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Using Wastewater Data to Communicate About Infectious Disease Dynamics in Communities

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There has been tremendous interest in monitoring wastewater for specific pathogens to support infectious disease surveillance for COVID-19 and other diseases in the United States and around the globe. Yet, for many audiences, the concept of wastewater surveillance is new, and the terminology is likely to be unfamiliar. This document describes how to communicate about wastewater-based surveillance to a variety of audiences and includes information on types of messages, data visualization, and modes of communication. It also provides guidance on explaining how the information from wastewater surveillance can support responses to infectious disease outbreaks at local to global levels. Evidence from multiple sources indicates that including wastewater data in public health messages about the COVID-19 pandemic is associated with greater acceptance by the public (Keshaviah et al. 2022; Centers for Disease Control and Prevention 2022). This document will use wastewater-based surveillance for COVID-19 as an example, but this guidance can also be applied to other infectious diseases.

How to determine your audience

Many stakeholders are interested in viewing and understanding wastewater data, just as a variety of stakeholders are interested in viewing case numbers during an outbreak. These stakeholders include government and public health leaders, health care providers, school administrators, and community members. Anyone who has benefited from viewing case information can also benefit from viewing wastewater surveillance data, but it is important that messages about wastewater data are tailored to each audience. The following section lists some key stakeholders.

Potential audiences for wastewater surveillance data

- Elected officials
- State and local public health authorities
- Health care providers and administrators
- Wastewater service providers
- School boards and administrators
- Institution leaders (corrections facilities, universities, and elder care facilities)
- General public (including special groups such as parents, community-based organizations, senior citizens, immunocompromised individuals, and other vulnerable populations)
- Media organizations

Effective communications outreach to lower-income communities is critical because these populations might be at greater risk of infection as a result of their employment (for example, frontline workers in essential industries who must physically be present in their workplace, who might need to interact with many people, or who might work in high-density settings). These same communities might also have limited access to vaccines, diagnostic testing, and health care.

Types of messages, message content, and functions

Communicating about wastewater surveillance might involve several types of messages with different functions, including the following:

1/ **Informational messages.** Informational messages, using nontechnical language, explain what wastewater surveillance is, how it is implemented (through collection of wastewater samples and laboratory analyses), who is involved, and what the results can tell us. It is important to be aware of sensitive terms and avoid their use for some audiences. For example, for the general public, the term "surveillance" can have negative associations with invasion of privacy and spying. However, for a public health audience, the term "surveillance" is commonly used to describe tracking infectious diseases, and it implies data collection responsibilities and using the data for public health responses. This is an example of an informational message designed for the general public: *"Wastewater monitoring involves collecting samples of sewage and testing them for genetic markers of the SARS-CoV-2 virus to understand if COVID-19 cases are rising or* *falling in a community.*" Messages might be more accessible if they include photos, or if they are disseminated in video form (see the Resources section for two examples). Also, having trusted local institutions deliver messages can lead to greater receptivity in the community.

Informational messages should also provide guidance about how to interpret the wastewater results. This guidance should explain how to understand the results in context of reported numbers of COVID cases and other relevant information about the COVID-19 pandemic in the geographic area covered by the surveillance. It is important to explain why wastewater results might differ from anecdotal evidence about COVID-19 in the community or trends in the number of reported cases. Informational messages should clarify that (a) at-home testing leads to underreporting of clinical surveillance data, (b) SARS-CoV-2 ribonucleic acid (RNA) concentrations in wastewater might rise before there is a corresponding increase in COVID-19 cases, and (c) people infected with SARS-CoV-2 can excrete the virus in their stools before they develop symptoms and get tested. Because the results from community samples of sewage are often available before diagnostic test results are compiled and reported, if they are reported at all, informational messages might need to clarify how wastewater data can provide an early warning of changes in the COVID-19 status of a community.

2/ **Correcting misinformation.** Correcting misinformation about wastewater surveillance is also a critical aspect of communication. Members of the public might be concerned about the risks of becoming infected with SARS-CoV-2 from exposure to wastewater; confused about wastewater versus drinking water; confused about the potential to identify individual COVID-19 cases from wastewater data, especially if the wastewater samples are from a small population; or concerned about testing wastewater for genetic markers that might have sensitive information. There is also potential for wastewater surveillance results to be used to spotlight and shame high-risk communities. It is important to emphasize that these wastewater data are used to understand the levels of disease in a community, and do not provide information about infection risk from wastewater. You can address these concerns through a frequently asked questions document or section of a website (such as the example provided at the end of this brief) and by discussing concerns in community meetings. Acknowledging data limitations and uncertainty (in wastewater and case count data) is a critical element of honest communication with the public as our understanding of the virus and pandemic is evolving rapidly.

3/ **Recognizing community.** Wastewater surveillance programs require cooperation among multiple sectors of government, wastewater utilities, health authorities, and the community. Accordingly, communications about wastewater surveillance should acknowledge the contributions of the various partners involved, as this creates a sense of shared community responsibility for the results and builds greater trust among the public. Highlighting stories from or about local communities that demonstrate how the surveillance results are providing more accurate information about trends in COVID-19 cases can encourage greater trust and interest in the results.

4/ **Call to action.** The overall goal of wastewater surveillance is to provide information on population-level disease dynamics to the public, community leaders, and government and health authorities at multiple levels and in various sectors to guide decisions about how to respond to the pandemic. This information can also be useful for health care providers, schools, local businesses, workplaces, corrections facilities, and individuals to make informed decisions about potential activities and risks. Communication about wastewater surveillance results can be linked to messages whose main purpose is to prompt specific behaviors or actions, such as encouraging social distancing, effective hygiene practices, and other harm-reducing behaviors. For example, a message to the public might state: *"SARS-CoV-2 spikes in wastewater this week are a warning sign that COVID-19 cases in your community are rising. Consider wearing a mask when you are in crowded indoor public spaces."* A message to local health care providers might state: *"SARS-CoV-2 spikes in wastewater this week are a warning sign that COVID-19 cases in your community are rising. Be prepared for additional demands on the health care system in your area in the coming one to two weeks."*

Key principles for clear data visualization and communication

- Try to communicate results in as **simple** and relevant a manner as possible. Tailor communications for various stakeholders, and start with a summary before communicating more complex, detailed information. Information that is too detailed or technical might prevent users from effectively engaging.
- Use **color coding** to communicate results in a simple manner. People often associate red with negative outcomes, so using it to indicate the presence or severity of a pathogen can be intuitive. However, about 4% of the U.S. population has some degree of color blindness. Make sure to avoid colors that can be difficult to differentiate, such as red versus green. Color scales can be an effective alternative. In these scales, darker shades indicate higher concentration or presence of the pathogen or disease, and lighter shades indicate lower concentration or presence.
- Wastewater surveillance information can be more effective when **combined with other information to provide context**—such as number of reported COVID-19 cases in the geographic area, diagnostic test positivity rate, number of COVID-related hospitalizations, percentage of hospital beds occupied, school or workplace absenteeism, and so on. With this approach, wastewater data can supplement information from other health indicators. These other sources might also help users disentangle day-to-day variation from more meaningful trends (for more information, see Anderson et al. 2022).
- Communicate clearly **which geographic areas are represented** by wastewater monitoring. People might not know the name or location of sampling sites that service their community. They also might not understand the different geographic scales represented by the results, and they are more likely to be interested in wastewater results from areas closest to them. If possible, tailor wastewater messages geographically to the populations represented by the results from specific sampling sites.
- **Interactivity** can make the information more relevant and improve retention. For example, enable users to click on a map to see data for various communities in their region, such as this map with wastewater results for Houston. Consider putting more detailed information for deeper understanding in tool tips and drop-down menus.

Sharing key messages and visualizations

The main uses of wastewater monitoring data are to provide information on the presence of COVID-19 cases (or other target diseases) or new outbreaks in a community, and to demonstrate changes in infection levels over time. Depending on how wastewater samples are collected and analyzed, the data might also be used to compare infection levels across communities or neighborhoods. To achieve this goal, there are many ways to visualize and share data with the public.

1/ **Assessing the presence of a health threat.** To assess if an infectious disease or new variant of a pathogen is present in a community, it can be helpful to visualize the presence or absence (detection or nondetection) of the targets associated with this disease (usually genetic markers of a specific pathogen) in wastewater over time in various geographic regions (example in Figure 1).

2/ **Assessing changes in the prevalence of a health threat over time.** Research has shown that changes in wastewater concentrations of pathogen genetic markers (such as SARS-CoV-2 RNA) over time align well with changes in the number of cases reported in the geographic areas covered by wastewater monitoring (example in Figure 2). Note that in order to compare pathogen measurements over time, it might be necessary to adjust the concentrations of pathogen markers to account for sample-to-sample differences in the wastewater flow rate, fecal content of the wastewater, population size represented by the wastewater sample, or assay sensitivity.

3/ **Assessing the overall magnitude of a health threat in a community.** Generally, the absolute concentration of a pathogen marker in wastewater cannot be used to directly estimate the number of cases in the community. However, observing differences in concentrations and comparing concentrations with those recorded during previous surges or declines in cases can provide context (example in Figure 2). Concentrations of a pathogen marker are especially helpful when used in conjunction with other information, such as case reports or the percentage of diagnostic tests that are positive.

