Public Health Laboratory Guide to Microbial Wastewater Surveillance

June 2026

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Overview

The purpose of this document is to provide recommendations for implementing and sustaining microbial wastewater surveillance programs in public health laboratories (PHLs) to support public health action. It expands upon prior Association of Public Health Laboratories (APHL) guidance developed during the COVID-19 pandemic, which focused on SARS-CoV-2, to include a broader range of DNA- and RNA-based targets of public health importance, including viral, bacterial, fungal, parasitic and other microbial indicators (i.e., antimicrobial resistance genes).

The landscape of microbial wastewater surveillance in the United States has continued to evolve since the previous guidance. PHLs at state, tribal, local and territorial levels remain central to the implementation of wastewater surveillance programs and continue to collaborate across multidisciplinary networks including wastewater utilities, health departments, federal and other national, commercial and academic partners to yield insights into community health.

This document describes PHL and partner key functions and updates the roles of the US Centers for Disease Control and Prevention (CDC) National Wastewater Surveillance System (NWSS) and the NWSS Centers of Excellence (CoE) in supporting robust wastewater surveillance infrastructure in the United States. Topics covered include onboarding new targets, selecting appropriate laboratory methods, ensuring high-quality wastewater data and describing core components of common microbial-based wastewater surveillance workflows, data and reporting practices and ethical considerations. Throughout, this guide emphasizes flexibility, recognizing that wastewater surveillance programs vary in capacity, goals, local infrastructure and available resources.

Microbial Wastewater Surveillance

Microbial wastewater surveillance is the systematic collection, processing and testing of untreated wastewater (hereafter referred to as wastewater) to detect, quantify or characterize microbial targets that might reflect human infections or other public health-relevant exposures within a defined community. A typical microbial wastewater surveillance workflow begins with human waste entering a municipal wastewater system, followed by wastewater sampling at a potential range of sewershed sites (e.g., community wastewater utilities, sub-sewershed systems, and facility-level). Samples are then transported to a laboratory for processing and testing, with results interpreted, reported and integrated with other information by health departments to inform public health action. By measuring microbial

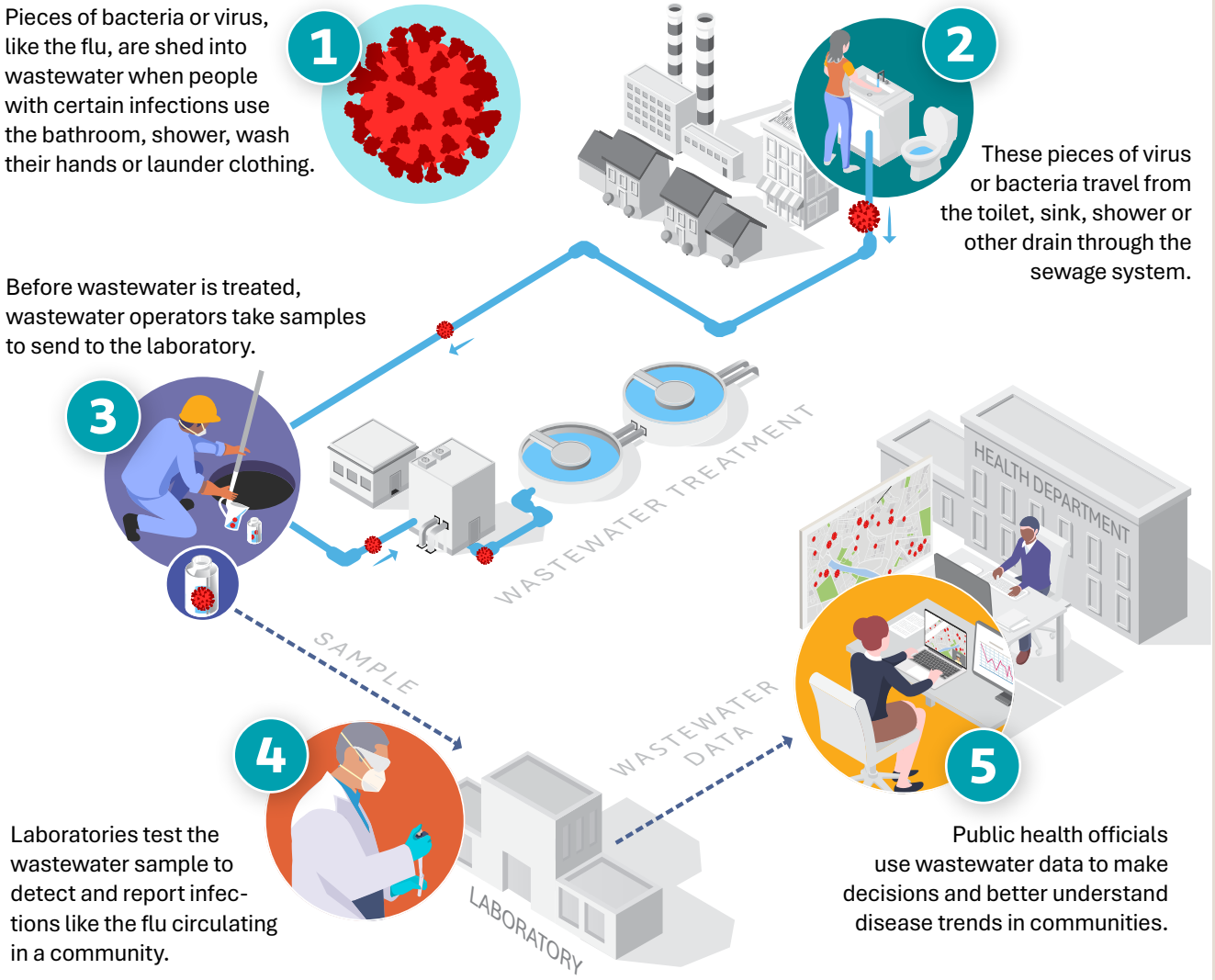
Key Advantages of Microbial Wastewater Surveillance

- **Early Warning:** May detect community infections before clinical indicators arise.
- **Community Coverage:** Captures population-level data independent of healthcare access, healthcare-seeking behavior, testing availability or symptom presentation.
- **Efficiency:** Contains input from hundreds to millions of individuals in a single sample that can be tested for multiple microbial targets.
- **Scalability:** Implementable wherever wastewater collection systems exist.
- **Complementarity:** Enhances and contextualizes other public health data sources.
- **Preserves Privacy:** Microbial wastewater data alone cannot be used to identify infected individuals.
- **Cost Effective:** Relatively low cost compared to individual clinical testing and provides opportunities to maximize limited resource allocation.

genetic material shed in human waste (e.g., feces or urine), wastewater surveillance provides community-level information on target trends that are independent of symptomatic status, clinical testing access or healthcare-seeking behavior (**Figure 1**).

Microbial wastewater surveillance supports a range of public health objectives (referred to as use cases in this document), and programs may focus on one or multiple intended use cases depending on capability, capacity, target characteristics and the information needs of public health partners (**Table 1, page 6**). Microbial wastewater surveillance complements but does not replace clinical and other public health surveillance systems, and when integrated with epidemiologic and environmental data can provide a more complete and timelier picture of community-level health. Wastewater surveillance data can be particularly valuable when other public health surveillance systems are limited, when individuals cannot or do not wish to access healthcare, when testing rates decline or home testing use is prominent, when infection status may carry stigma or when infections are predominantly asymptomatic.

Figure 1: Overview of microbial wastewater surveillance from human waste inputs through sample collection, laboratory testing and interpretation to inform public health action*



* Source: CDC, How Wastewater Monitoring Works

Table 1: Example microbial wastewater surveillance use case scenarios

Use Case	Description	Example
Early Detection of Emerging Targets	Some targets may be detectable before increases are seen in clinical data, offering critical lead time for preparedness, response planning and resource allocation.	Wastewater Surveillance for Measles Virus During a Measles Outbreak — Colorado, August 2025
Community-level Trend Monitoring	Repeated sampling provides insight into temporal trends, enabling tracking of increases, decreases or sustained levels of infection or exposure within a community.	Notes from the Field: Genomic and Wastewater Surveillance Data to Guide a Hepatitis A Outbreak Response — Los Angeles County, March 2024–June 2024
Variant and Strain Identification	Detection of variants of concern, lineage tracking, vaccine/antimicrobial resistance gene occurrence and identification of mutations that may influence public health risk.	Early Evidence of the SARS-CoV-2 B.1.1.529 (Omicron) Variant in Community Wastewater — United States, November–December 2021
Targeted Pathogen Sequencing and Detection	Wide-scale targeted approaches, such as targeted amplicon sequencing or hybrid capture of many known pathogens, can provide broad detection capabilities and help characterize pathogen diversity.	Aircraft Wastewater Surveillance for Early Detection of SARS-CoV-2 Variants — John F. Kennedy International Airport, New York City, August–September 2022
Agnostic or Broad Microbial Community Monitoring	Metagenomic sequencing enables detection of a wide range of microbial taxa and their genes without prior knowledge of specific targets, supporting early warning of novel pathogens or other public health-relevant targets.	Potential applications emerging in PHL settings, but currently exploratory. See APHL *Lab Matters* Magazine: Metagenomics May Offer Value Across Many Areas of Public Health, Summer 2025

