200 mL)
- Biohazard bag in which to place sample bottle(s), sometimes with an absorbent pad; usually two bags are used so that the sample can be double-bagged (one plastic bag inside the other), with the absorbent pad placed with the sample bottle(s) in the inner plastic bag
- Ice pack(s) to keep the samples cool during shipment or transport (note: if you are out of ice packs or don't have any, you can consider putting water in a Ziploc bag or a sample bottle and freezing ahead of time to create a makeshift ice pack) or ice (placed inside a plastic bag)
- Small Styrofoam cooler or bubble mailer with an outer cardboard shipping box or a reusable cooler that can be sealed and shipped on its own

How quickly does the sample need to arrive at the lab for SARS-CoV-2 (or other virus)

results to be valid?

It's preferable to send wastewater samples priority overnight so that they arrive at the lab the day after sample collection. It's also preferable to keep the wastewater sample cool (close to 4°C). Nucleic acid signals in wastewater degrade over time, and the rate of degradation differs across pathogen targets and depends on the temperature at which the sample is stored. For SARS-CoV-2, <u>Markt et al. (2021)</u> found little degradation of the SARS-CoV-2 signal in wastewater samples stored at 4°C for 9 days. <u>Brunet et al. (2023)</u> compared the persistence of different SARS-CoV-2 RNA biomarkers (N1, N2, E, ORF1ab, and RdRp genes) and found that all biomarkers were consistently detected after six days when stored at 4°C, but complete signal loss occurred after 13 days at 12°C and 6 days at 20°C. Freezing samples is not recommended for wastewater samples: it's possible that freeze/thaw cycles can lead to substantial loss of viral signal from wastewater.

What are the most common challenges associated with sample collection?

The initial identification of sampling locations—if those locations are not at the influent to a water resource recovery facility (WRRF)—can be challenging due to uncertainties related to the extent of the sewershed captured by the sampling location and access and safety considerations associated with sampling at manholes or pumping stations. The management of sample transportation can also be challenging because it requires tracking coolers and bottles and taking time to get the samples where they need to be, whether at a shipping (FedEx, UPS) drop-off location or a nearby laboratory. For rural areas, a shipping drop-off location may be a long drive from the WRRF. The management of portable autosamplers, especially if deployed in manholes, is another challenge due to the potential for clogged intake tubing, dead batteries, and programming issues.

What are some strategies for managing shipment logistics?

Although a laboratory -- whether a commercial laboratory or a public health laboratory -- will work with its partner utilities to finalize sample shipment logistics, some strategies employed to facilitate the successful, ongoing shipment of wastewater samples include:

- Preprinting a stack of chain of custody (CoC) forms with most information already filled in; see this example form that can be used as a template.
- Pre-freezing enough ice packs for an entire week or two of sample collection.
- Setting up recurring pickups with the shipping company. Here are links with more information on how to set regular pickups with common shippers, including <u>DHL</u>, <u>FedEx</u>, and <u>UPS</u>.
- Using a sample courier to transport samples either to a nearby laboratory or to a shipment drop-off location. Local public health and environmental laboratories are likely to have the name and contact information for local courier options.

Is wastewater considered a hazardous material?

When shipping wastewater domestically within the US, sewage is not considered a hazardous material. Hazardous materials are regulated under the <u>Resource Conservation and Recovery</u> <u>Act (RCRA)</u>, passed in 1976. <u>According to the EPA</u>, RCRA "... created the framework for America's hazardous and non-hazardous waste management programs. Materials regulated by RCRA are known as 'solid wastes'. Only materials that meet the definition of solid waste under RCRA can be classified as hazardous wastes, which are subject to additional regulation." <u>Domestic Sewage and Mixtures of Domestic Sewage</u> are specifically excluded from

the definition of solid waste under $40 \text{ CFR } \underline{\$261.4(a)(1)}$ and, therefore, aren't considered hazardous wastes under RCRA.