Figure 1. Tile graph of SARS-CoV-2 detection in wastewater samples from schools over time

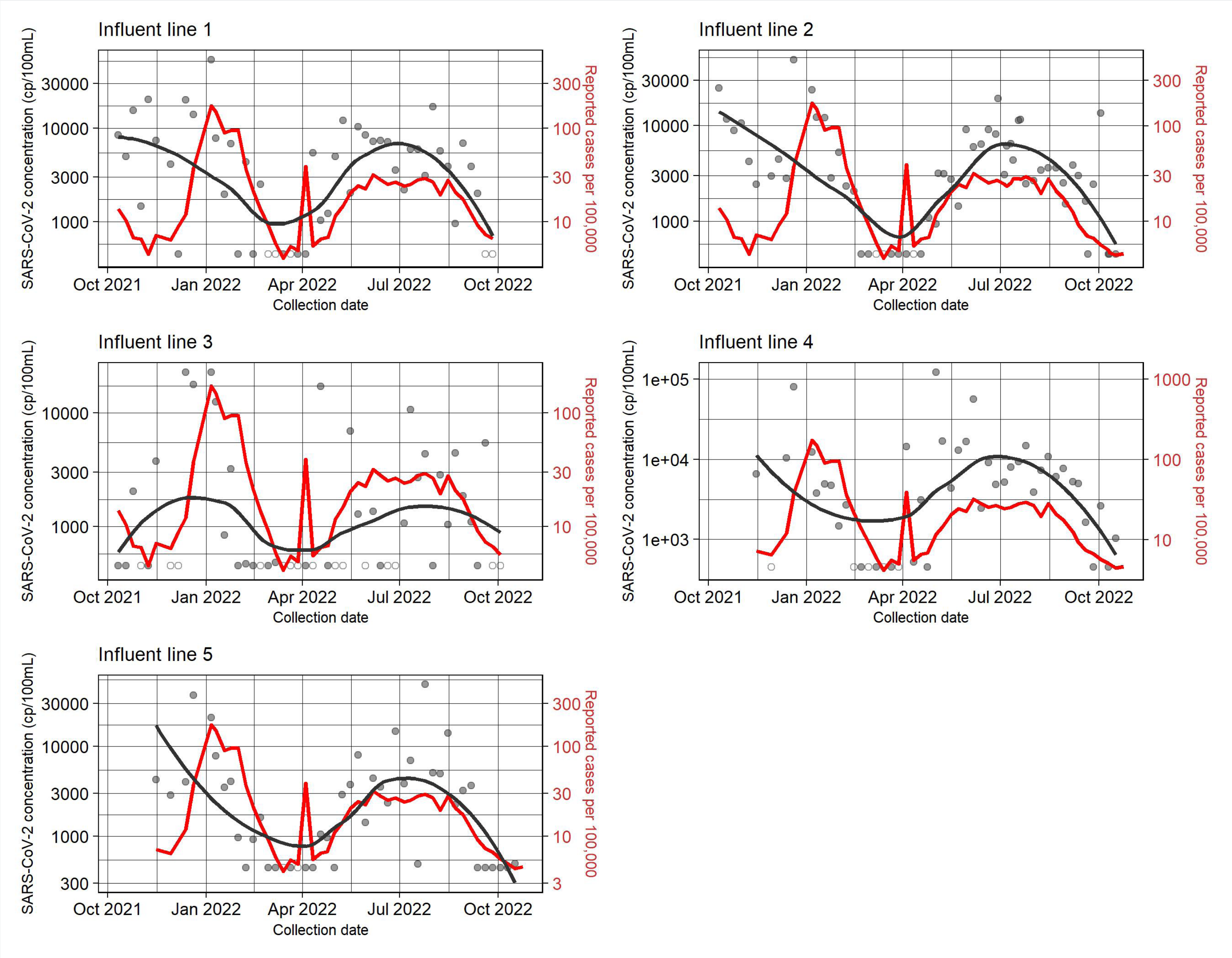
Notes: Each block represents the results of a wastewater sample collected at a school in a large metropolitan area. Samples were collected weekly for an entire school year, starting with 2 schools and gradually increasing to 11 schools per week. The color of the block indicates the strength of the pathogen detection signal (that is, SARS-CoV-2 RNA detection by polymerase chain reaction [PCR] from the wastewater sample). White blocks indicate that no sample was collected during that week.

4/ **Assessing the geographic distribution of a health threat in a community.** Mapping wastewater metrics across various geographic locations can help users see spatial patterns in the data and understand how the data that is most relevant to their location compares with data from neighboring communities. One challenge most users face with these messages is understanding what data are most relevant for them. Maps that show which areas are covered by the wastewater monitoring are particularly useful for visualizing information at a snapshot in time. When wastewater data are collected at different scales, it might be useful to use polygons to represent the results from samples that monitor large areas and overlay points to represent data from specific neighborhoods or institutions within those areas (Figure 3).

Modes and frequency of communication

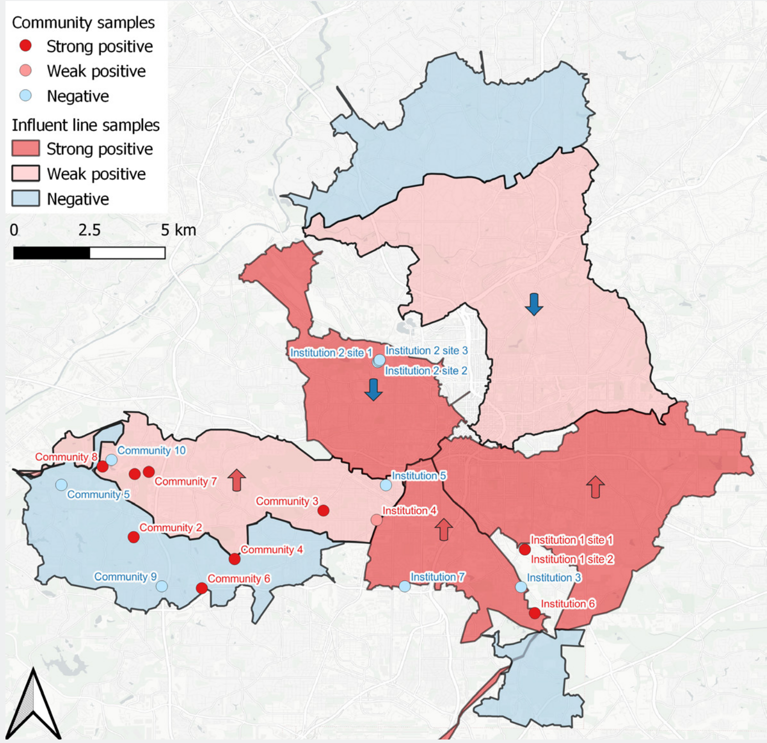
Because infectious disease case rates and wastewater pathogen concentrations can change rapidly, communications about wastewater surveillance need to provide real-time information in order to be useful for public health responses. For example, planning the allocation of health care resources based on wastewater surveillance data from the previous month is not likely to be relevant. Modes of communication that can be quickly updated to present the latest results, such as websites and dashboards, are well suited to this purpose. However, it is important to recognize that different audiences require different outreach strategies informed by what people have access to or feel comfortable with, and which sources they trust in their community. Taking time to understand information flows within a community can guide communication strategies and promote more effective outreach.

Figure 2. Reported COVID-19 prevalence at a county level (cases per 100,000 people) and SARS-CoV-2 concentrations (genome copies per 100 milliliters [mL]) in wastewater samples from influent lines at wastewater treatment facilities over time in one metropolitan area



Notes: The y-axis indicates estimated concentration of SARS-CoV-2 RNA (copies per 100 mL of wastewater). The x-axis indicates time. The gray points are measured concentrations of SARS-CoV-2 RNA in wastewater samples at specific time points. The black line represents the concentration estimates smoothed over time, and the red line indicates numbers of COVID-19 cases per 100,000 people reported to the county health authorities over time. Influent lines are large sewer pipes that collect wastewater from the sewerage system in a specific geographic area and transport it into the wastewater treatment facility. Comparing the SARS-CoV-2 concentrations trends over time among various influent lines enables the user to examine changes in COVID-19 cases in various geographic areas of the city. For example, from April to July 2022, there was a rise in the prevalence of COVID-19 cases reported by the county and a corresponding rise in the SARS-CoV-2 RNA concentration in wastewater samples in all five influent lines; the smallest increase in SARS-CoV-2 RNA concentration during this period was observed in samples from Influent Line 3—suggesting that there were fewer new COVID-19 cases in the geographic area served by this influent line.

Figure 3. Spatial differences in SARS-CoV-2 detection in influent line sewerage areas and arrows indicating change in virus detection signal from the previous week



Notes: Each polygon represents the sewerage area served by a specific influent line entering a wastewater treatment facility. The color of the polygon indicates the strength of the pathogen detection signal (that is, SARS-CoV-2 RNA detection by PCR) from a wastewater sample collected from the influent line, and the arrows indicate the change in the signal strength compared with wastewater samples from the same location during the previous week. The points represent the results from specific community wastewater samples collected at manholes or institutions (for example, schools). The color of the point indicates the strength of the pathogen detection signal (that is, SARS-CoV-2 RNA detection by PCR).

For example, analyzing social media tweets about COVID-19 in one city revealed that the public school system was a trusted source of information, as tweets from the school system had more engagement by the public than other community groups we identified.

We recommend working with local community-based organizations, faith-based groups, school systems, local city council members, and others who have the trust of communities that have historically been underserved to determine the types and modes of communication that are best suited for quickly and appropriately sharing wastewater surveillance information. Local news media is also an important partner. In the past two years, the news media at the national and local levels has played a critical role in explaining wastewater surveillance to the public and occasionally reporting the results of wastewater surveillance when a new SARS-CoV-2 variant or other pathogen (for example, mpox or poliovirus) was detected in local wastewater. Working closely with the news media can help ensure that wastewater data are used to encourage the public to take appropriate action (for example, vaccination) rather than instill panic.

Examples of public service announcements, social media toolkits, and websites with resources for COVID-19 communications are available in the resources section at the end of this document.

Periodic evaluation of communication strategies

The impact of a communications campaign might vary by the characteristics of the population and the status of the infectious disease pandemic. To guide the design and implementation of a communications strategy, it can be helpful to understand the key characteristics of the populations you want to reach and what public health communication tools have been effective for these populations in the past. To date, there is limited data on the effectiveness of communication campaigns specifically about wastewater surveillance for COVID-19 and other diseases. However, the following general principles and best practices can help you evaluate public health communication campaigns.

- Incorporate evaluations into the communication program at inception.

Modes of communication

In-person events (community gatherings, health fairs, parent–teacher association meetings, and faith groups)
Websites and dashboards
Email lists and direct reports to stakeholders
Electronic newsletters
Social media (Twitter, Instagram, TikTok, and so on)
Radio and television
Podcasts

Main communication points

Areas monitored by wastewater surveillance
Wastewater results at each location
Quick analysis of whether or how trends are changing
Recommended response – if appropriate

- Define the levels of influence you want to examine in your evaluation, such as the intrapersonal level, interpersonal level, and community level. These levels might encompass institutional factors, community factors, and public policy factors.
- Define the outcomes of interest (such as: knowledge, attitudes, risk perception, self-efficacy, and behavior change). Studies indicate that health communications tend to have a greater effect on knowledge and attitudes than on behaviors.
- Collect pre- and post-communication campaign data to measure changes in knowledge, attitudes, and practices related to the target infectious disease over time. Tools for data collection include interviews, focus groups, and surveys. However, given the dynamic nature of infectious disease epidemics (for example, COVID-19 and mpox), this approach might be confounded by other factors that change over time. A better approach might

- be to compare changes in knowledge, attitudes, and practices related to the target infectious disease between two communities with similar demographic characteristics, where one community received the communication campaign and the other community did not. Finding an appropriate comparison community might be challenging because even communities with similar demographic characteristics might have sharply different experiences during a disease epidemic that affect their perspectives about the disease.
- Collect information on implementation success. Are the messages reaching target audiences? Are audiences understanding the messages? Do the messages trigger appropriate action?