More Information

- APHL: National Trends in Wastewater Surveillance 2023 Survey Report
- APHL: National Trends in Wastewater Surveillance 2025 Survey Report
- CDC: Wastewater Monitoring Fact Sheet
- European Union: Wastewater Observatory for Public Health
- National Academy of Sciences: Community Wastewater-based Infectious Disease Surveillance
- National Association of County and City Health Officials (NACCHO): Wastewater Surveillance Resource Library
- WHO: Wastewater and environmental surveillance for one or more pathogens: guidance on prioritization, implementation, and integration
- Aircraft Wastewater Surveillance for Early Detection of SARS-CoV-2 Variants — John F. Kennedy International Airport, New York City, August–September 2022 | Notes from the Field, *MMWR*
- Early Evidence of the SARS-CoV-2 B.1.1.529 (Omicron) Variant in Community Wastewater — United States, November–December 2021 | Notes from the Field, *MMWR*
- Genomic and Wastewater Surveillance Data to Guide a Hepatitis A Outbreak Response — Los Angeles County, March 2024–June 2024 | Notes from the Field, *MMWR*
- Metagenomics May Offer Value Across Many Areas of Public Health | APHL *Lab Matters*, Summer 2025
- Wastewater Surveillance Data as a Complement to Emergency Department Visit Data for Tracking Incidence of Influenza A and Respiratory Syncytial Virus — Wisconsin, August 2022–March 2023 | Notes from the Field, *MMWR*
- Wastewater Surveillance for Measles Virus During a Measles Outbreak — Colorado, August 2025 | Notes from the Field, *MMWR*

Wastewater Surveillance in the US

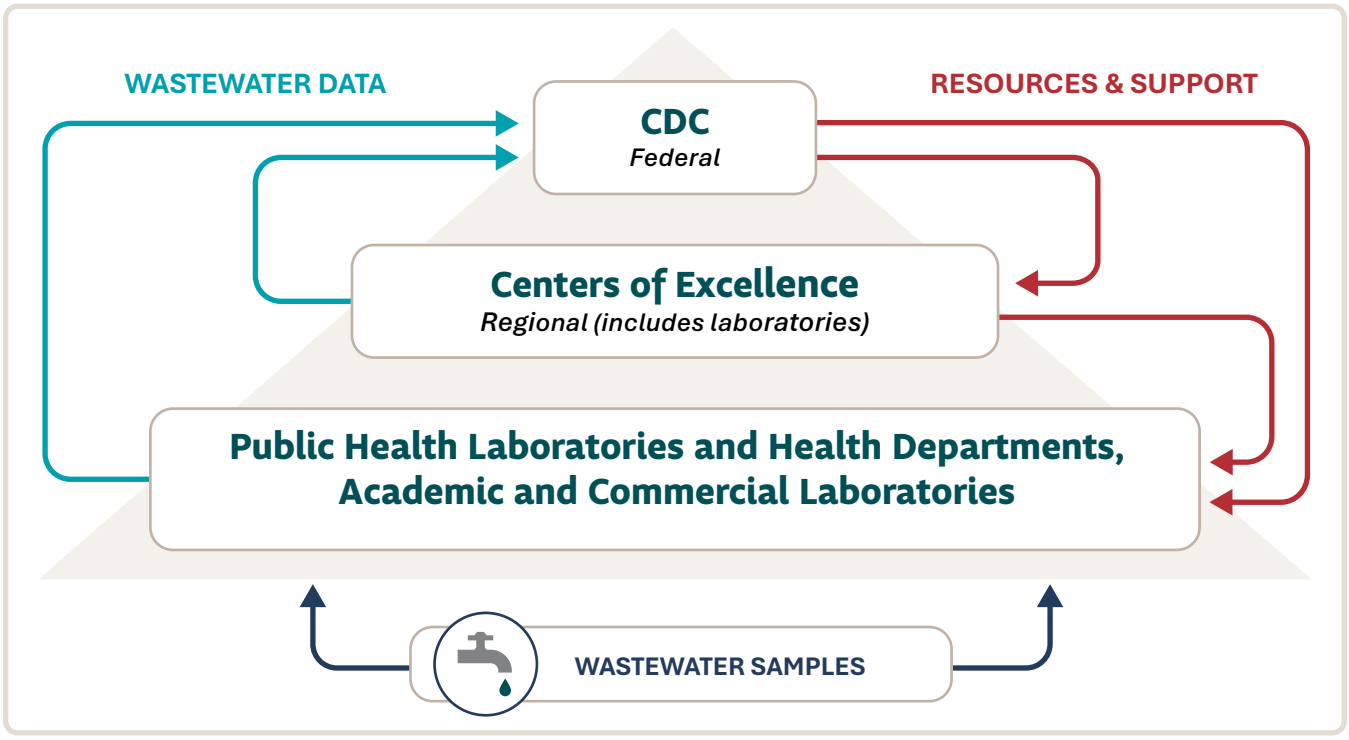
Community-level wastewater surveillance for microbial targets has gained momentum in the years since the pandemic. Capability and capacity for testing sewersheds has expanded through the development of NWSS, a collaborative effort bridging public health laboratories, utilities, academics and federal partners. Through NWSS, CDC provides technical and financial resources to support PHLs in testing priority microbial targets in wastewater to inform public health practice.

Wastewater surveillance outside of NWSS is conducted for research and jurisdictional purposes, in coordination with other key partners (**Table 2, page 11**).

National Wastewater Surveillance System

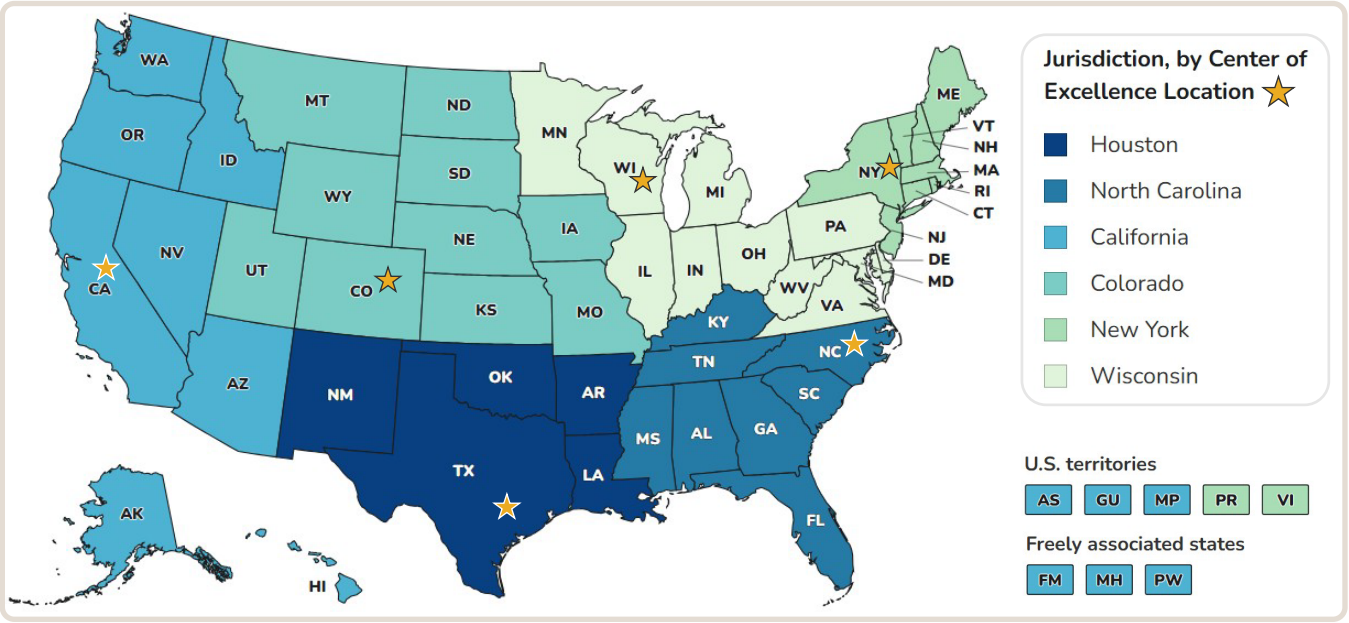
NWSS is designed to support the use of national wastewater data for monitoring infectious diseases and informing public health action. The system enables data collected by participating jurisdictions (e.g., public health departments and laboratories) to be integrated and presented in a consistent manner, allowing clinicians, public health officials and the public to better understand infectious disease activity within communities. PHLs and health departments, as well as commercial and academic laboratories, collect and report wastewater data to CDC (**Figure 2**). Through national coordination and in collaboration with PHLs and partners operating at state, tribal, local and territorial levels, NWSS aggregates wastewater surveillance data from approximately 1,280 communities, representing more than 146 million people, roughly 43 percent of the United States population. A key aspect of the program is flexibility. The system can be adapted to changing public health priorities, guiding emergency response, responding to emerging infections and preparing for bioterrorism and future pandemics. In addition, NWSS provides guidance on sampling strategy, testing methods, data reporting and analytics as well as public health interpretation and use of wastewater monitoring data to promote the accuracy and quality of wastewater data.

Figure 2: NWSS



To further strengthen microbial wastewater surveillance capacity, effectiveness, coordination and innovation, NWSS established a network of collaborative CoE partnerships among public health agencies, PHLs, academic institutions and wastewater utilities to serve as regional leaders in wastewater surveillance implementation. Currently, the CoE network consists of six consortia strategically located across the continental United States, each providing support to jurisdictions in their region, while collectively contributing data and expertise at a national level (**Figure 2, page 7**). The areas of coverage for each CoE are illustrated in **Figure 3**.