That said, many programs are designating wastewater samples as "UN3373, Biological substances, Category B" for shipment. Category B substances are infectious substances, including those transported for diagnostic or investigational purposes (such as for public health wastewater monitoring), that are <u>"not in a form generally capable of causing permanent</u> <u>disability or life-threatening or fatal disease in otherwise healthy humans or animals when</u> <u>exposure to it occurs"</u>. The Association of Public Health Laboratory's (APHL's) <u>Packing and</u> <u>Shipping Guidance for Biological Substances, Category B Specimens</u> offers instructions on how to package Category B substances for shipment.

What metadata are required for NWSS samples?

As described on <u>the CDC's NWSS website</u>, and specifically <u>in the NWSS metadata file</u>, health departments are required to include the following sample collection metadata when they upload SARS-CoV-2 wastewater data to the DCIPHER database. Utilities can provide most of this information once at the outset of the program but would need to provide some items for each sample collection event.

- **reporting_jurisdiction** The CDC Epidemiology and Laboratory Capacity (ELC) jurisdiction, most frequently a state, reporting these data (2-letter abbreviation)
- county_names 5-digit numeric FIPS codes of all counties and county equivalents served by this sampling site
- zipcode ZIP code in which the sampling site is located
- **population_served** Estimated number of persons served by this sampling site (i.e., served by this wastewater treatment plant or, if 'sample_location' is "upstream", then by this upstream location)
- **sample_location** Sample collection location in the wastewater system, whether at a wastewater treatment plant (or other community level treatment infrastructure such as community-scale septic) or upstream in the wastewater system
- **sample_location_specify** If 'sample_location' is "upstream", specify the collection location in the wastewater system; an arbitrary name may be used if you do not wish to disclose the real name
- institution_type If the sample represents wastewater from a single institution, facility, or building, specify the institution type; otherwise, specify "not institution specific"
- wwtp_name The name of the Wastewater Treatment Plant (WWTP) to which this wastewater flows. If this wastewater does not flow to a WWTP, specify an identifiable name for the septic or other treatment system to which this wastewater flows. An arbitrary name may be used if you do not wish to disclose the real name.
- wwtp_jurisdiction State, DC, US territory, or Freely Associated State jurisdiction name (2-letter abbreviation) in which the wastewater treatment plant provided in 'wwtp_name' is located
- **capacity_mgd** Wastewater treatment plant design capacity. This should be the capacity for which the plant is permitted.
- **sample_type** Type of sample collected, whether grab or composite. If composite, also provide the duration of sampling and type of composite, as listed in the Value Set (e.g., "24-hr flow-weighted composite"). A grab sample is defined as an individual sample collected without compositing or adding other samples, regardless of whether the sample matrix is liquid wastewater or sludge
- sample_matrix Wastewater matrix from which the sample was collected

- **sample_collect_date** The date of sample collection; for composite samples, specify the date on which sample collection *began*; note that this may be different than samples are dated for permit compliance: permits may require that sample dates correspond to the date sample collection *ends*
- **sample_collect_time** The local time of sample collection; for composite samples, specify the time at which sample collection *began*
- flow_rate Wastewater volumetric flow rate at the sample collection location over the 24-hr period during which the sample was collected. If only an instantaneous flow measurement is available, it may be reported in units of million gallons per day.

ANALYSIS

What is the most common analytical technique used for public health wastewater monitoring?

Public health wastewater monitoring can encompass a wide range of targets, including high risk substances (e.g., illicit drugs), pharmaceuticals and personal care products, counterfeit medications, population biomarkers, industrial chemicals, antimicrobial resistance genes, viruses, and other pathogens. The analytical technique(s) used for a given public health wastewater monitoring program, then, will depend on the type of substance being tracked. For high risk substances, pharmaceuticals, population biomarkers, and industrial chemicals, mass <u>spectrometry</u> (MS) approaches are often used due to the method's high sensitivity and selectivity for chemical targets. For pathogens, such as SARS-CoV-2, and antimicrobial resistance genes, <u>PCR</u> is the most common analytical technique. See the answer to <u>What</u> <u>exactly is PCR and how does it work?</u> for more information.