In summary, wastewater monitoring for specific pathogen markers of disease is a low-cost, efficient, sensitive tool for infectious disease surveillance at the population level. Use of this powerful approach is rapidly expanding to more geographic regions and multiple disease targets. Effective communication about wastewater-based surveillance of infectious diseases is critical for the communities where this approach is being implemented. We provide the following recommendations for using wastewater data to communicate about infectious disease dynamics in communities:

- Know your target audiences
- Develop clear messages with language and data visualizations tailored to specific audiences
- Explain how wastewater data can be useful for various audiences
- Be transparent about areas of uncertainty and the strengths and limitations of wastewater data
- Use appropriate dissemination channels for each of your audiences
- Periodically evaluate whether the messages are reaching the target audiences and are having the intended effect on knowledge, attitudes, and actions (at a personal level or institutional level) related to the disease highlighted by the campaign

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Videos

- Dr. Amy Kirby: Sewage Surveillance for COVID
- Testing Wastewater for COVID-19: The Clearest Path to Understanding Community Infection

Useful websites

- COVID-19 Testing Communications Toolkit: A platform that has free downloadable images custom-made for COVID-related communications
- Canva: A website that helps users create professional designs for presentations, videos, and social media
- Public Service Announcements: National Association of Broadcasters website where users can download radio, TV, and podcast public service announcements and scripts that highlight how your community can help prevent the spread of COVID-19
- Centers for Disease Control and Prevention (CDC) Social Media Tools, Guidelines & Best Practices: CDC resources for reaching audiences on social media
- Ohio Coronavirus Wastewater Monitoring Network – COVID-19 Dashboard: Example of a website with COVID-19 wastewater surveillance data for Ohio, developed by the Ohio Department of Health, that includes dashboards, interactive maps, and general information on wastewater monitoring and how to interpret the results

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Frequently Asked Questions

Adapted from Healthy Davis Together handbook

FOR THE GENERAL PUBLIC

Question	Answer
1. Why test and monitor wastewater?	Wastewater testing is a way of sampling for circulating diseases in an entire community, anonymously, without behavior change, and regardless of access to the health care system. Thus, it is an inexpensive way to get information on population-wide infection trends over time. This means that wastewater data can indicate where COVID-19 cases might be on the rise. Locating spikes helps inform where additional resources, such as diagnostic testing, vaccination resources, and increased health care capacity, might be required.
2. What does wastewater monitoring detect—just SARS-CoV-2 or other pathogens also?	Wastewater is widely used for monitoring SARS-CoV-2, but health authorities can monitor a wide variety of other pathogens in wastewater to contribute to infectious disease surveillance. Researchers continue to develop tools and supporting evidence for interpretation of new targets. Testing is now also widely available for influenza, respiratory syncytial virus (RSV), and mpox. These tests typically work by isolating and identifying the genetic material of the pathogen, rather than isolating an infectious virus.
3. Can wastewater monitoring detect drug use in a home?	Wastewater monitoring is valuable because it can used to sample an entire community at the same time. Thus, wastewater monitoring should not be used for detection of biological or chemical targets in individuals or individual households. Some organizations might conduct testing for drugs and other chemicals, but this use is distinct from wastewater monitoring for pathogens such as SARS-CoV-2.
4. Will my neighborhood ever be quarantined because of SARS-CoV-2 detection in the wastewater?	Wastewater data should be used to supplement the information that public officials use to take public health actions within their jurisdiction. The commonly used or permissible public health actions in response to outbreaks differ across U.S. states and the world. Regardless of local laws regarding quarantines and lockdowns, we recommend that wastewater data alone should not be considered sufficient to enact quarantine measures.
5. Is my drinking water contaminated if the wastewater tests positive for SARS-CoV-2? Should I worry about my drinking water?	No. Wastewater and drinking water systems are entirely separate. Wastewater is tested to ascertain disease in the community precisely because it is contaminated with many bodily fluids that could contain pathogens, and therefore it is taken to a treatment plant so that it can be made safe to release to the environment. Drinking water is cleaned and distributed to a community using separate systems and resources. SARS-CoV-2 is not transmitted by drinking water and is not likely to be infectious even in wastewater. Furthermore, wastewater treatment processes inactivate SARS-CoV-2 and most other pathogens.
6. If the wastewater monitoring results in my neighborhood are negative, does that mean I can stop wearing a mask?	Wastewater monitoring can provide some information about the presence, magnitude, and trend of the target infectious disease cases in a general geographic area and can help inform personal decisions about activities. However, it is possible that there are cases below the detection limit (which varies based on the approach to monitoring) or people whose infections are not captured in the wastewater (for example, because they did not use the bathroom during the time when wastewater samples were collected, because they might not shed the pathogen into their stools even if infected, or because they might contribute to a wastewater treatment plant not currently being monitored).
7. Where can I get more information about the wastewater monitoring results in my area?	CONTEXT-SPECIFIC ANSWER

FOR THE PUBLIC HEALTH AUTHORITIES

Question	Answer
1. How can I use wastewater monitoring data? How would this information change what I am already doing?	How you use wastewater monitoring information depends in part on the locations and frequency of wastewater sample collection. If many locations in a community are sampled simultaneously, wastewater surveillance data can provide information on geographic hot spots of SARS-CoV-2 infection and other diseases, and health authorities can use this information to target diagnostic testing, health messaging, and vaccination campaigns to these areas. Because wastewater data can capture the infections of people who are asymptomatic and those who might not otherwise get tested, wastewater surveillance data can also provide early warning of a surge in COVID-19 cases that enables health authorities to ramp up capacity at health care facilities and prepare for increased health care needs. Finally, with increased use of at-home testing and declines in clinical surveillance, which create gaps in reported case count data, wastewater monitoring can provide additional unbiased, population-level information about COVID-19 and other disease trends in the community that can support public health decisions regarding disease response.
2. What about surveillance needs for other diseases?	Depending on the community, wastewater monitoring is in place for other pathogens, including influenza, respiratory syncytial virus (RSV), mpox virus (MPXV), and others. SARS-CoV-2 is still the most commonly monitored pathogen, but you can use the same samples and testing platforms to test for additional disease targets. Any pathogen that is regularly shed in secretions that travel down the drain (toilet, sink, shower, and so on) is a potential candidate for wastewater monitoring.
3. Are there any long-term benefits to wastewater surveillance?	Because effective wastewater surveillance requires close collaboration between health authorities and water/wastewater utilities, the working relationships established through wastewater monitoring can have secondary benefits. In addition, establishing wastewater surveillance systems can enhance outbreak preparedness, as seen in the rapid addition of mpox testing to many wastewater monitoring sites at the beginning of the 2022 outbreak in several locations around the world. This data can also provide long-term information on trends of seasonal diseases that improves seasonal predictive modeling to inform public health response.
4. Who will pay for wastewater surveillance in my community?	Many state health departments and public health labs have received funding from the Centers for Disease Control and Prevention to establish wastewater surveillance for COVID-19. Sustained funding from national, state, and municipal governments will be needed to institutionalize wastewater surveillance.

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Developing Measurements of Health Markers in Wastewater

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When someone uses the bathroom, they excrete bacteria, viruses, and chemical metabolite markers that reflect the state of their health. These markers end up in wastewater, which represents a pooled sample from everyone that contributed to it and can provide a way to monitor the health of that population; this practice is called wastewater-based epidemiology (WBE). Researchers and public health officials have developed different assays, which are tests to determine the quantity of health markers in wastewater such as pathogenic viruses, bacteria, and chemical metabolites (See Table 1). Assays can also be developed for specific hormones or metabolic indicators of a particular disease. In this brief, we focus on the development and validation of assays designed to detect viral and bacterial pathogens.

What is an assay, and what are the differences between a clinical and wastewater assay?

An assay is a test that is used by researchers and clinicians to detect and quantify how much of a specific component is present in a sample. Many assays for pathogen detection in clinical and wastewater samples work by recognizing and amplifying genomic signatures (e.g., DNA and RNA) unique to those target pathogens so that researchers can detect and distinguish them from the vast background of other microbes in a sample. The assays designed for clinical use might only work for a particular type of sample (that is, urine, feces, sputum, or saliva) and not be able to tolerate the wide variety of compounds that end up in wastewater. Assays developed for wastewater must be sufficiently robust to resist potentially inhibitory substances that may interfere with the detection of a given target in the complex wastewater matrix. Clinical samples also often have relatively few types of organisms present compared with wastewater, so assays suitable for wastewater must be able to detect and distinguish pathogens among a more diverse background of molecules. Clinical assays frequently can be applied to wastewater samples, but this desirable outcome is not a given and may require assay modification followed by empirical testing and validation. Challenges in validating assays for wastewater arise from the need for having access to the pathogen of interest (as a positive control) and a target-free wastewater sample (negative control) that then can be fortified with the pathogen to evaluate assay detection limits. Absent of a standard of the pathogen of concern, experimentalists may resort to collecting wastewater from a location (for example, daycare, school, or a long-term care facility) with an active, known outbreak.

What makes for a good target (or biomarker) when monitoring diseases in wastewater?

- It's excreted in urine or stool in concentrations high enough to enable detection
- It's stable in wastewater (i.e., it's not appreciably degraded during transport in the sewer)
- It's directly related to infection, disease, or the human behavior monitored

How do you develop successful assays?

A reliable assay is **specific** (few false positives), is **sensitive** (few false negatives), and yields **timely results**. Assays based on amplifying targeted genetic markers (for example, PCR-based assays such as RT- qPCR/ddPCR) align well with those criteria. Yet these molecular assays require prior knowledge about the genomes of targeted pathogens, their variants, and all close relatives. The assays being developed must also be tested against as many known closely related organisms or viruses that are non-pathogenic, non-targeted, or both, to determine specificity. This way, the assay will reliably be interpreted as detecting and quantifying the targeted pathogen and not a similar but non-pathogenic cousin. Another important practice to implement when developing an assay is to use internal controls. These controls can be either cultivated target organisms or an analogous organism with distinct genetic targets but similar properties that are added to wastewater samples in a known concentration. When added just prior to the detection step, they can provide assurance that the assay is providing positive results when expected. When added before sample manipulation (e.g., filtration of samples to concentrate a target), they can provide important information on the recovery efficiency, consistency, and sensitivity of the assay.

Repurposing existing assays

There are many assays designed to detect known pathogens in individual clinical patients or other environmental settings (e.g., in contaminated food or processing facilities). The development and validation of these assays can be leveraged by repurposing them for WBE. This was almost universally done with WBE for SARS CoV-2 by using the existing assays approved by the Centers for Disease Control and Prevention and World Health Organization. The genomes of many SARS CoV-2 viruses from patient samples were sequenced and multiple unique regions were identified, and the resulting assays were tested for specificity and sensitivity. Building on previously developed clinical assays thus can accelerate the successful deployment of WBE assays for new threats: for example, clinical test kits applied to pre-processed (i.e., concentrated/purified) wastewater can increase assay efficacy, sample throughput, assay availability and scale-up.