Figure 3: CDC-supported NWSS CoE areas of coverage*



The CoE network plays a critical role in addressing shared challenges and advancing best practices across the wastewater surveillance community, including PHLs. CoEs develop and share protocols, provide training and curated resource repositories and support access to data analytics and modeling expertise. They offer troubleshooting assistance, and some provide access to control materials that support method development, validation and quality management. The CoE network also includes focused work groups to address technical and operational challenges on topics such as laboratory methodologies, epidemiology, data analytics and visualization, pathogen genomics and engagement. Through these functions, CoE activities help harmonize approaches across jurisdictions, encouraging innovation and adaptation.

PHLs play a central and evolving role in the design, implementation, innovation and sustainment of microbial wastewater surveillance programs. PHLs are well positioned to lead microbial wastewater surveillance programs alongside partners at

More Information

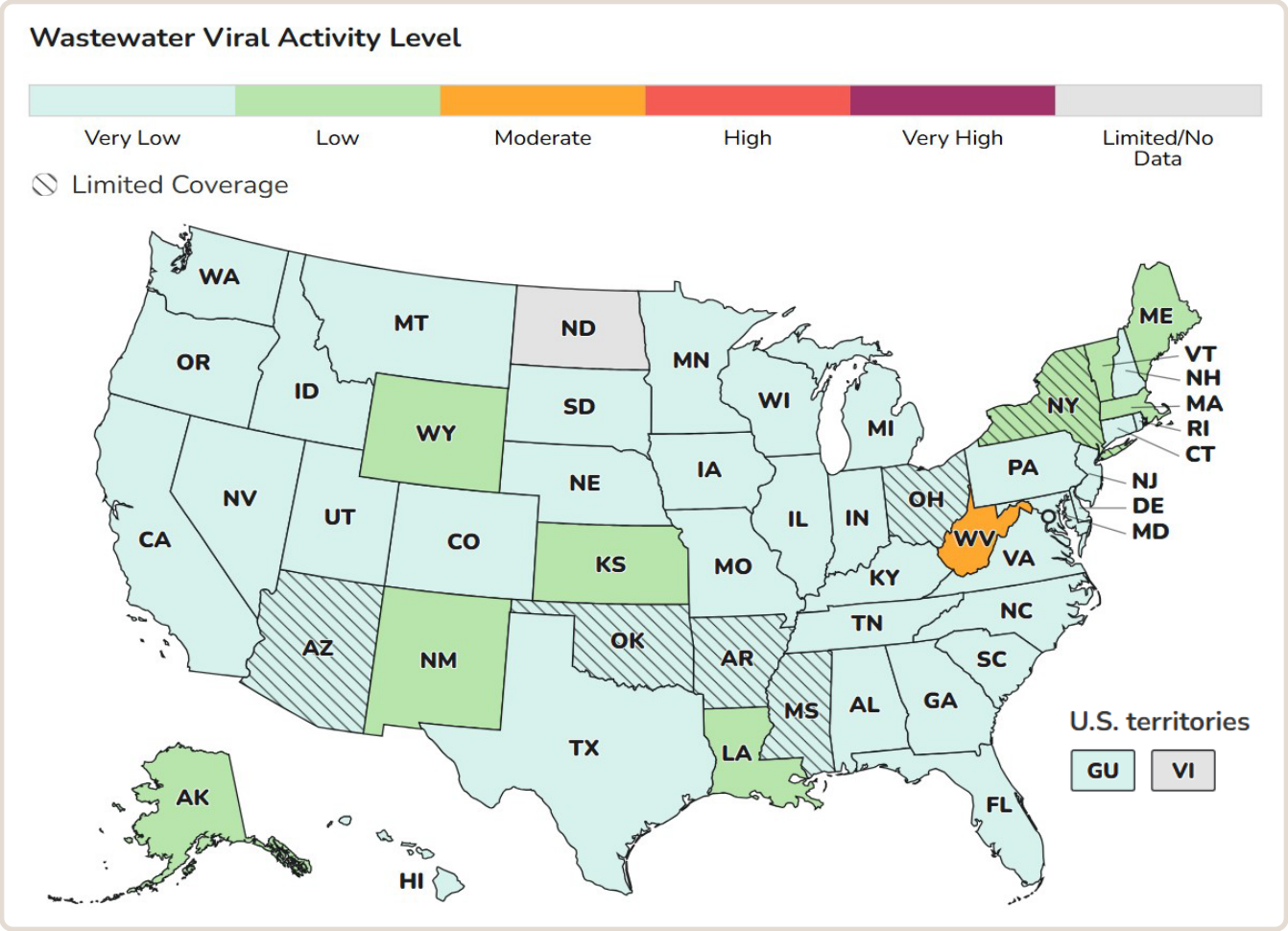
- CDC National Wastewater Surveillance System website
- CDC NWSS Centers of Excellence
- APHL Wastewater Surveillance
- California Wastewater Surveillance Program
- Colorado Center of Excellence
- Houston NWSS CoE: Houston Wastewater Epidemiology
- New York Center of Excellence
- North Carolina Center of Excellence
- Wisconsin Center of Excellence
- WastewaterSCAN Public Wastewater Monitoring Project

* Source: CDC Wastewater Monitoring Centers of Excellence

state, tribal, local, territorial, regional and national scales because they possess the following foundational capabilities. PHLs are integrated into the public health system, including networks focused on public health surveillance, disease detection and emergency readiness and response. They are staffed with professionals experienced in many of the tools and techniques required for microbial wastewater surveillance and have access to analytical instrument platforms needed for wastewater testing workflows. PHLs maintain workforce development programs that support staff training and retention. They have active quality management systems to ensure accurate and reliable results. Laboratories also typically have established communication channels that support interpretation and dissemination of results to public health decision-makers and the public.

A central feature of NWSS is the publicly accessible dashboard (**Figure 4, page 10**), which allows users to explore near real-time wastewater surveillance laboratory data for common respiratory viruses, including targets such as influenza, respiratory syncytial virus (RSV) and SARS-CoV-2, as well as select emerging or outbreak-associated pathogens such as avian influenza (H5), Monkeypox virus and measles. This dashboard is particularly valuable during rapidly evolving situations, enabling timely data dissemination at the national level while state and local jurisdictions build or scale reporting capacity. These visualizations also complement state and local dashboards and, in many cases, provide additional context or comparative insight across regions. By applying standardized data submission requirements and displaying data from all submitting jurisdictions in a consistent manner, the system works to improve comparability while still allowing jurisdictions flexibility in how programs are implemented locally.

Figure 4: CDC NWSS Wastewater Monitoring Dashboard, depicting RSV viral activity levels*



Other Partners

In addition to state, tribal, local, territorial and federal government-led efforts, wastewater surveillance capacity in the United States is supported by other independent large-scale initiatives. These programs can contribute complementary datasets, expand geographic coverage and explore alternative surveillance models that may inform future public health practices. One such initiative, WastewaterSCAN, represents an academic-led, philanthropically-funded initiative focused on routine testing (three to seven times per week) of more than 16 pathogens at 147 sites situated across 40 states. Together with key public health, laboratory, utility, academic, commercial and community partners, NWSS, the CoE network and independent national initiatives form a wastewater surveillance ecosystem that enhances situational awareness, supports methodological innovation and provides multiple lines of evidence to inform public health decision-making. The success of wastewater surveillance programs hinges on effective partnerships and coordination across these key partners and the integration of multidisciplinary expertise from wastewater systems operation to public health interpretation and communication (**Table 2, page 11**).

* Source: NWSS Wastewater Monitoring Dashboard (depicting RSV activity levels as of May 16, 2026)

Table 2: Key partners in microbial wastewater surveillance

Partners	Description
Wastewater Utilities	Provide access to wastewater samples, lead understanding of infrastructure and sample metadata and support data interpretation by providing sewershed system maps and operations details. Enhance jurisdictional acceptance through established community relationships.
State, Tribal, Local and Territorial Health Departments	House each jurisdiction wastewater surveillance program. Prioritize pathogens for incorporation into surveillance system, help interpret data in a public health context by combining findings with epidemiological information, develop response plans and interpretation guides to support data-driven public health actions (e.g., alerts, community outreach) and communicate findings to leadership, communities and healthcare partners.
Public Health Laboratories	Analyze and process wastewater samples, support data analysis and interpretation and provide technical expertise and guidance. Maintain staff, quality assurance systems, methodologies, and laboratory infrastructure (e.g., laboratory information management systems, or LIMS) needed to ensure data quality. Coordinate with wastewater utilities and health departments to collect samples and share, interpret and communicate data and findings.
Academic Institutions	Support for method innovation, pilot testing, collaborative research, testing capacity, modeling and forecasting, high-level synthesis, recommendation documents (e.g., National Academies of Sciences, Engineering and Medicine reports) and source of trained workforce.
Commercial and Contract Laboratories	Support rapid method development during emergency events, provide high-capacity or routine high-throughput testing and offer access to high-cost instrumentation and proprietary laboratory data analytic pipelines.
Healthcare Providers and other Health Professionals	Contribute to design and implementation of sampling plans, share insights with public officials and support integration of findings into existing health care systems. Clinical information can provide valuable confirmation of wastewater data.
National Associations*	Coordinate training, best practices, resource sharing and communities of practice. Strengthen quality, consistency, communication across jurisdictions and advocate at the national level.
CDC National Wastewater Surveillance System (NWSS)	Provides national coordination, centralized data reporting platform, technical assistance, laboratory method validations, trend testing and interpretation guidance.
NWSS Centers of Excellence (CoE)	CDC-funded regional leaders that have formal partnerships between public health departments, PHLs, academic institutions and wastewater utilities. They support and advance wastewater surveillance at the state, tribal, local, territorial and national scales within assigned regions, as well as collaborate with each other and CDC.