What exactly is PCR and how does it work?

Polymerase chain rection (PCR) and reverse transcriptase (RT)-PCR (for the detection of RNA targets) are molecular methods suitable for the detection of low levels of nucleic acids specific to public health targets (e.g., SARS-CoV-2) in wastewater samples. PCR involves amplification of specific gene sequences followed by detection of the amplified gene. Quantitative PCR (qPCR), droplet digital PCR (ddPCR), and digital PCR (dPCR) are the most widely used PCR methods for pathogen monitoring (<u>Girones et al. 2010</u>) because they allow for detection **and** quantification of a specific target. To date, PCR-based methods have been used for quantification of a wide range of viral pathogens (<u>Blinkova et al. 2009</u>; <u>Hellmer et al. 2014</u>; <u>Boehm et al. 2023</u>), including SARS-CoV-2 (<u>Alygizakis et al. 2021</u>), a diverse range of antibiotic resistance genes (<u>Karkman et al. 2016</u>; <u>Pazda et al. 2019</u>), *Candida auris* (<u>Barber et al. 2023</u>) in wastewater.

Relative to qPCR, ddPCR and dPCR may reduce interferences from PCR inhibitory substances (<u>Sedlak, Kuypers, and Jerome 2014</u>) and be particularly useful for quantifying low levels of pathogens in wastewater (<u>Jahne et al. 2020</u>). Another benefit of ddPCR and dPCR is their ability for absolute quantification, rather than depending on separate standard curves for relative quantification.

What is next generation sequencing and how does it work?

Sequencing, in general, is the "process of determining the nucleic acid sequence – the order of the nucleotides" in DNA (or RNA). Next generation sequencing (NGS), specifically, involves sequencing millions of nucleic acid fragments in parallel and then using <u>bioinformatics</u> to piece

together the fragments. As a result, NGS is beneficial in public health wastewater monitoring for identifying specific pathogen strains or variants (those with slightly differing nucleic acid sequences) responsible for outbreaks (<u>Gwinn et al. 2019</u>). NGS was crucial in identifying the emergence and spread of SARS-CoV-2 variants of concern (e.g., Alpha, Delta, Omicron) during the COVID-19 pandemic.

It may be helpful to think of NGS in terms of an analogy to music. Think of a band as a type of pathogen (e.g., the SARS-CoV-2 virus), the band's songs as different SARS-CoV-2 variants, the complete notes for each song as the complete SARS-CoV-2 genome, and different measures (short sequences of notes) in a song as nucleic acid fragments, or genes. NGS is the process of rapidly reading those measures (genes), and specifically differences in those measures (mutations), to differentiate between songs (variants) and understand how the band (virus) is changing over time. Even if all the measures for "Hey Jude" aren't present, the detection of characteristic measures (e.g., the chorus) may give sufficient confidence to identify the song as "Hey Jude" (the Omicron variant) by the Beatles (SARS-CoV-2). Detecting critical mutations within a genome not only indicates that a pathogen is changing over time but can also provide information about changes in its virulence, ability to evade immunity, and resistance to medical treatment. Thus, NGS can be a valuable complement to PCR-based detection and quantification for both public health surveillance (i.e., clinical testing) and public health wastewater monitoring.

INTERPRETING RESULTS

Who is supposed to interpret the wastewater results?

Ultimately, health departments—a state or county public health agency, a city health department, a tribal health entity, or a territorial health organization—are responsible for interpreting and using public health wastewater monitoring data to inform public health decisions. For NWSS, much of this interpretation is based on the data analytics embedded into CDC's DCIPHER platform. Expand the answer to the next FAQ below to find out more about NWSS data.