Experience gathered during the COVID-19 pandemic can serve as a WBE road map for developing and validating novel assays

The goal of developing or adopting an assay for WBE is to collect actionable information where it does not otherwise exist. An ideal assay is sensitive to a low number of targets, specific for the pathogen and its variants, and provides a quantifiable signal that corresponds proportionally to target concentration (e.g., SARS-

Table 1. Characteristics of assays used for different wastewater targets

Target	Type	Assays	Maturity
Respiratory disease targets			
SARS-CoV-2 and variants	RNA virus	RT-qPCR, RT-ddPCR, targeted amplicon sequencing	High
Influenza	RNA virus	RT-qPCR, RT-ddPCR	Low/Mid
Respiratory syncytial virus	RNA virus	RT-qPCR, RT-ddPCR	Low
Enteric disease targets			
Norovirus	RNA virus	RT-qPCR, RT-ddPCR	High
Rotavirus	RNA virus	RT-qPCR, RT-ddPCR	Low
Salmonella	Bacteria	qPCR, ddPCR, culture	Mid
Campylobacter	Bacteria	qPCR, ddPCR, culture	Mid
Shigella toxin-producing E. coli	Bacteria	qPCR, ddPCR, culture	Mid
Vector-borne disease targets			
West Nile virus	RNA virus	RT-qPCR, RT-ddPCR	Low
Other emerging disease targets			
mpox virus	DNA virus	qPCR, ddPCR	Low/Mid
Poliovirus	RNA virus	RT-qPCR, RT-ddPCR, targeted amplicon sequencing	High
Pharmaceuticals and drugs			
Prescription drugs	Metabolite	Mass spectrometry	High
Illicit drugs	Metabolite	Mass spectrometry	High
Ingested chemicals			
Caffeine	Metabolite	Mass spectrometry	High
Nicotine	Metabolite	Mass spectrometry	High
Human biomarkers			
Creatinine	Metabolite	Mass spectrometry	Mid
crAssphage	DNA virus	qPCR, ddPCR	Mid
Stress hormones	Metabolite	Mass spectrometry	Mid

CoV-2 virus counts indicative of infected people). The COVID-19 pandemic was unique in that individual testing was, at least for a period, widespread for symptomatic and non-symptomatic people. The value of WBE for COVID-19 was evident early in the pandemic, when it was possible to compare the concentration of SARS-CoV-2 in wastewater and the number of recorded infections within sample sizes ranging from individual buildings to wastewater treatment facilities serving hundreds of thousands of people. The pandemic provided the unusual duality of testing approaches, allowing the opportunity for WBE assays to be optimized for representative sampling, efficient extraction of genetic material, and the comparison of viral concentrations in wastewater to numbers of cases.

The connection between WBE and individual testing data has continued to shift throughout the pandemic. Currently, fewer tests are being administered, with a bias toward confirming likely infections. At home testing now increasingly supplants clinical testing efforts. As such, WBE for COVID-19 has become a primary source of disease surveillance at the population level.

It is important to align wastewater pathogen assay data with more traditional surveillance data, which include individual testing, syndromic monitoring from clinics, hospital admissions, and deaths. Integrating wastewater data with other traditional metrics can improve predictions of disease prevalence, rapidly identify communities experiencing active outbreaks and those coming out of infection surges, and improve the equitable and timely allocation of public health resources.

Potential roles for pathogen assays applicable to wastewater

Beyond the current pandemic, additional pathogen assays are beginning to become more prevalent (see Table 1). Without widespread individual testing, wastewater monitoring can provide valuable early warnings for new outbreaks of pathogens, such as mpox and poliovirus. Endemic or more common or seasonal diseases can also be monitored, thereby allowing public health entities to forewarn local hospitals and clinics about their prevalence and projected hospital bed capacity needs. More explorative, less directed approaches that involve high throughput sequencing of wastewater can provide research scientists and public health officials with a new lens to monitor emerging pathogens, variants, and novel threats (new genetic targets) that may be observed in wastewater before infected individuals present in healthcare settings for treatment.

Conclusions

The widespread adoption of WBE worldwide in response to the ongoing COVID-19 pandemic has accelerated the development of additional assays that can monitor human health at the population level. Table 1 lists the assays for various targets of human health and their characteristics. For a more extensive list, see Adhikari and Halden (2022). Aside from the ongoing pandemic and successive outbreaks caused by new variants, researchers can monitor other pathogens where only syndromic or limited data are available. Normally, people would have to seek health care and be tested to identify an incidence or outbreak of disease. By applying several assays for specific pathogens, public health entities can monitor the presence and spread of specific pathogens across geographic space and time.

Assays developed or adopted for use with wastewater can also help address an outbreak known only through syndromic data. For example, gastrointestinal distress could be linked to bacterial pathogens (such as *Campylobacter*, *Escherichia*, or *Salmonella*) or instead by viral pathogens (such as Norovirus). Infection dynamics and treatment options differ between pathogens (e.g., bacteria, viruses, fungi), as do the sources and transmission routes for the associated infections, which can inform strategies for testing for contaminated food or environments. For example, an increase in respiratory illnesses in a school or school district may be quickly identified as, for example, another outbreak of SARS-CoV-2, seasonal influenza, or RSV. Broadening the repertoire of assays suitable for WBE and refining the case for their uses will provide public health officials with better, more informative data on which to base best mitigation decisions.

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Suggested citation: Stevenson, B.S., L.B. Stadler, K.B. De León, and R.U. Halden. "Developing Measurements of Health Markers in Wastewater" Washington, DC: Mathematica, 2022.

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ISSUE BRIEF

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Integrating Wastewater and Public Health Data

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By bringing together data from multiple sources in a structured way, public health officials can fill gaps when one data source is weak or see nuanced insights that might otherwise go unnoticed. Data integration allows for analysis that makes it easier to act on data. For instance, plots that show trends across wastewater and clinical data and risk scores that show where to prioritize resources can serve as justification for decisions. To be successful, integration for wastewater and public health data should be timely, consistent, disseminated broadly, and enable public officials to make more informed decisions.

Integrate data to fill gaps

Individually, wastewater data and public health data provide insights into only a portion of the community-level burden of COVID-19, seasonal flu, gastrointestinal bugs, and other diseases. For example, with individual case count data, infections are only registered if people first have symptoms and then visit a doctor or testing site. If either of these steps does not occur, we miss important data that helps us understand the full scope and context of illness. Although wastewater cannot provide local health agencies with the type of individual-level data contained in administratively reported case counts from hospitals, it can help officials understand viral infection levels and spread across communities without relying on individual testing practices. When paired with complementary data (see Box 1), wastewater data provide a more comprehensive view of disease burden in a community and can be more useful as a public health surveillance tool.

Box 1. Complementary data sources

Wastewater data (from sanitation agency or wastewater laboratory): target pathogen levels (SARS-CoV-N1, Influenza, etc.), human biomarkers (PMMoV, CRASSPHG, etc.), toxins, volatile organic chemicals, GIS sewer network attributes, effluent flow, service population

Public health data from public health departments or hospital systems): test positivity rate, COVID-19 incidence, hospitalizations, intensive care unit bed occupancy, mortality, vaccination rates

Other data (from census, ACS, or ArcGIS databases): age, income, race, urban or rural classification, underlying health conditions, workforce participation, community features (apartments versus standalone housing, presence of prisons, universities, nursing homes, or other communal living facilities), land use, population mobility, community events, and tourism data

Assess and access available data

The first step in integrating wastewater data with other public health data is to assess what local data are already available (see Box 1 for examples) and understand the processes that enable sharing the data.

1/ **Characterize existing data.** Public health agencies might have access to a variety of health metrics, such as positive cases, hospitalization rates, intensive care unit bed occupancy, vaccinations, and deaths. It is important to know how often each data set is collected and at what scale (for example, by address, zip code, or county) to assess the potential added value of wastewater data.

Tips to keep in mind when acquiring and assessing data:

- Weekends and holidays can cause lags in data reporting that result in artificial variability of certain measures, such as case rates and hospitalizations. Smoothing the data (for example, by calculating rolling seven-day averages) can help you better interpret data.
- Timing (date) and scale (location) are the two basic shared characteristics that allow you to merge distinct data sets.
- Knowing where data originate and what entities have ownership or interest in the data (for example, hospitals, states, or other elected officials) is important.
- Special attention must be given when data includes personal health identifiers such as names, addresses, dates of treatment, birth dates, and so on. Even if no single data set includes personal health identifiers, take care when overlaying or combining multiple data sets together to ensure that no one can triangulate identifying information from the component data sources.
- If public health data must be aggregated to neighborhoods, GIS shape files need to be shared with the health agency during initial partnership discussions.

2/ **Determine what insights you hope to gain.** For instance, you might ask whether there is a relationship between viral concentrations in wastewater and clinical measures, such as the number of positive tests, positivity rate, or hospital admissions. By plotting these metrics together, you can uncover patterns. For example, if the number of hospitalizations spike two weeks after wastewater viral concentration levels spike, then officials can make health care staffing decisions informed by this relationship (that is, the potential early warning from the wastewater data). Meeting with biostatisticians and epidemiologists at the outset will be helpful because they can share limitations for data use or suggest ways to handle missing data.

3/ **Access the data.** If the data are already publicly available (for example, through a dashboard), you must understand any restrictions on reuse of the data. If the data are not publicly available, data sharing agreements must be established before sharing any data. These agreements can take several weeks or up to several months to be fully executed. Memorandums of understanding or data transfer agreements outline what data will be received, processes for receiving the data, data storage, and how the data will be used, including later publication. Keep in mind that there are many levels of stakeholders in any given institution, and you should create a stakeholder list in the initial partnership meetings. For example, personnel from legal and risk departments often review data agreements before they are executed. And if the wastewater activity is being undertaken in partnership with a research study— particularly if personal health identifiers are being shared— the completed data sharing agreement must be submitted to and accepted by the university's institutional review board. One key to success and speedy execution is to have buy-in from a high-level champion, such as a medical director at the public health agency, who can help get the stakeholders to agree to data sharing. Finally, data managers from the organizations sharing data should know about the data sharing agreement and what is expected of them. After all agreements are signed, the data transfer can begin by making a formal request to the public health data manager.

Case studies from Louisville, Kentucky

In Louisville, the fact that both wastewater and clinical testing results rose shortly after the 2021 holiday season provided decision makers with confidence in the wastewater data. Examining wastewater data alongside clinical testing data also highlighted the increased virility of the novel Omicron variant compared with the Delta variant. Further, by pairing wastewater and clinical testing data, researchers could identify neighborhoods where under-testing was likely occurring by looking for areas where wastewater viral concentrations were elevated but case counts were not. Louisville's public health agency used such information to deploy targeted testing resources to those areas.