* Examples: APHL, Association of State and Territorial Health Officials, Council of State and Territorial Epidemiologists, National Association of County and City Health Officials, Water Environment Federation

New Target Selection Considerations

Microbial wastewater surveillance expanded rapidly during the COVID-19 pandemic, resulting in a large national network of PHLs with capabilities to quantify SARS-CoV-2 in wastewater. As programs mature, many laboratories are implementing additional microbial targets of public health relevance (**Table 3**).

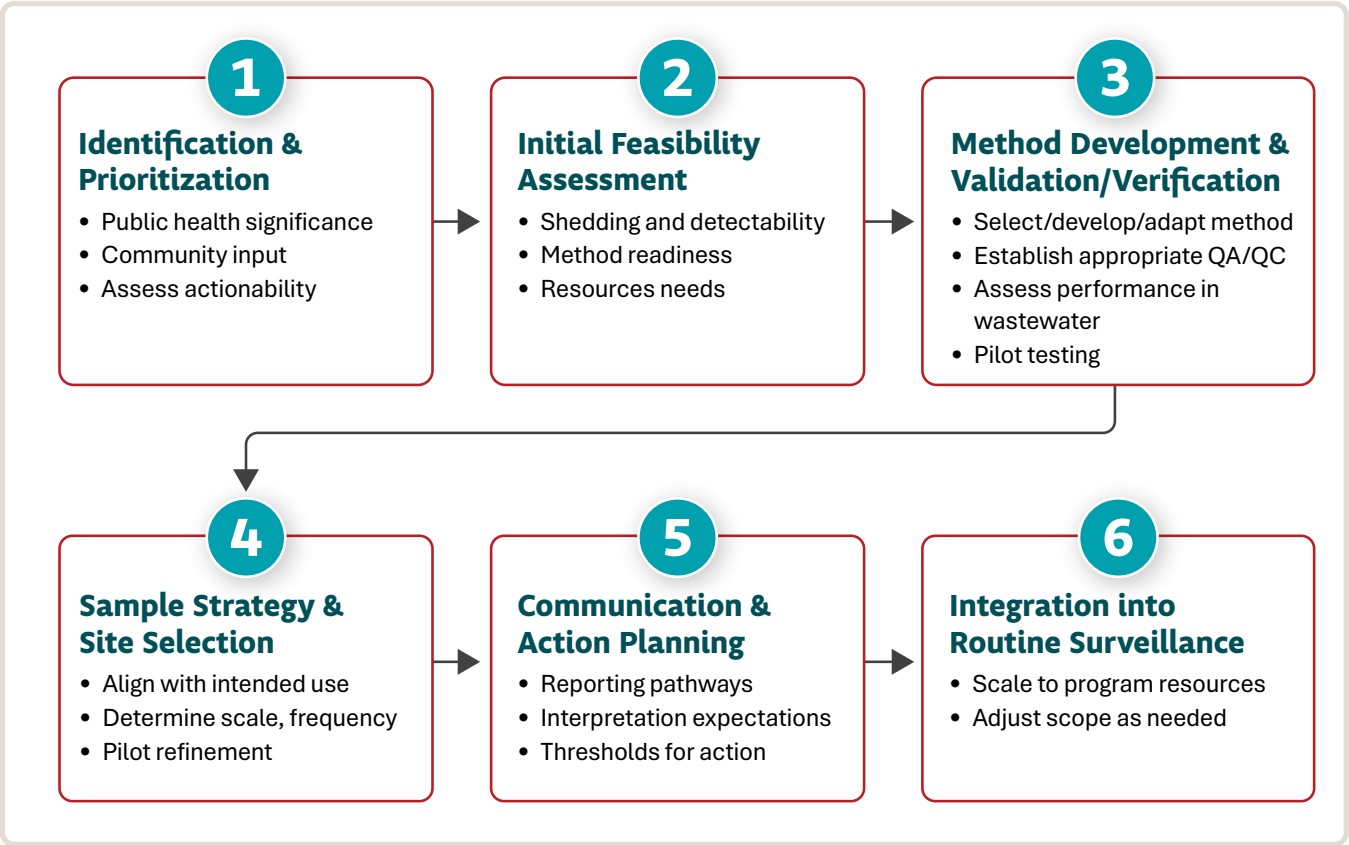
These pathogen molecular targets can differ substantially in biological, physical and chemical properties. Differences in size, structure, surface charge and genomic composition can influence human shedding dynamics, environmental persistence, partitioning between liquid and solid fractions of wastewater and efficiency of concentration and extraction during sample processing. Consequently, methods optimized for SARS-CoV-2, an enveloped RNA virus, may not perform adequately for other targets. These variations underscore the need for a structured, target-specific onboarding approach. This section describes key considerations and decision points for onboarding new microbial wastewater surveillance targets (**Figure 5, page 13**).

Table 3: Microbial wastewater surveillance targets currently* reported to the public on a national scale through CDC NWSS and/or the WastewaterSCAN initiative

Target Category	Category-specific Pathogen	CDC NWSS	WastewaterSCAN
Enteric Virus	**Norovirus**	-	Yes
	Hepatitis A	-	Yes
	Acute flaccid myelitis enteroviruses (e.g., EV-D68)	-	Yes
Fungal	***Candida auris***	-	Yes
Pox Virus	**Monkeypox clade I specific**	Yes	Yes
	Monkeypox clade II specific	Yes	Yes
	Monkeypox non-clade specific (NVO)	Yes	-
Respiratory Virus	**SARS-CoV-2**	Yes	Yes
	Influenza A (Typing and subtyping: H1, H3 or H5)	Yes	Yes
	Influenza B	Yes	Yes
	Respiratory syncytial virus (RSV) (A, B or non-specific)	Yes	Yes
	Human metapneumovirus (HMPV)	-	Yes
	Measles (wild type, vaccine derived, non-specific)	Yes	Yes
Vector-borne Virus	**West Nile Virus**	-	Yes

* As of March 2026

Figure 5: Key steps for onboarding a new microbial target for wastewater surveillance



Identification and Prioritization

The onboarding process begins with identification and prioritization of candidate targets that address a clearly defined public health need. From a public health lens, targets are typically prioritized based on the ability to support outbreak detection, quantify community burden, assess intervention impacts and/or complement existing surveillance systems. Prioritization may reflect regional considerations, such as localized transmission patterns and vectors, or broader factors, including international outbreaks or mass gatherings.

Target selection should emphasize actionability. Wastewater data should support clearly defined response pathways, such as increased clinical testing, enhanced vaccination awareness, development of public communication campaigns or vector control activities. Targets with unclear or speculative use cases may be deprioritized. Ethical implications of data generation should also be explored and explained, with safeguards clearly defined prior to implementation (see Ethical Considerations section for more information). Additional factors such as the scale of surveillance (e.g., community-level, sub-sewershed or facility-level monitoring), availability of resources and the capacity to generate timely and actionable information should also be considered.

Programs should explore the use of structured prioritization mechanisms, such as ranking exercises, surveys and advisory committees, to support transparent decision-making. In addition, community expert consultation is essential during this phase and commonly involves collaboration with epidemiologists, academic experts, state and local health departments, wastewater utilities, healthcare partners and public health leadership. Engagement helps ensure alignment between surveillance use cases and decision-making needs.

Initial Feasibility Assessment

Following prioritization, an initial feasibility assessment evaluates whether wastewater surveillance is appropriate and achievable for the selected target. A foundational consideration is whether the target is shed in feces, urine, saliva and/or skin at concentrations that are likely to be detectable in wastewater. Quantitative shedding data may be limited, particularly for emerging pathogen targets, requiring reliance on estimates, surrogate data or expert judgment. Target stability in wastewater, including susceptibility to degradation, temperature sensitivity and mutational potential should be considered, if information is available.

Feasibility assessments should also evaluate the availability of reference and control materials, compatibility with existing laboratory workflows, staffing and training needs, equipment and space requirements, as well as biosafety considerations (e.g., if target is a select agent). The CoE consortium can be an excellent resource for target feasibility and method guidance.

Some targets may have CDC validated digital PCR (dPCR) wastewater-specific protocols already available such as SARS-CoV-2, RSV, wild-type measles virus, influenza A with subtyping (H1, H3, H5) and influenza B. These Instructions for Use (IFU) are available through the APHL Wastewater Surveillance Community of Practice on ColLABorate. ColLABorate is open to laboratories contributing data to NWSS, their partners and APHL partner organizations. Additional CDC-validated IFU methods are being rolled out continuously. Targets for which only clinical protocols exist should be subject to wastewater-specific validation prior to use.

Method Development and Validation/ Verification

Once a new target has been prioritized and if an initial feasibility assessment suggests that wastewater surveillance is appropriate, laboratories should undertake structured method development and validation or verification before routine implementation. A detailed, standardized framework for microbial wastewater surveillance method development, validation and verification is outside the scope of this document. However, key conceptual considerations and decision points relevant to target onboarding are described here. The CoE consortium has convened a work group to develop comprehensive guidance, including step-by-step protocols, statistical approaches and recommended performance criteria. That guidance will be shared with the APHL ColLABorate community and linked to this document upon publication.