However, some public health wastewater monitoring programs opt to make their data public and downloadable, which gives the opportunity for school districts, universities, or other entities to use wastewater data to help inform their own public health responses. Here are some examples of how health departments are sharing wastewater data with the public:

- California Department of Public Health
- <u>City of Tempe, Arizona</u>
- Colorado Department of Public Health and Environment
- Florida Department of Health
- Houston Health Department
- Illinois Department of Public Health
- Indiana Department of Health
- Maryland Department of the Environment
- Massachusetts Department of Public Health
- Michigan Department of Environment, Great Lakes and Energy with Michigan Department of Health and Human Services
- Missouri Department of Health and Senior Services

- Nevada Empower Program
- New York State Department of Health
- North Carolina Department of Health and Human Services
- Ohio Department of Health
- Oregon Health Authority
- <u>Utah Department of Environmental Quality</u>
- <u>Virginia Department of Health</u>

Where does NWSS data go and how can the public get access?

NWSS public health wastewater monitoring data—whether from ELC-funded efforts or from the commercial wastewater testing contract—are uploaded to a shared portal (known as the Data Collation and Integration for Public Health Event Response, or DCIPHER for short) where they are evaluated by the NWSS analytic engine. The analyzed wastewater data are disseminated to the health departments via DCIPHER for public health action. While DCIPHER access is limited to health departments, NWSS data are available to the public via download from the NWSS dashboard or by making a public data request. To download data from the NWSS dashboard, you can either download metric-specific data by expanding the data table under the map or you can download all the historical metric data or all the historical concentration data. To make a public data request for historical wastewater concentration data and COVID incidence data. email the NWSS team with the subject line 'NWSS public data request.' Before you receive the data back from the NWSS team, you'll be required to sign a data use agreement. Consistent with typical privacy protections related to public health data, some NWSS data are excluded from NWSS data download and the public data request. This includes data from sewersheds with fewer than 3,000 people; data from sewersheds with missing population estimates; facility- or institution-specific data; sewersheds being monitored but without data being submitted to DCIPHER; data with quality issues; and data from Tribal sewersheds, unless they opt in to sharing their data. It is also possible for utilities to request that their data be excluded from public data requests and the NWSS dashboard. Utilities can do this by communicating their preference to their health department partner or by emailing the NWSS team.

The table below summarizes which types of NWSS data are available to the public through either a data request to the NWSS team or by downloading from the NWSS dashboard.

Type of Data	Released via Public Data Request to NWSS Team	Released via Download from COVID Data Tracker	Not Released Publicly
Anonymized Sample Location Name (WRRF Name or Other Non- Facility/Institution Sampling Location Name)			
County Name and FIPS Code			
Wastewater SARS-CoV-2 Concentrations			
Wastewater SARS-CoV-2 Metrics, Including Trends, Percentiles, And Percent Change			
COVID-19 Case Incidence (Number of New Reported Cases) For Locations With 5 Or More Cases			
Sewersheds With < 3,000 People or No Population Data			V
Facility- Or Institution-Specific Data			V
Sewershed Shapefiles			V
COVID-19 Case Incidence (Number of New Reported Cases) For Locations With Fewer Than 5 Cases			V
Laboratory Protocols			
Any Data for A Utility That Has Opted Out of Public Data Release			V

What is normalization and how is it used by NWSS and ELC-funded jurisdictions?

