



## Questions and answers about wastewater surveillance

### Basics

#### 1. What are the National Wastewater Surveillance System (NWSS) Centers of Excellence (CoEs)?

NWSS established Centers of Excellence (CoEs) in August 2022 to advance wastewater surveillance for public health. Currently, NWSS funds four CoEs:

- California (est. 2023), led by California Department of Public Health with Stanford University and Emory University
- Colorado (est. 2022), led by Colorado Department of Public Health and Environment with the University of Denver ([Colorado NWSS CoE website](#))
- Houston (est. 2022), led by Houston Health Department with Houston Public Works and Rice University ([Houston NWSS CoE website](#))
- Wisconsin (est. 2023), led by Wisconsin Department of Health Services, Wisconsin State Laboratory of Hygiene at the University of Wisconsin–Madison, and the University of Wisconsin–Milwaukee School of Freshwater Sciences

### Starting and implementing a Wastewater Surveillance Program

#### 2. What factors should be considered when selecting target areas or communities for wastewater surveillance?

The process requires careful consideration of epidemiological, logistical, and technical factors. It involves assessing geographic dispersion, accessibility, and available epidemiological data. Centers for Disease Control and Prevention assert that state, tribal, local, and territorial public health needs should drive wastewater surveillance sampling, with active engagement from wastewater treatment plants ([CDC, 2023](#)). Strategic selection of sampling points within accessible wastewater infrastructure is vital, aligning with laboratory capabilities. A sampling strategy should balance resources, testing capacity, and public health data needs, potentially needing adjustments over time as scientific understanding and public health needs evolve.

#### 3. How do health departments work with other agencies or partners to collect and analyze wastewater samples?

Health departments collaborate with agencies and partners to collect and analyze wastewater samples. This includes forming partnerships with local wastewater utilities, public health agencies, and academic institutions to access sampling points. Health departments actively participate in and lead regular meetings to discuss logistics and results. Collaborations with state and federal environmental agencies ensure regulatory compliance, and working with academic institutions contributes to method development. Cooperation with equipped laboratories is essential for sample analysis, and stakeholder involvement helps in interpreting and communicating surveillance findings effectively to the public ([CDC, 2023](#)).

## Sample collection

### **4. How frequently should wastewater samples be collected to provide meaningful and actionable information for public health interventions?**

Ideally, wastewater surveillance teams should collect samples at least twice weekly at the community level to capture variations in viral shedding patterns over time, with more frequent sampling recommended in less populated areas or during dynamic public health situations ([Holst et al., 2022](#)). Collaborating with stakeholders to determine the optimal sampling frequency is important to establish a robust dataset for capturing trend variations, new variants, and assessing public health interventions. When determining the frequency, health departments should consider factors such as virus prevalence, RNA stability in wastewater, and sample analysis capacity.

### **5. How do you determine the effective level of surveillance (i.e., building-level, upstream, or sewershed)?**

According to the CDC, sewershed-level surveillance may be prioritized for capturing trends ([CDC, 2023](#)). Conversely, in areas with diverse wastewater sources, a focus on individual buildings may provide more detailed information. Building-level surveillance can be better suited for identifying localized outbreaks. Considering spatial and temporal resolution, collaborative discussions with health departments, academic partners, and stakeholders tailor the surveillance level to community needs. Additionally, integrating upstream surveillance methods, such as monitoring wastewater samples from various points in the sewage system, can complement these efforts by providing early indicators of disease prevalence and potential outbreaks.

### **6. What's the difference between a composite and grab samples?**

A grab sample is a single collection of wastewater at a specific time and location, offering a snapshot of conditions. In contrast, a composite sample, facilitated by an autosampler, is automatically collected and mixed with multiple grab samples taken at predetermined intervals over a specified duration. This automated process provides a more comprehensive representation of temporal variations in wastewater characteristics. Surveillance objectives determine the choice between grab and composite sampling, with grab samples suitable for specific events and composite samples offering a broader view of trends over time in wastewater characteristics. ([CDC, 2023](#))

### **7. What is passive sampling?**

Passive sampling offers an alternative to traditional methods. Operators place absorbent materials, such as cotton gauze, cellulose sponges, or filters, in housings or hang them from strings within the wastewater flow to collect indicators or markers that may signal the presence of pathogens ([Schang et al., 2021](#)). This method is simpler and more cost-effective to implement



compared to composite and grab sampling. However, unlike those methods, it does not provide quantitative results.