Method development begins with defining the intended use case and anticipated performance level required. The intended use case can influence needed performance parameters such as sensitivity, specificity, reproducibility, sample processing time and cost. For example, early detection applications may prioritize maximum sensitivity to detect a target at trace levels, whereas routine trend monitoring may emphasize higher precision of target concentration measurements and opt for higher throughput, but lower cost methods to enhance longitudinal comparability.

Laboratories should then select or adapt a method compatible with the new target and anticipated wastewater sample type (e.g., influent, primary sludge). Ideally, a validated method for wastewater surveillance application will already be available (i.e., via APHL ColLABorate Community of Practice or equivalent). However, if a validated method does not exist, a clinical protocol (e.g., assay) may be adapted for wastewater surveillance or a completely new method developed. Like new methods, clinical protocols should be subject to formal method validation because wastewater samples often contain inhibitors and other substances that can interfere with concentration, extraction and unbiased target characterization (e.g., qPCR, dPCR, sequencing). Method selection should also consider

compatibility with the current PHL sample handling, concentration, extraction and quality control management practices to determine if modifications are needed to adopt the new target for routine surveillance.

For new or adapted (clinical to wastewater surveillance) methods, a wastewater-specific performance evaluation is an essential aspect of method validation. Performance evaluations assess whether the method reliably characterizes the target in wastewater samples and is suitable for the intended use case. Performance evaluation success relies on using wastewater samples that are representative of typical anticipated monitoring conditions that contain the new target. In some cases, it may be difficult to obtain appropriate wastewater samples, and it may be helpful to use archived or shared samples from other jurisdictions.

In addition, any new workflow implemented by a laboratory should be subject to pilot testing prior to routine surveillance implementation (verification). A pilot test should include repeated analysis of representative, real wastewater samples from multiple sites and time points, if possible. Pilot testing verifies that the entire workflow (e.g., wastewater collection through data interpretation) produces reliable results that support expectations for the intended use case. Findings may reveal the need for adjustments (e.g., allowance of more time for sample collection and transport to laboratory, wastewater sample pretreatment) or identify site locations that consistently yield poor quality outcomes. If significant modifications are needed, it may be necessary to repeat pilot testing until an acceptable outcome is achieved.

Finally, laboratories should document method details (e.g., target nucleic acid sequence, primer/probe sequences, PCR thermocycling conditions) and operational readiness. A summary of key observations from method development and pilot testing should be created that includes strengths, limitations and known caveats. In addition, conditions that warrant re-evaluation, such as emergence of a target variant or workflow changes should be clearly documented.

Sampling Strategy and Site Selection

When onboarding a new target, it may be necessary to re-evaluate sampling strategies and site selection based on pilot testing results, funding and laboratory capacity. In addition, programs should confirm with partners (e.g., utilities, health departments) that existing sampling locations adequately represent communities of interest and consider whether additional or alternative sites are needed. During early implementation, sampling frequency may need to be temporarily increased to improve detection probability and establish baseline trends. Coordination with wastewater utilities is also essential to ensure operational feasibility, identify any site-specific constraints and clearly communicate any changes to sampling procedures. This is also an opportunity to accommodate utility operator safety priorities and concerns.

Communication and Action Planning

Clear communication pathways and predefined action plans are essential to ensure microbial wastewater surveillance data for new targets are interpreted consistently and linked to public health decision-making. Close coordination among all partners is critical and should be initiated as early as possible. Key considerations for communication and action plan development are described in **Table 4**.

Table 4: Key considerations for development of microbial wastewater surveillance communication and action plans

Plan Element	Description
Define and Develop Communication Pathways	• Establish how results will move from the laboratory to public health agencies, utilities, local leadership and the public. • Define who will have access to data, which results will be public-facing and the timing and cadence of release. • Identify responsible parties and decision authority at each step of data generation and reporting (e.g., laboratory staff, epidemiologists, local/state decision-makers, communication leads).
Align Interpretation Expectations Before Implementation	• Discuss anticipated range of normal variability for new target. • Clarify how trace detections, intermittent signals and unexpected findings should be interpreted under all anticipated scenarios. • Incorporate data and reporting lags. • Include considerations for uncertainty and known method limitations. • Draft talking points and messaging that can be customized as needed.
Define Thresholds or Conditions for Action	• Clearly define decision points that may prompt additional investigation or response (e.g., trace detection after prolonged non-detection, rapid increase in concentration, or emergence of new variant).
Ensure Timely and Coordinated Reporting	• Agree on routine reporting intervals (e.g., weekly, bi-weekly) and mechanisms for urgent communication of unexpected results. • Align reporting timelines with laboratory capacity to avoid delays or misinterpretation. • Coordinate messaging with epidemiology, communications and leadership teams to ensure consistent interpretation across audiences.
Maintain Transparency and Plan Refinements	• Explore how to communicate data transparently and respectfully. • Ensure action plan accessibility to all partners. • Identify any ethical concerns that can arise and plan how to address them. • Reassess and refine action plans as methods mature, new data become available, or public health priorities change.

Integration into Routine Surveillance

As pilot testing activities clarify target behavior and method performance, programs can transition to sustainable routine surveillance. Integration should balance public health value with available resources, such as staffing, laboratory throughput, sampling capacity and budget. Strategies such as PCR-based multiplexing and batch sample processing may be considered to improve efficiency and reduce costs. Programs may also establish scale-up and scale-down plans that adjust sampling frequency or site coverage in response to detections or changing epidemiologic conditions. Regular reassessment supports a flexible surveillance system that delivers actionable information while remaining operationally feasible.

Considerations for Offboarding a Target

Microbial wastewater surveillance priorities evolve over time as public health needs and resource availability change. Just as structured onboarding is important, planned approaches for offboarding or pausing target testing supports program efficiency and transparency. Reasons for offboarding may include diminished public health utility, shifts in priorities, persistent methodological limitations, or resource constraints (e.g., staff, laboratory capacity, funding). Key considerations for offboarding a target are described in **Table 5**.

Table 5: Key considerations for offboarding a microbial wastewater surveillance target

Consideration	Description
Data Continuity and Documentation	Clearly document the rationale, timing and conditions of discontinuation to support transparency and future reference.
Communication Planning	Coordinate with public health departments, utilities and other partners to ensure a shared understanding of why surveillance is being reduced or stopped and what information gaps remain.
Archiving and Re-evaluation	Preserve samples, data and method documentation where feasible to support retrospective analyses and future re-implementation if conditions change.
Ethical and Equity	Ensure that offboarding decisions do not disproportionately affect specific communities or undermine trust in wastewater surveillance programs.

More Information

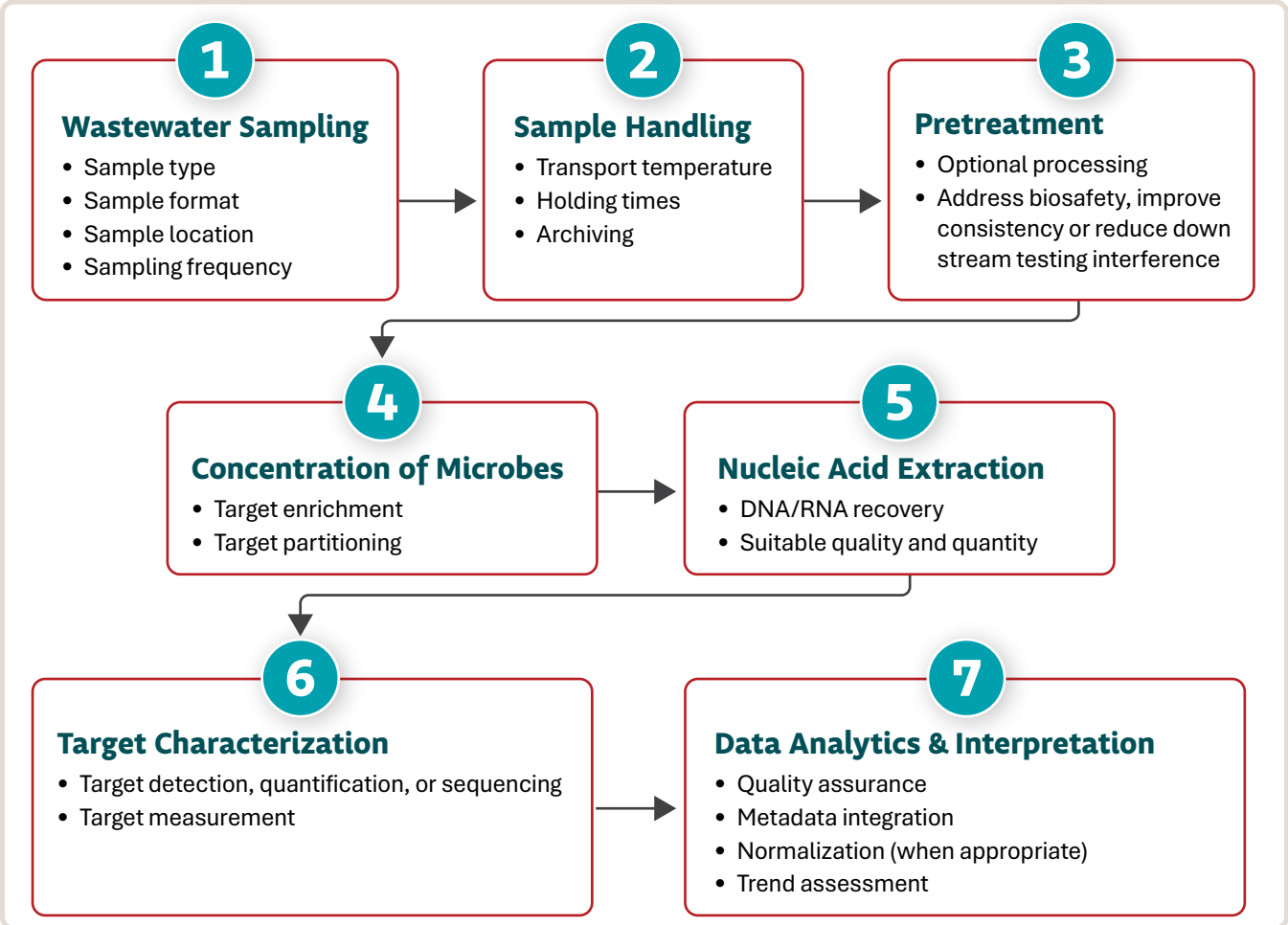
- APHL: National Trends in Wastewater Surveillance 2025 Survey Report
- ASM: Wastewater Surveillance for Bacterial Targets: Current Challenges and Future Goals
- ASTHO: Framework for Addressing Ethical Considerations in Infectious Diseases Public Health Wastewater Surveillance
- CDC: Wastewater Target Pathogens of Public Health Importance for Expanded Sampling, Houston, Texas, USA
- WastewaterSCAN Public Wastewater Monitoring Project
- WHO: Wastewater and environmental surveillance for one or more pathogens: guidance on prioritization, implementation, and integration