For COVID-19, public health wastewater monitoring involves the quantification of nucleic acid sequences ("genes") that are specific to the <u>SARS-CoV-2</u> virus using PCR-based methods (see What is PCR and how does it work?), with results reported as the number of gene copies (gc) per volume of wastewater (or, for some public health wastewater monitoring programs, the number of gene copies per mass of primary solids). Thus, final concentrations can be reported as gc/L for liquids-based analyses, or gc/g for solids-based analyses. Within a particular sewershed, wastewater gene copy concentrations can go up or down over time due to changes in COVID-19 prevalence in the community, but also due to dilution with non-sewage flows or other conditions in the sewer. There may also be system-specific (geographic) differences that result in different gene copy concentrations for the same level of COVID-19 in the communities being served (e.g., a community with no industrial flows vs. a community with significant industrial flows). Therefore, it may be helpful to adjust the wastewater SARS-CoV-2 concentration data to account for influences other than changes in disease prevalence so wastewater data can be meaningfully compared over time, and potentially across different locations as well. As described on the NWSS website, one way to normalize wastewater concentration data is to multiply the concentrations (gene copies per volume) by wastewater flow (volume per time), to obtain gene "loads" (gene copies per time, where time is usually in days). This flow normalization (i.e., converting gc/L to gc/day) is the approach recommended by NWSS. A flow-normalized concentration can also be population-normalized by dividing by the population of the service area, resulting in a per capita flow-normalized concentration in gc/person-day. Alternatively, NWSS wastewater concentrations can be normalized to the concentration of a fecal indicator. Fecal indicators are organisms [e.g., pepper mild mottle virus (PMMoV)] or compounds specific to human urine and feces that can be measured and expressed as a concentration, thereby giving an indication of how much human fecal material is present in the wastewater. The concentration of the public health target (e.g., gc/L of SARS-CoV-2) can then be divided by the concentration of the fecal indicator (e.g., gc/L of PMMoV) to calculate the normalized SARS-CoV-2 concentration.

The jurisdictions receiving ELC NWSS funding approach normalization in slightly different ways, as shown in this <u>table</u>.

ETHICS

What privacy concerns are inherent to public health wastewater monitoring?

Because wastewater data are not collected at the individual level, individual privacy is preserved in public health wastewater monitoring programs. Group privacy, however, can be at risk in monitoring programs that capture wastewater from small sewersheds or facility-level sampling, with ethical concerns becoming more apparent as "the number of contributing individuals in a community or institution is smaller" (Canadian Water Network 2020). For this reason, NWSS wastewater data collected from sewersheds with fewer than 3,000 people are not available to the public.

Are there ethical guidelines available for public health wastewater monitoring?

Public health wastewater monitoring is an example of a public health surveillance activity (see the answer to <u>Why is the word 'surveillance' used?</u> for more information). And in any public health surveillance activity, public health goals must be achieved without imposing harm on 22

individuals or groups. In 2017, the World Health Organization (WHO) published <u>public health</u> <u>surveillance guidelines</u> built around four ethical principles:

- Common good: sufficient oversight from public health agencies is required to ensure that the benefits of surveillance are shared
- Equity: public health surveillance can help highlight the health concerns of disadvantaged communities
- Respect for persons: information about individuals and groups needs to be protected to minimize harm
- Good governance: governance structures need to be accountable and transparent and foster community engagement

The <u>Canadian Water Network's COVID-19 Wastewater Coalition</u> adapted 14 of the 17 original WHO guidelines to public health wastewater monitoring (see the <u>Canadian Water Network</u> 2020 and <u>Hrudey et al. 2021</u>) and determined that the following WHO guidelines, *using the original WHO numbering*, are applicable to public health wastewater monitoring:

1. Countries have an obligation to develop appropriate, feasible, sustainable public health surveillance systems. Surveillance systems should have a clear purpose and a plan for data collection, analysis, use, and dissemination based on relevant public health priorities.

3. Surveillance data should be collected only for a legitimate public health purpose.

4. Countries have an obligation to ensure that the data collected are of sufficient quality, including being timely, reliable, and valid, to achieve public health goals.

7. The values and concerns of communities should be taken into account in planning, implementing, and using data from surveillance.

8. Those responsible for surveillance should identify, evaluate, minimize and disclose risks for harm before surveillance is conducted. Monitoring for harm should be continuous, and, when any identified, appropriate action should be taken to mitigate it.

9. Surveillance of individuals or groups who are particularly susceptible to disease, harm, or injustice is critical and demands careful scrutiny to avoid the imposition of unnecessary additional burdens.