Prepare the data for integration

Before integrating data, check the quality of the received public health data and normalize wastewater results based on human biomarkers. Then, integrate the data based on shared basic characteristics such as date or location.

4/ **Confirm data quality.** Ensure that the received data are reliable (that is, complete, accurate, and timely), and track any gaps, potential data inaccuracies, limits of detection, and suspicious outliers (see Box 2). Ensure that column headers and cell formatting across spreadsheets match before beginning integration. For example, transform all dates into the short date format (for example, 12/12/2022) from long or free formats. Ensure that there are no spaces in column headings or spreadsheet titles. The public health data set should come with a data dictionary that explains heading titles and notes about the data. Layering data might require integration across software, such as from spreadsheets to GIS files.

Box 2. Data quality

According to the Centers for Disease Control and Prevention, there are three key elements of data quality for public health surveillance: completeness, accuracy, and timeliness. Data are considered complete when they capture all the cases of interest. Data are accurate when the information they reflect is true. Data are timely when they are available within the period of time when it is useful. The Centers for Disease Control and Prevention offers simple tools to improve data quality.

5/ **Normalize wastewater data.** Normalization is the process of structuring data so that you can compare measures across sites or data sets. Normalizing wastewater data is important because the amount of viral material in samples fluctuates with the number of people contributing to the sewer system, wastewater flow rate, dilution from rainfall, and other environmental factors. Fecal indicators, such as Pepper Mild Mottle Virus (PMMoV), can serve to normalize or adjust the results based on the amount of human-made material in the sewer system. The University of Louisville normalizes SARS-CoV-2 concentrations in wastewater samples by dividing the number of SARS genetic copies (N1) by the number of PMMoV copies in the sample (using the formula N1/PMMoV). Read more about how fecal indicators can serve to calibrate epidemiological models for pathogen surveillance. See Box 3 for other normalization approaches.

Box 3. Normalization approaches

For wastewater data to be included on the Centers for Disease Control and Prevention's National Wastewater Surveillance System Dashboard, data must be normalized by flow rate and service population size. Flow rate and service population size normalization are easier because they can be calculated through demographic and spatial data. Not all sewer collection points have flow rate data. Population size normalization will not yield accurate results if the number of people contributing to the sewershed changes because of tourism, weekday commuters, and so on. Learn more about normalization for the Centers for Disease Control and Prevention's National Wastewater Surveillance System Dashboard.

Integrate wastewater and public health data

With two prepared data sets that share a common merge field (usually date or location), you can begin integration.

6/ Use Excel, ARCGIS Pro, or other software to merge the data sets together.

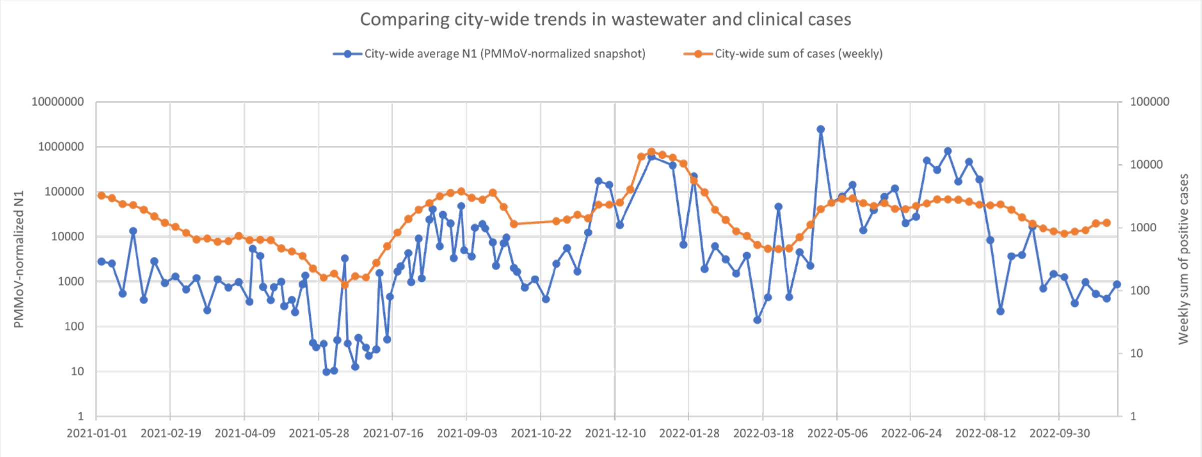
7/ **Create visualizations.** Now that you have merged the data, you should create easily understandable graphs to help public health leaders identify patterns and relationships in the wastewater and public health metrics. There are many ways to visualize the data, at varying scales, such as citywide (Figure 1), within individual neighborhoods (Figure 2), or by sewersheds served by water quality treatment centers. Each data point can reflect daily counts, moving averages, or weekly averages.

Share integrated data

One way to leverage integrated data is through a decision framework that assigns relative risk to areas based on several metrics, wastewater level, wastewater trends, case rate, case trends, and vaccine rate. This epidemiological and geographically contextual model is the vehicle for translating integrated wastewater and public health data to city officials.

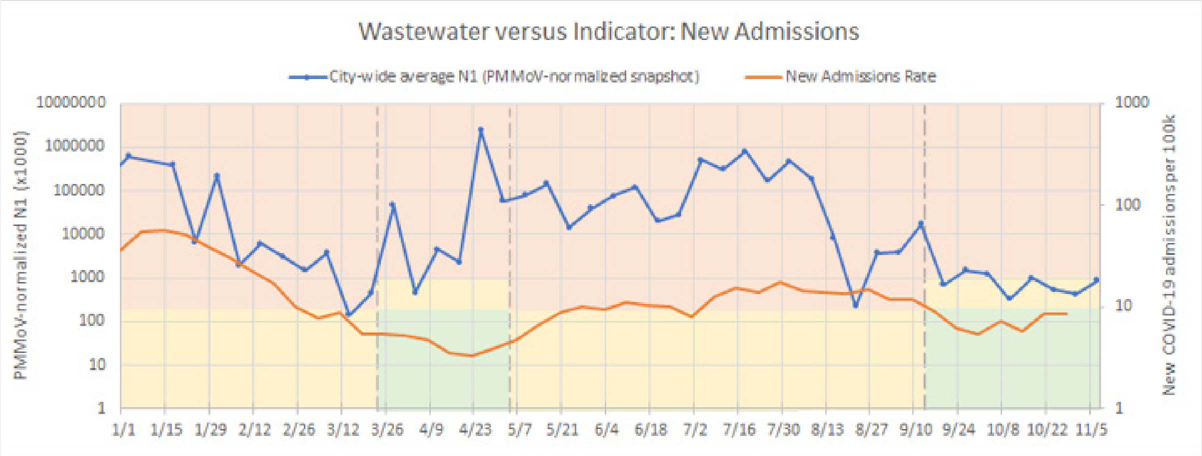
8/ **Develop an integrated risk score.** Decide collaboratively with your public health agency what key metrics are important to track. In Louisville, case rate was the most important metric for decision-making at the city level. Because of the wave pattern of COVID-19 infection, we included increasing and decreasing trends for wastewater and positive cases. After you decide on metrics, you can assign scores based on data and rank areas by relative risk of infection.

Figure 1. Plot of wastewater viral concentrations overlaid with clinical case counts across Louisville



Here, we plot wastewater and positive clinical cases across Louisville-Jefferson County together. When the wastewater (N1) line is much higher than the clinical case line, this might warrant further investigation. For example, when we see this pattern, we might ask whether wastewater is a more reliable measure of community infection than clinical testing alone.

Figure 2. New hospitalizations plotted alongside wastewater results for the entire city of Louisville



Notes: Color coding is based on the Centers for Disease Control and Prevention's [Indicators for Monitoring COVID-19 Community Levels](). Here, we plot wastewater and hospital metrics together. When we see a wastewater spike, such as the period from 4/1/2022 to 5/1/2022, and then a subsequent spike in new admissions around three weeks later, public health officials might advise health systems to increase staffing in the weeks after another wastewater spike based on this past pattern.

Figure 3 shows a visualization of integrated data and the scores for each of the key metrics for the Cedar Creek neighborhood. Trends are apparent in the three graphs at the bottom. Figure 4 shows the standardization (see Box 4) of all the key metrics and the weighting given to case rate. By using Figures 3 and 4, we can calculate the risk score for Cedar Creek:

Wastewater level: 3/5 = 0.60
Wastewater trend: 2/3 = 0.67
Case rate: 5/8 = 0.62 x 2 = 1.25
Case trend: 1/3 = 0.33
Vaccine coverage: 3/3 = 1
Sum for risk score: 3.85

Box 4. Standardization

When combining multiple metrics, such as wastewater level, wastewater trend, case rate, case trend, and vaccine coverage into a single score, it is important to standardize each metric. In this case, we put each metric on a 0 to 1 scale and then assigned double weight to the metric that was most important to Louisville's health department: case rate. By standardizing each of the five metrics to 1, the resulting risk scores will range from 0-6, allowing for ranking based on risk.

Conclusion

Combining information from multiple sources provides decision makers with a more holistic picture of disease dynamics and greater confidence to act. The discrete data sets can act as validators for each other or alert decision makers to changes during evolving public health situations. Wastewater monitoring should be viewed as another public health tool to understand population health, especially in light of its ability to illuminate patterns of infection when integrated with demographic and geographic data.