## Laboratory techniques

### 8. Which certifications are required for wastewater testing?

For wastewater testing, laboratories prioritize biosafety training and adherence to Good Laboratory Practices (GLP) for a secure testing environment and reliable results. While certifications like Collaborative Institutional Training Initiative (CITI) or College of American Pathologists (CAP) accreditation aren't mandatory, they contribute to maintaining testing integrity. Although specific certifications such as Environmental Protection Agency (EPA) or International Organization for Standardization (ISO) 17025 may become expected, current best practice standards should be followed ([APHL, 2022](#)).

### 9. Which biosafety level is wastewater considered?

Wastewater is generally classified as Biosafety Level 2 (BSL-2) for surveillance purposes, acknowledging the potential presence of microorganisms. BSL-2 practices include employing standard microbiological techniques, wearing personal protective equipment, and implementing containment measures to minimize risks to laboratory personnel. Adhering to BSL-2 guidelines, including proper handling and decontamination, is crucial for safe laboratory analysis and sample integrity.

### 10. How is wastewater handling different from other specimens?

Handling wastewater for surveillance requires adherence to Biosafety Level 2 (BSL-2) precautions due to potential infectious agents. Use specific techniques, such as grab or composite sampling, along with proper container and preservation methods. Take careful measures to prevent cross-contamination during sample processing, which may include concentration methods like centrifugation or filtration, followed by nucleic acid extraction. Ensuring the safety of lab personnel and result reliability involves using appropriate personal protective equipment and decontamination procedures throughout the process.

### 11. What equipment is used for processing wastewater samples?

Wastewater testing uses specialized tools for accuracy and precision, including refrigerated centrifuges, filtration, bead capture, or precipitation methods for sample concentration. Automated liquid handlers ensure consistent and efficient processing. For nucleic acid target quantification, real-time quantitative polymerase chain reaction (qPCR), digital polymerase chain reaction (dPCR), or digital droplet polymerase chain reaction (ddPCR) machines are employed, enhancing result sensitivity and specificity.

### 12. How is sample integrity maintained during transportation to the laboratory?



Sterile containers, immediate refrigeration, or ice storage prevent contamination and preserve sample integrity. For longer transit, use stabilization techniques. Secure packaging and adherence to Biosafety Level 2 (BSL-2) protocols ensure samples reach the laboratory intact, guaranteeing reliable testing.

### **13. What is typically analyzed in wastewater testing?**

Analyzing a community's wastewater often focuses on the presence of pathogens like viruses, including SARS-CoV-2, as well as bacteria, antimicrobial resistance (AMR) genes, and fungal targets. This comprehensive approach enables tracking not only the spread of infectious diseases, but also the potential emergence and dissemination of antimicrobial resistance and fungal infections. By offering an aggregate snapshot of community health and environmental conditions, wastewater testing serves as a complementary surveillance system to existing clinical surveillance systems for pathogens of public health concern.

### **14. How is the accuracy and precision of test results ensured in the laboratory? (Related: What quality control measures are in place to monitor the reliability of test results?)**

Laboratories ensure the accuracy and precision of test results by following their quality manual guidelines and developing validated wastewater assays. Relevant personnel, such as laboratory managers and quality assurance officers, define and approve validation study plans with specific acceptance criteria. Guidelines from organizations like the Association of Public Health Laboratories offer best practices for testing quality assurance measures ([APHL, 2022](#)).

### **15. How long does it take to receive test results for wastewater samples?**

Depending on the method, you can perform wastewater testing and report results within 24 hours after receipt of the sample. However, expectations for turn-around times for sample reporting may vary. Factors such as laboratory capacity, volume, holidays, weekends, and chosen method may impact the timeline.

### **16. Are there specific guidelines for sample disposal after testing is completed?**

Yes. Specific guidelines ensure the safe disposal of wastewater samples after testing. Disinfect wastewater with a 10% bleach solution and leave it for 30 minutes before diluting it tenfold with water and disposing of it in the laboratory sink. Wear appropriate personal protection equipment and take precautions to minimize splash-back. Thoroughly rinse sample containers and properly dispose of them to prevent contamination and ensure environmentally responsible disposal.

### **17. What is the laboratory's policy on confidentiality and data security for test results?**

Wastewater testing data security should meet the standards set for all testing within a laboratory. Though there is no protected health information or personally identifiable information associated with testing samples, additional reasons for keeping results confidential may apply to

vulnerable populations and/or the need to assess and review results before they are released to a private or public dashboard. For building-level surveillance, the laboratory may implement additional precautions, such as restricting result dissemination to authorized personnel only.