10. Governments and others who hold surveillance data must ensure that identifiable data are appropriately secured.

11. Under certain circumstances, the collection of names or identifiable data is justified.

12. Individuals have an obligation to contribute to surveillance when reliable, valid, complete data sets are required and relevant protection is in place. Under these circumstances, informed consent is not ethically required.

13. Results of surveillance must be effectively communicated to relevant target audiences.

14. With appropriate safeguards and justification, those responsible for public health surveillance have an obligation to share data with other national and international public health agencies.

15. During a public health emergency, it is imperative that all parties involved in surveillance share data in a timely fashion.

16. With appropriate justification and safeguards, public health agencies may use or share surveillance data for research purposes.

17. Personally identifiable surveillance data should not be shared with agencies that are likely to use them to take action against individuals or for uses unrelated to public health.

Adherence to ethical principles during public health wastewater monitoring efforts can be achieved by engaging social scientists and public health professionals throughout the duration of the program and performing extensive outreach to communities to promote meaningful knowledge exchange (<u>Coffman et al. 2021</u>).

In addition to the Canadian Water Network's adaptation of the WHO guidelines, the European Monitoring Centre for Drugs and Drug Addiction published <u>Ethical Research Guidelines for</u> <u>Wastewater-Based Epidemiology and Related Fields</u> in 2016. This document identifies the potential ethical risks in wastewater research and suggests strategies to mitigate those risks. Additional ethics guidance for public health wastewater monitoring is available in the publication by <u>Bowes et al. (2023)</u>, while both ethical and legal considerations are discussed by <u>Ram et al. (2022)</u>.

How can public health wastewater monitoring support sustainable development goals and equity?

Public health wastewater monitoring has the potential to help achieve the <u>UN Sustainable</u> <u>Development Goal 3: Ensure healthy lives and promote well-being for all at all ages</u>, specifically Target 3.3 (By 2030, end the epidemics of AIDS, tuberculosis, malaria, and neglected tropical diseases and combat hepatitis, water-borne diseases and other communicable diseases) and Target 3.5 (Strengthen the prevention and treatment of substance abuse, including narcotic drug abuse and harmful use of alcohol) because of the wide range of substances that can be measured in wastewater. Although CDC's initial focus for the <u>National Wastewater Surveillance</u> <u>System (NWSS)</u> was on COVID-19, NWSS will continue to asses other priority targets for future expansion.

CASE STUDIES

What are some examples of successful application of public health wastewater monitoring?

A few examples for COVID-19 and other health concerns are shown on our <u>Wastewater Data</u> <u>Use Examples</u> page (Do you want your program highlighted? Please fill out <u>this form</u> or <u>email</u> <u>WEF</u>). A great place for case studies from early in the COVID-19 pandemic is the U.S. EPA's <u>Compendium of U.S. Wastewater Surveillance to Support COVID-19 Public Health</u> <u>Response</u>. The <u>2023 Consensus Study Report on Wastewater-based Disease Surveillance for</u> <u>Public Health Action</u> from the National Academies of Sciences, Engineering, and Medicine offers some more recent examples. In addition, our <u>podcast</u> provides interviews with water professionals involved in public health wastewater monitoring programs, while our <u>video series</u> highlights utility perspectives as well. And here are some dashboards showing examples of how health departments have implemented public health wastewater monitoring: 24

- California Department of Public Health
- City of Tempe, Arizona
- Colorado Department of Public Health and Environment
- Florida Department of Health
- Houston Health Department
- Illinois Department of Public Health
- Indiana Department of Health
- <u>Maryland Department of the Environment</u>
- Massachusetts Department of Public Health
- <u>Michigan Department of Environment, Great Lakes and Energy with Michigan</u>
 <u>Department of Health and Human Services</u>
- Missouri Department of Health and Senior Services
- Nevada Empower Program
- New York State Department of Health
- North Carolina Department of Health and Human Services
- Ohio Department of Health
- Oregon Health Authority
- <u>Utah Department of Environmental Quality</u>
- Virginia Department of Health