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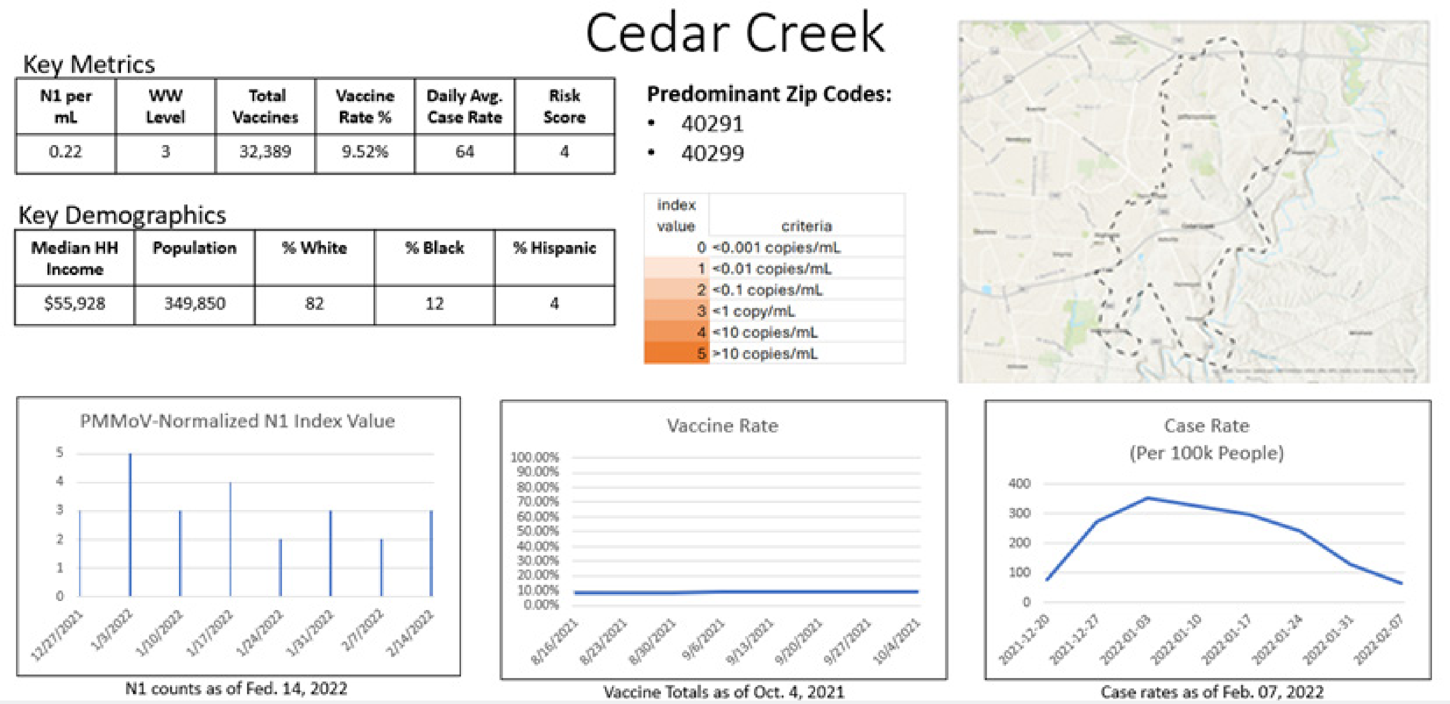
Figure 3. Sample risk-decision framework summary from the Cedar Creek neighborhood



Cedar Creek

Key Metrics

N1 per mL	WW Level	Total Vaccines	Vaccine Rate %	Daily Avg. Case Rate	Risk Score
0.22	3	32,389	9.52%	64	4

Predominant Zip Codes:
- 40291
- 40299

Key Demographics

Median HH Income	Population	% White	% Black	% Hispanic
$55,928	349,850	82	12	4

index value	criteria
0	<0.001 copies/mL
1	<0.01 copies/mL
2	<0.1 copies/mL
3	<1 copy/mL
4	<10 copies/mL
5	>10 copies/mL

Figure 4. The weighting scheme to assign risk scores to individual neighborhoods

Wastewater Level		Wastewater Trend		Case Rate		Case Rate Trend		Vaccine Coverage	
0-5/5 points based on PMMoV corrected N1 index value.		1-3/3 points based on declining, stable, or increasing N1 trend.		(0-8/8)*2 points based on current case rate.		1-3/3 points based on declining, stable, or increasing N1 trend.		1-3/3 points based on vaccine coverage.	
0	0, no detect	.33	Decreasing	0	>1	.33	Decreasing	.33	High (>61%)
.2	1, <10 copies/ml	.66	Stable	.25	1-10	.66	Stable	.66	Medium (41-60%)
.4	2, <100 copies/ml	1	Increasing	.5	11-25	1	Increasing	1	Low (<40%)
.6	3, <1,000 copies/ml			.75	25-37				
.8	4, <10,000 copies/ml			1	38-57				
1	5, >10,000 copies/ml			1.25	58-87				
				1.5	88-132				
				1.75	133-200				
				2	200+				

ISSUE BRIEF

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Developing Wastewater Sampling Plans to Monitor Public Health

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The choice of where, when, and how to collect wastewater samples should be based on the goals and purpose of the monitoring system you use. The choice of where to sample is critical because it determines the population represented and captured by the wastewater measurement as well as the sensitivity of the assay (the laboratory test that measures the amount of a specific substance), in terms of number of people who need to be infected to observe a positive signal. Although you can collect samples from anywhere in the sewer system, in practice, samples must be collected at locations where you can access wastewater safely and reliably, such as wastewater treatment plants, lift stations, or manholes. In addition, the frequency and type of sample collected will affect the resolution and quality of information generated from the wastewater system. This brief highlights the following key considerations when developing a wastewater sampling program: (1) Designing a sampling plan - where and when to collect wastewater; (2) Sample collection types; (3) Adaptive sampling; and (4) Data quality and variability.

Designing a sampling plan

Wastewater samples should be collected in a manner that aligns the resulting information with the public health goals of the monitoring program. This will depend on aspects of the public health goals, including the size of the community you want to monitor, the financial resources available for performing the sampling and acting on the results, and the layout of the community's sewage system. For example, if you want early detection of viral outbreaks at a specific school or nursing home facility, then you would have to sample that facility daily because doing so aligns with the goal of catching outbreaks as soon as possible at a specific facility. But if your goal is to understand the general trends of the virus in a region in which you are sampling multiple wastewater treatment plants within that municipality, then a weekly sampling plan at the geographically dispersed treatment plant level is suitable because it aligns with the goal of monitoring larger

Sample collection types

Sample type	Examples	Advantages	Disadvantages
Grab	Placing a 500mL collection bottle in wastewater and filling it.	· Low cost · Minimal equipment required · No external power or batteries required	· Diurnal variability affects sensitivity and representativeness · Less quantitative than composite samples
Passive	Suspending a Moore Swab, medical gauze on a string, for 24 hours in a pipe carrying wastewater. Once the Moore Swab is retrieved, collecting liquid that is squeezed out.	· Low cost · Minimal equipment required · No external power or batteries required	· Performance depends on material, sewage characteristics, and time deployed · Less sensitive and quantitative than composite samples
Composite	A battery powered, automated sampler's hose sits in a manhole and is automatically collecting 15 mL of wastewater every 15 minutes for 8 hours.	· More representative because each sample comprises aliquots collected at defined flow or time intervals. · Representative samples enable sensitive and quantitative measurements of disease targets in wastewater samples.	· Autosamplers are expensive, require external power, and require maintenance

community samples for an entire region. Another way to think of the sampling frequency is that large systems will change more slowly because they support more people, so you don't have to sample the treatment plants daily for you to understand the trends for a region.

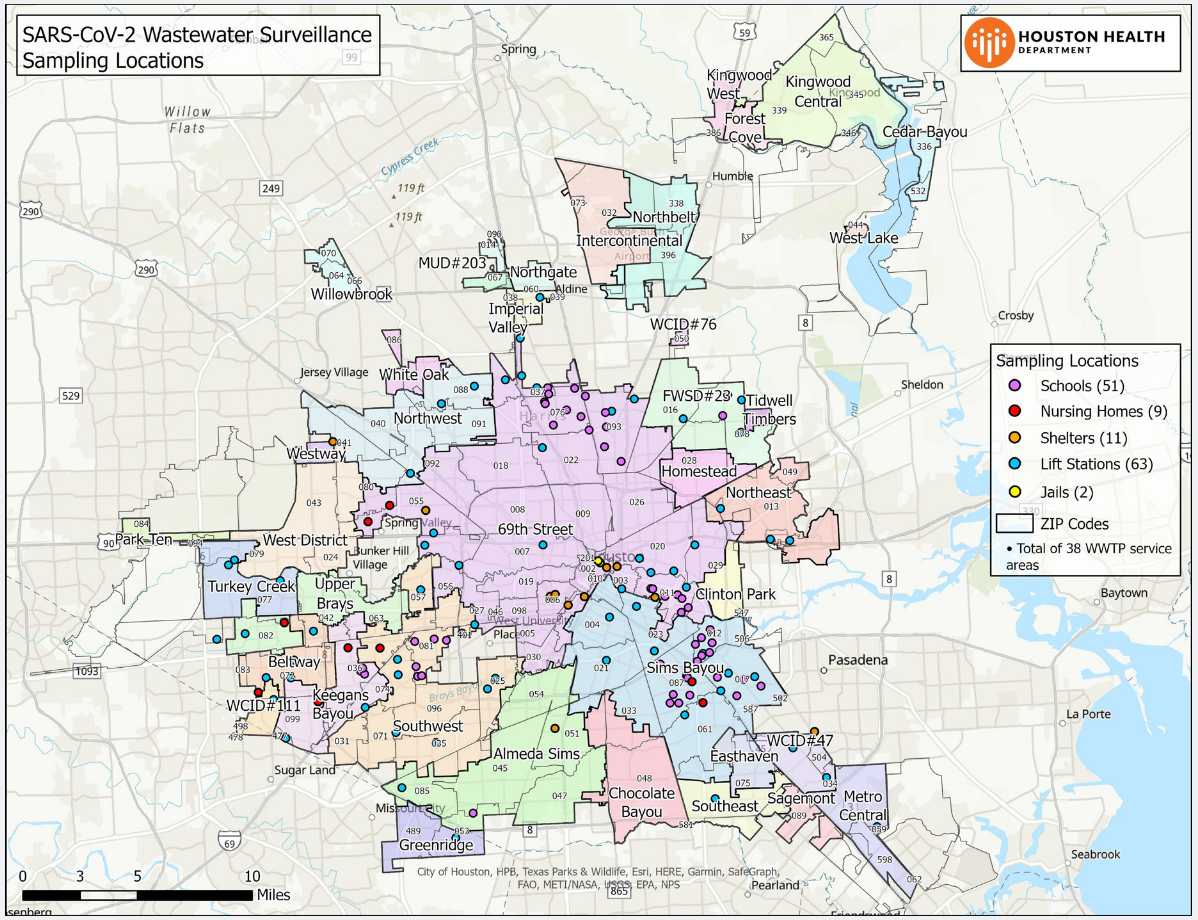
Sampling at the community level

Consider establishing a wastewater monitoring system for a city, municipality, county, or other communities served by multiple wastewater treatment plants with the two-pronged goal of developing regional trends and identifying hot spots of increased viral activity. A helpful first step is to collaborate with the wastewater utility entity (or entities) for the region and to map the sewage system. See Figure 1 for an example of mapping the wastewater treatment plants. Working with utility partners is crucial because they have the technical expertise to understand the abilities and limitations of the sewage system in your region and will typically be the team performing the physical sample collecting. Through your collaboration with the utility partner, you can decide the type of sample and the collection frequency. At the community level, a common sample design is a 24-hour composite measurement collected at wastewater treatment plants at least once a week. For sample site location, it is common to sample at all wastewater treatment plants in the region. At this point, decide what your wastewater monitoring goal is and how you will adjust a sampling plan to create a sustainable sampling plan for your program and its financial resources. There are many ways to iterate on the sampling plans that will still allow for high-quality data. The following are insights into selecting sites and sampling frequency:

Site selections

- Selecting sites based on largest population size will allow you to monitor the largest number of people in the region but will not provide insights into hot spot regions because the geographic area is too large. For example, if a region can only monitor one site, select the largest site to maximize the area monitored.
- Review the population covered by each wastewater treatment plant and decide whether any sites contain certain populations that align with your program's goals. Sampling sites that serve vulnerable communities and those without access to the health care can guide resources to help these communities. For example, if you are interested in monitoring areas with low testing rates, review the testing rates of the population served by each wastewater treatment plant and select accordingly.
- Understand the geographical makeup of the wastewater treatment plant so you can better decide on which sites to monitor. For example, if a site is in a remote location, consider incorporating extra resources to ensure the sample can be transported to the lab properly.
- Consider selecting lift stations, or other intermediary sites that wastewater travels through as it makes its way to a wastewater treatment plant, to obtain a more geographically refined approach. The sites allow for more granularity in geographic wastewater viral trends, but they often do not have the same resources available at wastewater treatment plants. For example, flow rate from wastewater treatment plants is easy to obtain and can be used by the data analysis team for normalization. Flow rate might not be as easy to obtain at sites such as lift stations, so they must be estimated based on population size.