Microbial Wastewater Surveillance Molecular Testing Methods

This section provides an overview of the major analytical steps and platforms used to characterize microbial genetic material in wastewater samples. Nucleic acid targets can originate from a wide range of microbes with different structure, size, genetic material type (RNA or DNA), genomic diversity, environmental persistence and human shedding behavior, all of which can influence method performance. As a result, no single wastewater molecular testing workflow is optimal for all targets or surveillance use cases.

A typical wastewater molecular testing workflow includes wastewater sampling and sample handling (e.g., transport, storage, pretreatment), followed by microorganism concentration and nucleic acid extraction. Target characterization is then primarily performed using molecular-based methods such as PCR-based or sequence-based platforms, with results subjected to data analytics and interpretation (**Figure 6**). Changes to any individual step can substantially influence analytical performance and interpretation of results. For each target and workflow combination, laboratories are strongly encouraged to select a protocol and apply it consistently over time to support reliable comparison of results.

Figure 6: Framework of a typical wastewater molecular testing workflow for microbial wastewater surveillance*



* Specific workflows may vary by target, platform and intended public health use case.

Wastewater Sampling

Wastewater sampling represents the first step in microbial wastewater surveillance molecular testing workflows and plays a critical role in determining data quality, interpretability and representativeness. This subsection describes common wastewater sample types, sample locations, collection practices, sample frequency and partnership considerations.

Common Sample Collection Types

Two wastewater sample types are commonly used for molecular testing including: 1) untreated wastewater influent and 2) primary sludge.

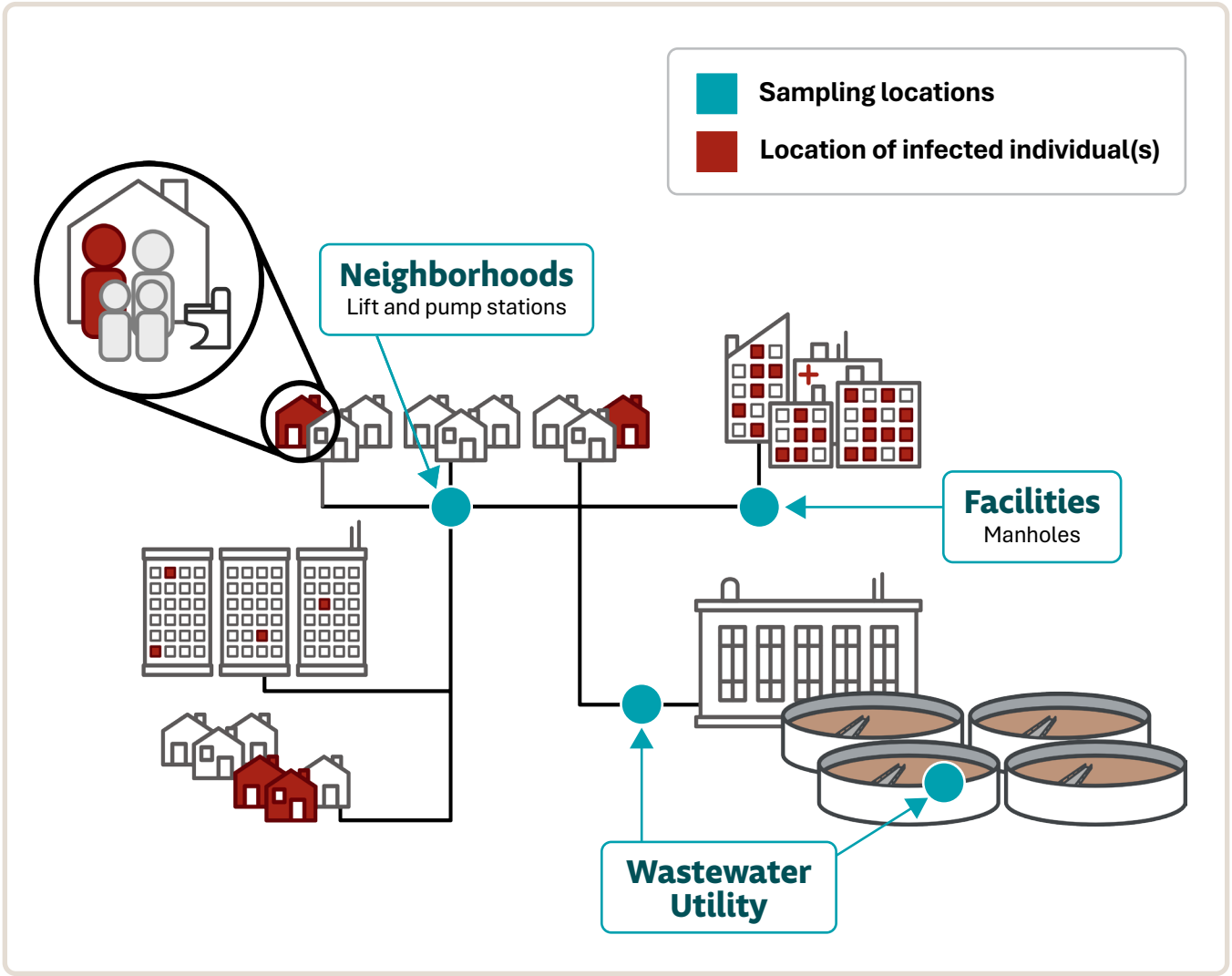
- **Untreated Wastewater Influent:** Untreated wastewater influent is typically sampled at the headworks (intake point) of a wastewater utility. This is the initial stage of treatment where raw sewage first enters the system and excludes side stream returns from the plant. It consists of pooled liquid waste generated from household and building use (e.g., toilets, showers, sinks) and typically includes fecal and urinary contributions, hygiene contributions (e.g., showering, brushing teeth), as well as potential non-household inputs such as stormwater, agricultural runoff and industrial discharges. Target genetic material may be closely associated with the influent liquid (soluble) fraction, suspended particulates within the untreated influent, or both. In some workflows, laboratories separate and analyze liquid and particulate fractions independently, as microbial targets may preferentially partition to one phase depending on biological properties and residence time in the wastewater system.
- **Primary Sludge:** Primary sludge is composed of suspended solids that settle during a primary sedimentation step employed by some wastewater utility facilities. Many microbial targets, including some viruses and bacteria, partition strongly with solids, resulting in potentially higher concentrations in primary sludge relative to untreated wastewater influent. Primary sludge sampling often requires additional homogenization prior to laboratory processing and may involve site-specific considerations related to access and sampling frequency. The optimal sample type depends on target behavior, intended surveillance use case and logistical feasibility.

Sample Location

Sampling location within the wastewater system (sewershed) should reflect the community of interest, the intended scale of surveillance and the expected behavior of the target. Community-level surveillance commonly relies on samples collected at wastewater utilities to represent larger populations. Upstream sampling sites situated prior to the wastewater utility headworks, such as lift or pump stations, may allow monitoring of smaller, defined sub-sewershed areas. Facility-based sampling, such as at schools, nursing homes, shelters, hospitals or correctional facilities, typically occurs at manholes and supports focused investigations of smaller communities.

Selection of sampling locations should consider access constraints, sewage flow and target persistence within the collection network. Common wastewater sampling locations and their relationship to target occurrence within a sewershed are illustrated in **Figure 7**.