### **18. Which testing methods are used to analyze wastewater samples?**

Two common testing methods for wastewater samples are qPCR (quantitative Polymerase Chain Reaction) and dPCR/ddPCR (digital PCR/ digital droplet PCR). Both detect target DNA presence and estimate amounts, but dPCR/ddPCR offers highly precise measurements by subdividing the sample into smaller reactions, akin to counting specific items in a large building. These methods are valuable for monitoring wastewater, aiding in pathogen or contaminant detection and quantification.

### **Data collection and analysis**

### **19. How does wastewater surveillance account for variations in population density and wastewater characteristics across different regions within a jurisdiction?**

Wastewater surveillance metrics can account for population variations through a process known as normalization. This involves adjusting the viral concentration data from diverse locations to a standardized scale, considering factors like flow rates and microbial composition in the wastewater sample (e.g., Pepper Mild Mottle Virus [PMMoV]). By doing so, surveillance programs can roughly compare data and trends between regions over time. Continuous refinement and validation of normalization techniques are essential to uphold the effectiveness of surveillance efforts.

### **20. Which software or analytical tools are commonly used for processing and analyzing wastewater surveillance data?**

Tools for statistical analysis and data visualization such as R, Python, Tableau, and SAS are instrumental in deriving meaningful insights from wastewater surveillance data. Geographic Information System (GIS) tools, including ArcGIS and QGIS, assist in spatial mapping and interpretation of surveillance results.

### **21. How is the design of a public-facing wastewater surveillance data dashboard tailored to effectively communicate information to the community?**

Public-facing wastewater surveillance data dashboards share charts and maps that are easy to understand. Plain language is used to explain trends and findings. Helpful features, like tooltips and legends, provide additional context. Dashboard data visualizations emphasize changes in viral concentration over time and comparison of trends between different sewershed areas. Collaboration with communication experts, community members, and laboratory partners ensures the dashboard is accurate and easy to use.

### **22. How do internal and public-facing dashboards differ? Why should you have both?**



Having both ensures that experts have what they need and that the community stays informed and engaged.

Internal dashboards are for professionals within health departments and utilities to provide detailed, real-time data and interactive analytic tools. Internal dashboards also help experts make decisions with detailed data, while public dashboards raise community awareness and trust.

The audience for public-facing dashboards is the general public, and they use an easy-to-understand design and plain language to communicate general trends. Public dashboards also include FAQs and straightforward narrative text to help the public understand the data

## **Public Health Action and Communication**

### **23. How is the data from wastewater surveillance used with other public health data to inform decision-making processes?**

Wastewater surveillance data is often paired with epidemiological information like reported clinical cases, hospitalizations, and testing rates to inform decision-making. This allows for comprehensive analysis to identify hotspots, assess outbreak dynamics, evaluate intervention effectiveness, optimize resource allocation, and refine public health strategies. Multiple sources of disease surveillance data facilitate timely and targeted interventions by providing nuanced insights into local disease dynamics.

### **24. How does the identification of specific pathogens in wastewater contribute to early detection and response to potential disease outbreaks in a community?**

Wastewater surveillance enables response to potential disease outbreaks by monitoring viral shedding or the presence or absence of a specific pathogen within the community, and it may provide a leading indicator of infection levels before clinical cases are reported. Identifying pathogens like SARS-CoV-2 in wastewater helps public health authorities detect emerging hotspots, assess the magnitude of viral circulation, and identify trends in viral activity. Wastewater surveillance systems may allow for proactive and targeted interventions, including increased clinical testing, contact tracing, vaccinations, and resource allocation to mitigate disease spread. Thus, wastewater surveillance enhances the timeliness and effectiveness of community-level responses to infectious diseases.

### **25. How is information from wastewater surveillance communicated to the public? What measures are taken to ensure clear, transparent, and understandable messaging regarding potential health risks?**

Information from wastewater surveillance is communicated to the public through state and local public health departments, who tailor communication strategies to their jurisdictions' needs.



User-friendly public-facing dashboards present key findings in visually intuitive formats with plain language explanations, emphasizing transparency about data limitations and the evolving nature of surveillance results. As advancements in the wastewater surveillance field develop, it is important for public health experts to regularly review and update communication strategies to align with evolving best practices and national guidance.