Figure 1. Map of sampling locations in Houston, Texas



Note: The Houston Health Department coordinated with the utility partner, Houston Public Works, to create the map of the wastewater treatment plants, lift stations, and facility sampling locations.

Sampling frequency

- Pick an acceptable data timeliness for your program and select sample frequency based on that. For example, the utility partner is able to sample three times a week, but the laboratories can only handle two samples per week. If the sample frequency abilities of different parts of the program do not match, then data timeliness will be an issue.
- Decide how important flexibility and consistency are to the program. For example, if the data analysis team compares the samples between weeks, collect the wastewater samples on the same day each week to maintain consistency.
- Consider time between samples and the corresponding days of the week. If sampling more than twice a week, it is common to allow at least one day between sample collection dates to gain a larger temporal scale. For example, if it is important to the goal of the program that you monitor the weekend trends and weekday trends in a region, sample on Saturdays and Wednesdays.

Sampling at the institution level

It is also important to consider monitoring at specific facilities, such as congregate living facilities, college dorms, schools, jails, or large community working environments. Generally, you will collect composite samples at manholes with flow coming from only the facility intended for monitoring. Unlike wastewater treatment plants, the facility composite samples can cover shorter time frames, overlapping with when the facility population commonly uses the restroom. For example, an elementary school only needs an eight-hour composite sample for the hours that schools are in session. The facilities selected for monitoring are commonly in areas of high viral load for the community or when the population is at high risk of poor health outcomes. See Figure 2 for an example of selecting school sites with school populations that draw from a ZIP code with high COVID-19 burden. There are many aspects you can tailor facility wastewater monitoring to, so review what aligns with your program's goal and financial resources.

Site elections

- Physically inspect the manholes associated with the site for sampling viability. The manhole will need enough flow for sample collection, to flow from the human waste (for example, from restrooms and not the kitchen sinks), and to be physically accessible for the team to safely sample.
- Consider what sites are associated with outbreaks in your region and the importance to your program of capturing those outbreaks. For example, if tourists and travelers are the first to bring the new variants to your region, select to sample at the airport.
- Collaborate with epidemiologists and public health outreach teams to identify facilities of vulnerable populations—for example, a large shelter has communal living instead of individual rooms and caters to people older than age 60.
- Consider deploying passive samplers to identify outbreaks in facilities where symptoms exist, but clinical sampling does not occur. On these types of deployments, it is important that the sampler is deployed in such a manner that it does not cause a clog in the pipe.

An environmental investigator from the Houston Health Department collects a sample from a manhole site in Houston, Texas.

Sampling frequency

- If the facility is in a highly affected area and interventions are underway, consider sampling multiple times a week because it is beneficial to catch changing environments early. Further outbreaks at specific locations might be an early indicator of outbreaks in the larger community.
- If the facility is part of a larger system (for example, it contributes to a wastewater treatment plan that is monitored), then weekly measurements provide a strong indicator of viral levels within each relevant subpopulation.
- If the decisions on daily testing of people in the facility are made based on the wastewater monitoring, then consider the importance of sampling daily. For example, the students in a college dorm will go to university testing sites based on the wastewater results.

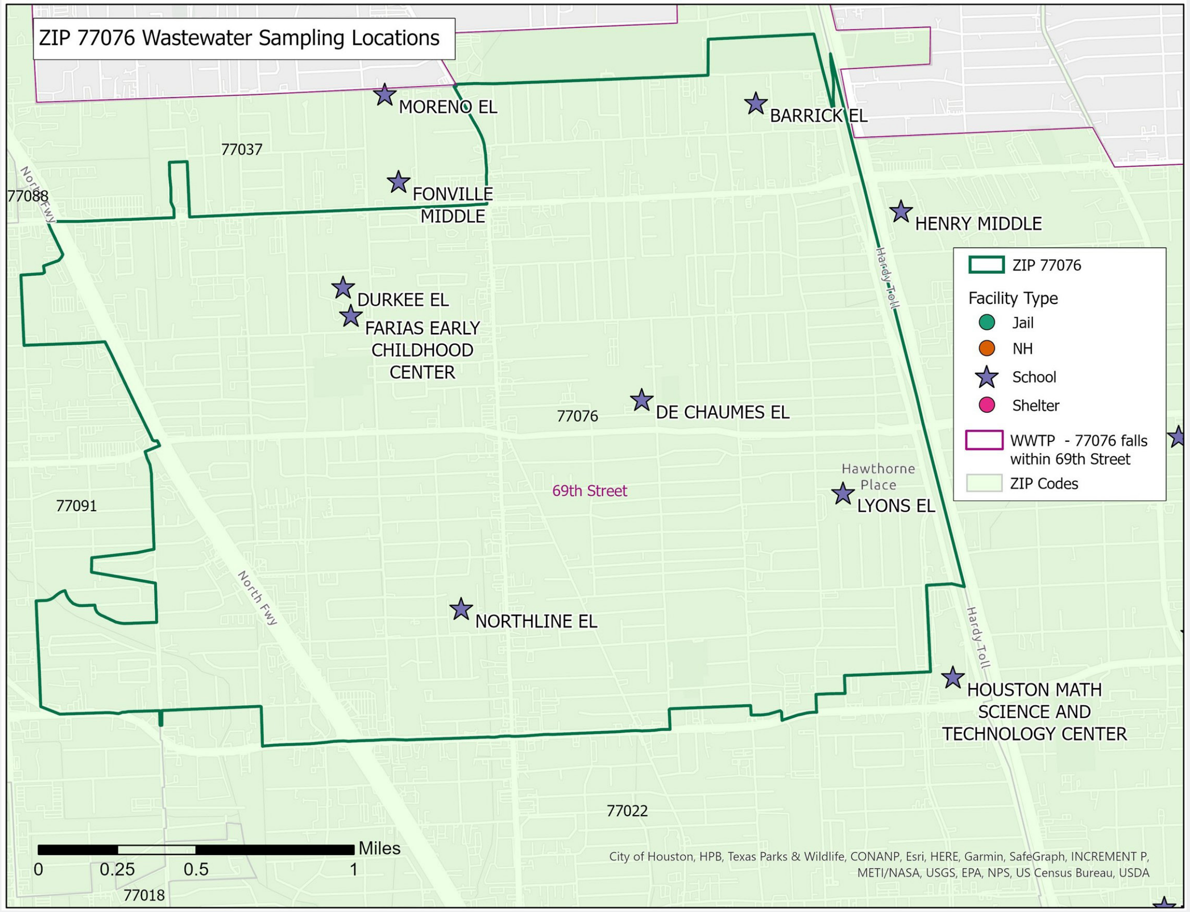
Adaptive sampling

Adaptive sampling provides the opportunity to optimize resources and maximize the information gleaned from the wastewater monitoring system. Take the last example of intense sampling for an institution, such as a university. Some universities rotated the locations of the limited number of autosamplers they had to cover all dorms within a week. This strategy kept them within budget and provided the necessary information to manage outbreaks at the university. For regional sampling, after you have established baselines, you can sample areas with low populations and low active virus levels less frequently and still maintain the integrity of the regional sampling program. Similarly, as virus levels trail off, less frequent sampling (for example, monthly) of individual facilities will still provide the opportunity to identify re-emergence of the disease at specific locations. Adaptive sample designs can be informed by other information regularly monitored by health departments, such as the community case counts, emergency room visits, or number of prescriptions issued. Adaptive sample designs can be simple, like deciding to decrease sampling frequency at all sites, or can entail more complicated models that optimize sampling frequency and location.

Data quality and variability

The are many ways to sample and quantify virus levels within wastewater, and the scientific and engineering community is quickly advancing this important technology. The current gold standard sample is a 24-hour flow-weighted composite sample from an autosampler, and the viable but inexpensive alternatives are grab, Moore swab, and tampon sample collection. For each sample collection method, there will be multiple sources of variability in the measurements that are independent of the viral load. For example, the flow at a wastewater treatment plant changes each day and hour depending on who contributes to the sewage system that day. This can lead to fluctuations in reported viral concentrations in the wastewater sample. For lower cost sampling approaches, such as grab samples, one would expect the variation from sampling to be higher because the variability of the snapshot of the sewage use will be higher

Figure 2. A map of ZIP code 77076 in Houston, Texas, and all nearby public schools with viable manholes for school-level wastewater monitoring



City of Houston, HPB, Texas Parks & Wildlife, CONANP, Esri, HERE, Garmin, SafeGraph, INCREMENT P, METI/NASA, USGS, EPA, NPS, US Census Bureau, USDA

Notes: A goal of the wastewater monitoring program in Houston, Texas is to focus public health monitoring initiatives in ZIP codes with higher COVID-19 burden. As such, all schools with viable manholes for wastewater monitoring were selected to be part of the Houston wastewater monitoring program.

for a grab sample (one moment in time) than a composite sample (15-minute intervals over a 24-hour period). Variability might also be introduced from to the nature of sewage systems, which can be unique across municipalities. For example, gray water additions and industrial inputs into a sewage system are different in different areas, and both can contribute to the variability of wastewater data.

Statistical methodologies estimating the virus level can overcome the inherent sampling and measurement error. See Figure 3 for an example of the variability seen in weekly 24-hour flow-weighted composite sample results at a sewershed in Houston, Texas and the statistical trend model fit to these results. If you are regularly sampling your wastewater system, the viral load trends will be apparent, but it is important to investigate the source of this change and not immediately assume that it indicates a real change in virus levels. To illustrate, a wastewater treatment plant in Houston went through a three-week process of purposefully re-processing the wastewater flowing through the plant. This in turn resulted in dramatically reduced virus levels in the weekly sample, falling well outside of what was expected for the wastewater treatment plant. Because of the sampling frequency and sampling locations of this regional wastewater monitoring system, the dramatic change was identified as a process change and not a change in the virus level for the community sampled, so the samples from that time could not be used.