Figure 7: Common wastewater sampling locations*



* Source: Houston CoE, image adapted from original figure

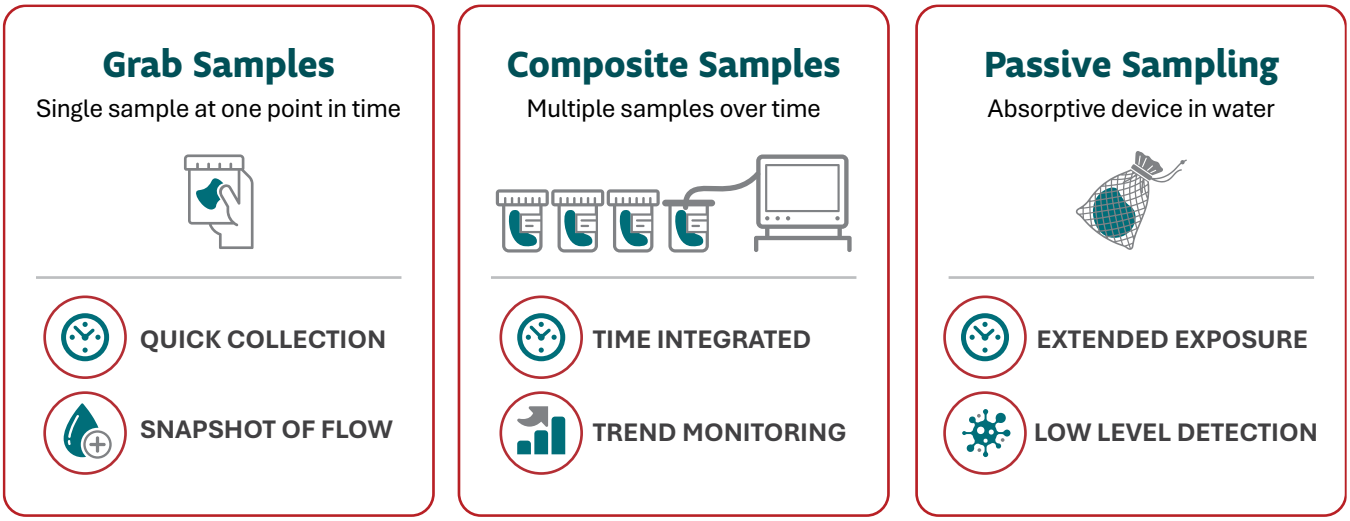
Sampling Practices

Selection of sampling practice should be guided by the intended use case, available infrastructure and target characteristics, including any expected temporal variability in target shedding. Three general sampling practices are commonly used in microbial wastewater surveillance applications.

- **Grab Sampling:** A grab sample represents a single point in time and can be collected rapidly without specialized equipment, making it most useful when rapid turnaround is needed, when access or wastewater flow conditions prevent composite sampling, or for short-duration investigations such as facility-level assessments. However, grab samples may not capture intermittent shedding and may be less representative of a community. Before collecting a grab sample, it may be helpful to review daily flow variation to identify an optimal sampling time.
- **Composite Sampling:** Composite samples are generated by pooling multiple subsamples collected over a defined interval (time-based) or in proportion to wastewater flow (flow-based). This approach provides a time-integrated representation of target presence and concentration, improves reliability for trend monitoring and increases the likelihood of detecting rare targets. Composite sampling is often preferred for community-level surveillance and is the most common sampling practice currently used by PHLs for wastewater surveillance, according to the APHL 2025 Wastewater Surveillance Survey. However, it may not be feasible at all locations due to access, infrastructure, or flow limitations. Automated samplers are commonly used, although manual pooling of grab samples may also be employed when automation is unavailable. Primary sludge may be sampled using either grab or composite approaches depending on wastewater facility operations and use case.
- **Passive Sampling:** Passive sampling involves placing an absorptive capture medium into wastewater for an extended period (typically < 24 hours) to accumulate microorganisms over time. Passive samplers can enhance detection of targets that are shed intermittently or at very low levels and may be useful during early detection efforts, facility-based investigations, or pilot studies. Because passive sampling does not capture a defined wastewater volume, quantitative interpretation remains challenging and is a topic of intense research.

PHLs frequently rely on wastewater utilities or environmental partners for wastewater sample collection, particularly when specialized equipment or site access is required. Clear communication regarding sample type, practice, timing and chain-of-custody procedures promotes consistent practices and high-quality data generation.

It is considered a 'best practice' for PHLs to provide a sampling kit including sample transportation (e.g., prepaid shipping or another scheduled courier). For some programs, stipends are provided to wastewater utilities to offset collection costs and support sustained collaboration. Coordination of collection with routine regulatory sampling practices may improve efficiency.



Wastewater Sample Handling

Proper sample handling is critical to preserving nucleic acid integrity and supporting accurate and timely characterization of microbial targets in wastewater. Handling practices should be designed to minimize degradation, reduce variability and maintain traceability from collection through analysis.

Key considerations include maintaining appropriate temperature control during transport and storage, typically by shipping samples on ice and storing at 4-8 °C until processing. Holding times should be defined and documented, as delays may disproportionately affect certain nucleic acid targets. Programs are also encouraged to collect sufficient sample volume or mass to support immediate analysis as well as archiving for re-analysis, method development, retrospective studies, or future target onboarding. Archiving practices should minimize repeated freeze–thaw cycles to minimize nucleic acid degradation.

Sample chain-of-custody procedures promote sample traceability and quality assurance throughout the workflow. Collection of associated metadata, such as flow, sample type, date and time of collection, temperature at receipt and environmental parameters (e.g., temperature, pH, total suspended solids), further supports downstream interpretation and data quality assessments.

Wastewater Sample Pretreatment

Wastewater sample pretreatment refers to optional processing steps performed after sample receipt and prior to concentration or nucleic acid extraction to improve processing consistency, manage biosafety considerations and/or reduce factors that may interfere with downstream testing. Pretreatment needs vary depending on the target organism and the wastewater sample type.

Common pretreatment activities include separation of solid and liquid phases, enzymatic or reagent-based treatments (e.g., proteases, surfactants) to improve downstream processing (e.g., amplification inhibitor removal) and homogenization to enhance sample representativeness. Some workflows incorporate inactivation approaches, such as pasteurization or heat treatment, to address biosafety concerns; when used, these steps should be evaluated for potential impacts on target integrity and measurement consistency. Removal of coarse debris through screening, filtering, centrifugation, or decanting can reduce both non-representative materials and substances that interfere with molecular analysis. Because pretreatment practices can influence downstream method performance, they must be evaluated during method development, applied consistently within a workflow and clearly documented.

Wastewater Sample Concentration

Wastewater sample concentration involves enriching microorganisms or nucleic acids of interest in the wastewater sample by reducing the volume or mass prior to analysis. Concentration efficiency can be influenced by microbial properties such as size, surface charge and association with solids.

A range of concentration approaches are currently used in microbial wastewater surveillance such as membrane filtration, ultrafiltration, polyethylene glycol (PEG) flocculation with centrifugation and magnetic bead-based methods. Each approach offers distinct advantages and limitations related to sensitivity, recovery efficiency, throughput, labor intensity, automation potential, cost and susceptibility to inhibition. Performance may vary by microbial target, wastewater sample type, wastewater composition and intended use, with no single method optimal for all applications.

Selection of an appropriate concentration approach should consider target partitioning and the characteristics of wastewater (e.g., targets that associate strongly with solids may enrich preferentially in filtration or centrifugation methods). Note that rare or emerging targets can benefit from larger sample volume processing to achieve sufficient sensitivity, although this approach can lead to co-concentration of substances that may interfere with downstream workflow steps. In rare cases, workflows may omit the concentration step and rely on direct nucleic acid extraction of wastewater, which can reduce processing time and cost but may be more susceptible to amplification inhibition and limit opportunities for re-testing or archiving.

Wastewater Nucleic Acid Extraction

Nucleic acid extraction isolates, concentrates and purifies nucleic acid material from wastewater samples. Extraction protocols typically rely on combinations of chemical lysis, mechanical disruption, size exclusion, charge-based binding and solubility partitioning to optimize recovery and purity.

Chemical lysis-oriented approaches disrupt cells and viral capsids using chaotropic salts (e.g., guanidine hydrochloride, guanidine thiocyanate), detergents (e.g., triton X-100, sodium dodecyl sulfate), or enzymes (e.g., lysozyme, proteinase K) to release nucleic acids. In contrast, mechanical disruption utilizes physical force to free nucleic acids, including treatments such as sonication and high-speed homogenization (e.g., bead beating). A common extraction approach, silica spin columns, combines the charge-based interactions between negatively charged nucleic acids and silica surfaces under high salt conditions with size-exclusion filtration to capture nucleic acids. In contrast, solubility-partitioning methods rely on differential solubility of nucleic acids relative to proteins, lipids and other wastewater components and includes protocols such as phenol-chloroform-isoamyl alcohol extraction, salt-induced precipitation (e.g., ethanol or isopropanol with sodium acetate) and PEG precipitation.

Microbial characteristics can strongly influence extraction performance. Organisms with robust cell walls, such as spore-forming bacteria or fungi, may require additional mechanical, thermal, or enzymatic disruption to release nucleic acids. RNA targets require workflows designed to preserve labile molecules and minimize exposure to enzymatic degradation (i.e., RNases). Because PCR-based platforms are susceptible to amplification inhibition, extraction methods often incorporate inhibitor removal steps to support unbiased target detection and quantification. Selection of extraction approaches should also account for factors such as cost per sample, compatibility with concentration approach, compatibility with automation, batch size and alignment with the characterization platform (e.g., PCR-based or sequence-based). The chosen method should consistently produce nucleic acid of sufficient quality and quantity to support reliable downstream analysis, as well as potential archiving needs.

Common Target Characterization Platforms

This subsection describes common analytical platforms used to characterize genetic material recovered from wastewater samples and to translate laboratory measurements into information relevant for public health decision-making (**Table 6, page 26**). In microbial wastewater surveillance, two broad categories of target characterization platforms are typically employed: PCR-based and sequencing-based platforms. These platforms differ in their underlying technological principles, analytical resolution, sensitivity to wastewater matrix effects and suitability for specific use cases. Selection of an appropriate platform should be guided by the intended use; expected target abundance, need for detection, quantification, or sequence-level information and the properties of the microbial target. Because microbial wastewater targets vary widely in abundance, stability and genetic diversity, laboratories may employ multiple platforms at different stages of target onboarding or for distinct use cases.