Conclusion

A wastewater sampling plan for public health use can be designed in numerous ways that differ in the number of sites sampled, sample collection method, or sampling frequency. This flexibility allows wastewater monitoring to be a practical tool for public health monitoring at the local level (facility or community) up to the state level. As more places are looking to implement wastewater monitoring, it is important for them to consider the breadth of sampling schema available and to tailor them to what fits for their community and its goals.

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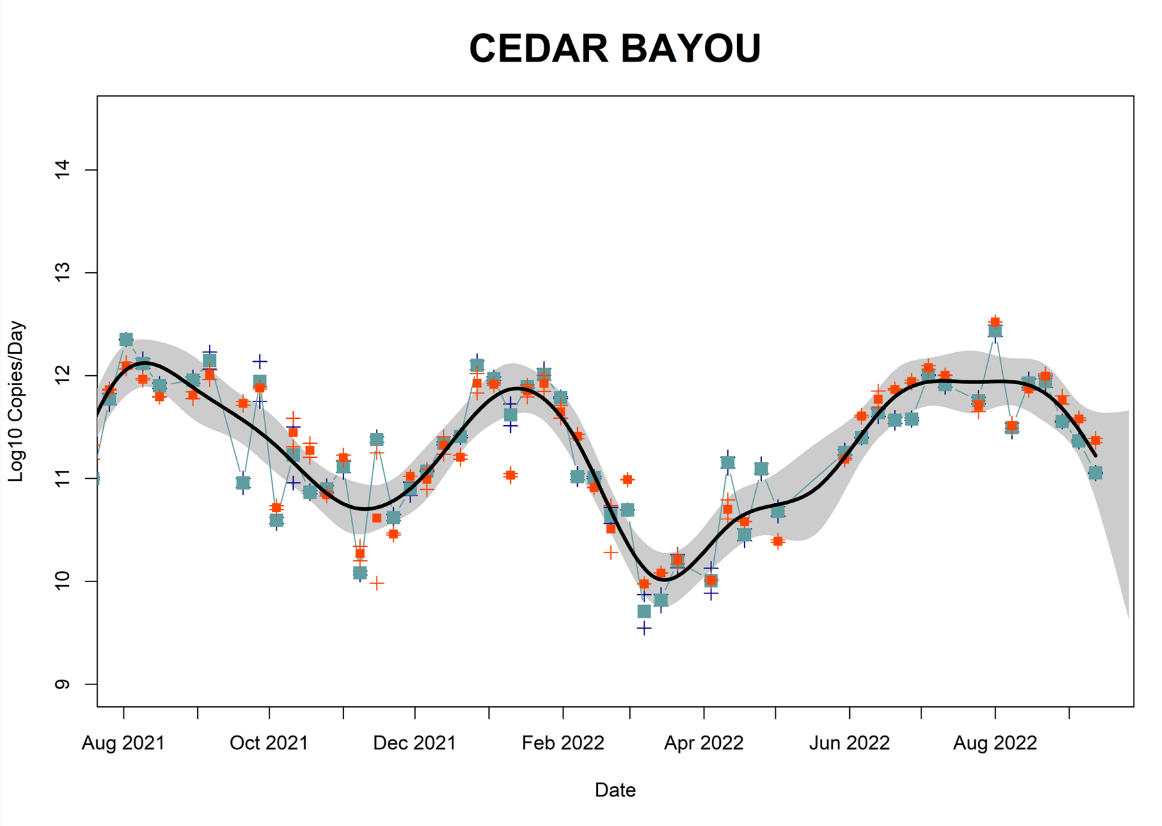
Figure 3. Measurements and trend from one sewershed in Houston, Texas



Notes: Teal and orange points represent aggregate measurements (in copies per day) from two different labs.

How to Monitor Wastewater Ethically to Benefit Vulnerable Communities

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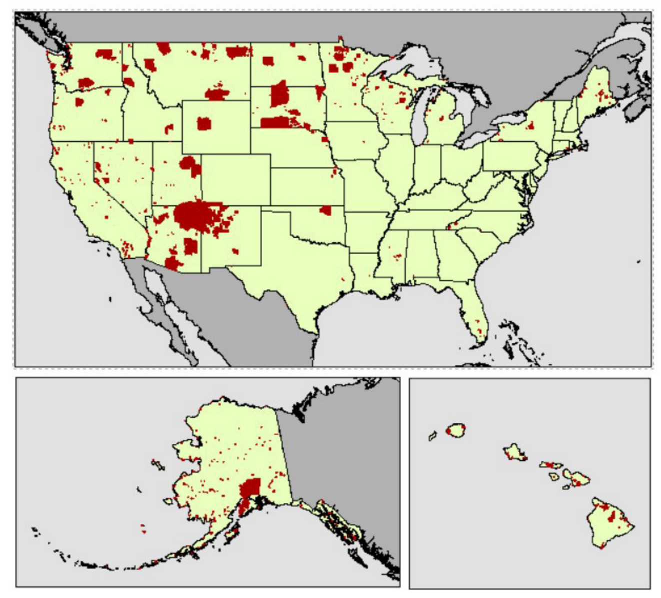
Case Study: WBE in tribal communities

There are 5.4 million American Indians and Alaska Natives in the United States, with 574 distinct tribes communicating in some 130 different languages. Because of historical exploitation and marginalization, public health research among tribal nations can be challenging. Successful application of WBE is possible, however, as an early demonstration study focusing on substance misuse has illustrated (Driver et al. 2022). Unique aspects include tribal autonomy, tribal organization and governance, and unconventional wastewater infrastructure that may necessitate the monitoring of lagoons rather than conventional wastewater treatment plants to assess community health. Tribal buy-in is essential and consent may have to be obtained at multiple levels, e.g., from a Tribal President or Chief as well as from individual Tribal Chapters and Villages, Research Review Boards, and tribal sub-committees.



Notes: Highlighted areas include American Indian reservations, off-reservation trust lands, Alaska Native Villages, and State-designated Tribal areas.

During the COVID-19 pandemic, analysis of community wastewater for biomarkers (that is, genetic, chemical, and biological signature compounds) emerged as a powerful public health tool that can save lives. Yet this method of sifting through the human waste communities excrete might appear threatening and invasive to some people and subpopulations (McClary-Gutierrez et al. 2021), particularly those who have been historically exploited by the Western medical and research establishments. Ethical concerns about wastewater-based epidemiology (WBE) include issues of privacy, data sharing, and potential stigmatization of communities or community members (Jacobs et al. 2021; Keeler et al. 2018). Ethical considerations in WBE are particularly important when monitoring the health of vulnerable populations, such as school children; senior citizens; Indigenous people, and communities of color; prisoners; and refugees. Achieving ethical and socially equitable WBE typically requires thoroughly engaging all community leaders and groups with a vested interest in the resulting data and using custom-tailored protocols and reporting procedures. Further, the perspectives of the community being monitored should be considered first and foremost.

Strategies for using WBE to benefit vulnerable populations

When determining whether to implement WBE in a particular setting or among a potentially vulnerable population, visioning exercises and focus groups can be helpful (Keeler et al. 2015). Answering the following questions can help clarify whether to pursue WBE and how to set it up for success:

- Why are we monitoring wastewater, and who will benefit from it?
- What are the histories, beliefs, religion, and cultural practices of the communities in which monitoring is envisioned and how can their interests and concerns be acknowledged and addressed?
- What information will be collect (and what can be considered optional)?
- Who will have access to the data?
- Who will be included and excluded in the monitoring given the local infrastructure?

- Can the information collected potentially lead to marginalization or stigmatization of individuals, groups, or geographic settings?
- Who are the appropriate entities, diverse communities, and their spokespersons to engage with, and how can a true partnership of equals be created that honors the needs and concerns of all?
- How will the information be communicated?
- Can it be delivered in the preferred methods and channels of communication of community members?

Answering these questions is an important first step in engaging and garnering buy-in from key community stakeholders beyond health agencies and public health researchers. Identifying, engaging, and responding to communities and their stakeholders is a time consuming but essential process. The immediacy required from a public health perspective must be carefully weighed and balanced with the need to gain the trust of community members and to fully understand their concerns and motivations. Anticipating and managing these timing issues and other tensions must be understood as an ongoing process that at times will be frustrating to one or more entities engaged in the partnership.

Implementation logistics

Different, marginalized or underserved communities will have varying informational needs and concerns, but the following steps can improve the chances of successfully implementing WBE for vulnerable populations:

- Determine the interest in and potential benefit of a future WBE campaign. The success and long-term sustainability will be greatest if the community fully supports and demonstrably benefits from the monitoring campaign (Bowes et al. 2022).
- Secure ample funding to perform the planned activities.
- Identify and invite stakeholders and spokespersons into a working group to explore the need for focus groups, visioning exercises, and how to avoid or successfully manage potential sensitivities and issues.
- Determine governance structures and obtain the necessary authorizations (for example, institutional review board review and approval), access to sanitation infrastructure, and data release strategies.
- Convene a public forum to lay out the project, reporting, and anticipated benefits and risks in plain, accessible language, and invite comments on the planned approaches. Doing so helps to build trust, creates buy-in, and aligns stakeholder expectations.
- Continuously share updates and documentation on data acquisition, data communication, and public health actions and benefits that might result from WBE activities.
- List resources and organizations that can help create successful and durable partnerships (for example, the Centers for Disease Control and Prevention's National Wastewater Surveillance System, OneWaterOneHealth, and AquaVitas).

Avoiding pitfalls

Implementation groups should engage in a constant dialogue with spokespersons from all stakeholder groups to learn about and address potential concerns early on and to enable a sustained, successful public health protection campaign. Challenges to WBE implementation can arise from the following areas:

- A lack of communication around the intents and purposes of monitoring
- Biased selection of monitoring sites, resulting in stigmatization of local communities
- Expanded or increased spatial resolution of the monitoring network and the list of target analytes without explicit consent from stakeholders
- Lacking, delayed, or selective communication of monitoring data
- Sharing data with entities that cause community concern (such as law enforcement)
- Overstating of the informational value of the data collected
- Lack of effort to translate obtained information into a culturally acceptable and accessible format
- Lack of strategies and funding to respond to health challenges detected by WBE, leaving communities with threatening information and no way to address it

Conclusion

Implementing a WBE monitoring system for vulnerable communities can be hugely beneficial (Driver et al. 2022) but is not without risk (Jacobs et al 2021; Keeler et al. 2018). Yet, with proper planning, thorough community engagement, and tailored, continuous information exchange between all communities and entities involved, WBE can be implemented and has been demonstrated to protect the health and interests of at-risk populations (Bowes et al. 2021).

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