PCR-based Platforms

PCR-based platforms, including quantitative PCR (qPCR) and digital PCR (dPCR), are widely used in microbial wastewater surveillance because they can selectively amplify and measure defined molecular targets within the highly complex mixture of nucleic acids present in wastewater. Target specificity and sensitivity are of key importance and are, in part, achieved using well-designed and tested primers and, when applicable, probes that bind to defined genetic regions, enabling detection and quantification even in samples containing diverse microbial communities.

Because PCR-based approaches require DNA for amplification, RNA targets must first be converted to complementary DNA (cDNA) through a reverse transcription step. Reverse transcription may be performed as part of a one-step PCR protocol to streamline processing or in two-steps when optimization or cDNA archiving is desired. PCR-based approaches support a range of microbial wastewater use cases, such as early detection of emerging pathogens, routine trend monitoring and quantitative tracking of established targets.

- **qPCR:** qPCR measures the accumulation of amplified target DNA or cDNA in real-time through fluorescence detection after each PCR cycle. Target concentrations are determined by comparing the measured quantification cycle (Cq) value to a standard curve generated from a dilution series of reference material at known concentrations. In microbial wastewater surveillance, probe-based assays are commonly used because fluorescence is generated only when a sequence-specific probe binds to and is cleaved from the target, providing an additional level of specificity in complex matrices like wastewater. In contrast, DNA-binding dye–based assays detect any double-stranded DNA molecules and may detect non-specific products, making them less suitable for wastewater applications. qPCR has a wide dynamic target measurement range, often spanning six to seven orders of magnitude, which can be advantageous when target concentrations fluctuate substantially over time, between locations, or when the same nucleic acid extract is used to test for multiple targets that occur across a wide range.
- **dPCR:** dPCR partitions each sample into thousands of small reactions prior to amplification, allowing each partition to be classified as a positive or negative result at the end of all PCR cycles. Following amplification, an absolute target concentration is estimated using Poisson statistics based on the proportion of positive partitions. Partitioning can reduce the influence of some amplification inhibitors and improves detection of low-abundance targets. These characteristics make dPCR particularly useful for early detection of emerging targets, confirmation of weak or borderline qPCR signals and method evaluation during new target onboarding. dPCR also offers several potential advantages over qPCR for testing multiple assays in the same reaction (multiplexing). Because targets are detected independently by partition level detection, dPCR can be less sensitive to amplification efficiency differences, assay competition and amplification inhibition effects that can complicate multiplex qPCR interpretation. dPCR can provide precise measurements, although throughput may be lower for some platforms and per-sample costs are generally higher than for qPCR. Like qPCR, dPCR applications can use probe-based or DNA binding-based chemistries.

Sequencing Platforms

Sequencing platforms characterize genetic material by determining the nucleotide sequence of targeted regions or, in some cases, entire genomes present in wastewater samples. Unlike PCR-based platforms, which are designed to detect or quantify predefined targets, sequencing approaches can provide additional resolution for variant identification, lineage assignment and broader microbial community composition assessment. Based on the 2025 APHL survey, PHLs most commonly use next generation sequencing (NGS), but also employ Sanger sequencing and third generation sequencing (TGS) for microbial wastewater surveillance applications on occasion.

Sequencing platforms vary in throughput, resolution, complexity, cost and suitability for specific intended uses. In general, sequencing methods require higher nucleic acid quality and quantity compared to PCR-based approaches and can be more sensitive to sample matrix complexity and target abundance. Because sequencing platform selection can influence data interpretation, consultation with subject matter experts is recommended when integrating sequencing into microbial wastewater surveillance workflows.

Sanger Sequencing

Sanger sequencing (first generation sequencing) determines the nucleotide sequence of a defined nucleic acid region by generating fragments of different lengths that are subsequently resolved to reconstruct the sequence. This approach provides high per-base accuracy for single amplicons or short genomic regions but has significantly lower throughput compared to newer sequencing technologies.

In addition to the PCR amplicon, Sanger sequencing requires sequencing primers that bind within the amplified region or to standardized primer binding sites when amplicons are cloned into plasmids. Changes in primer binding sites or the need for additional molecular processing can introduce uncertainty and/or additional effort. Because Sanger sequencing cannot resolve complex mixtures of variants, it is not suitable for characterizing multiple targets in the same reaction (multiplexing). As a result, Sanger sequencing is commonly used in wastewater surveillance to confirm specific mutations, verify a PCR amplicon sequence, or support troubleshooting and method development rather than for large-scale genomic surveillance.

NGS

NGS uses massively parallel sequencing to analyze thousands to millions of DNA fragments simultaneously. Typical read lengths range from approximately 100 to 1,000 base pairs, depending on the platform and library preparation approach. By combining sequence barcoding with parallel sequencing, NGS can simultaneously analyze hundreds to thousands of samples in a single instrument run. Amplicon-based NGS enables sensitive detection of multiple variants within a target population, while shotgun metagenomic NGS supports broader, agnostic profiling of microbial communities, allowing for the discovery of unexpected or emerging targets.

NGS is well suited for variant of concern identification and more comprehensive genomic investigations but generally requires substantial laboratory infrastructure, bioinformatic expertise and longer turnaround times compared to PCR-based platforms. Performance can be limited when target concentrations are low or when primer mismatches reduce amplification efficiency in amplicon-based workflows.

TGS

TGS platforms read long DNA or RNA fragments in real-time, often exceeding 10,000 base pairs, enabling near full-length genome characterization and improved assembly of complex genomic regions. Using sample barcoding and parallel sequencing of individual molecules, these platforms can analyze dozens to hundreds of samples and genetic targets within a single instrument run, while preserving linkage among genes or variants on the same molecule.

However, higher read error rates for some platforms, variable throughput and increased bioinformatic complexity for error correction may limit routine application for trend monitoring or precise variant frequency estimation. As these technologies continue to evolve, they may play an expanding role in specialized microbial wastewater surveillance applications where long-read information provides clear added value (e.g., structural variant detection and plasmid characterization).

Table 6: Strengths and limitations of common microbial wastewater surveillance target characterization platforms

Platform	Strengths	Limitations
Quantitative PCR (qPCR)	• Wide instrument availability • Cost effective and high throughput • Wide dynamic range (6-7 orders of magnitude)	• Reduced reliability at very low concentrations (< 10 copies per reaction) • Requires high-quality standard curves and stable precisely quantified control materials • Less tolerant of amplification inhibition
Digital PCR (dPCR)	• Superior sensitivity • More tolerant of amplification inhibition • Robust multiplexing protocols available	• Higher cost per sample • Narrower dynamic range (4-5 orders of magnitude) • Less high throughput
Sanger Sequencing	• High per-base accuracy • Useful for confirming mutations or characterizing short regions • Effective for verifying PCR amplicon sequence during method development or troubleshooting	• Low throughput • Requires relatively abundant target material • Not suitable for detecting mixtures of variants or low-frequency mutations
Next Generation Sequencing (NGS)	• High-throughput and scalable • Sensitive detection of multiple targets • Metagenomic profiling of microbial communities	• Resource intensive • Performance declines with low abundance targets • Primer mismatches or genome mutations can reduce coverage for targeted approaches
Third Generation Sequencing (TGS)	• Near real-time preliminary results • Read flexibility (~50 bp up to ~4 Mb) • Useful for structural variant detection and plasmid characterization • Some platforms offer potential for portability to support field deployment	• Higher read error rates for some platforms, though steadily improving • May require higher quality and concentrations of nucleic acid input, depending on application • Increased bioinformatics complexity for error correction • Throughput varies by platform

Data Analytic Considerations

Microbial wastewater surveillance generates complex datasets that require analytical pipelines aligned with the selected target characterization platform to ensure results are reliable, interpretable and suitable for public health decision-making. Key analytic considerations include incorporation of robust quality control checks, normalization approaches (where applicable), privacy safeguards and consistent data processing methods that account for wastewater system variability and analytical uncertainty. Where possible, quality benchmarks and acceptance criteria should be integrated into automated data review and flagging processes to support timely identification of anomalous results.

Normalization strategies, such as adjustment for flow, estimated human fecal content with endogenous markers, or solids mass, may help contextualize results and improve comparability across sites and over time. Version-controlled analytic and bioinformatic pipelines support reproducibility, transparency and traceability as methods evolve. Publishing sequencing results on public databases helps ensure data quality and helps inform others of potential target mutations that may influence public health risk. Programs should also ensure that metadata are complete and retained alongside analytical outputs and that archiving and re-analysis capacity is maintained to support retrospective interpretation, method refinement and integration with epidemiologic or clinical data sets.

Benefits and Considerations of Workflow Automation

Automation options are available across multiple steps of the wastewater molecular testing workflow:

- **Wastewater sampling:** Automated composite samples can support time- or flow-weighted sampling to improve temporal representativeness.
- **Nucleic acid extraction:** High-throughput platforms can increase consistency, reduce hands-on time and support scale-up or multi-target testing.
- **PCR reaction setup and plate loading:** Automated liquid handling systems can reduce pipetting variability, lower contamination risk and improve reproducibility.
- **Sequencing workflows:** Automation is increasingly used for library preparation and pooling, improving throughput and standardization.
- **End-to-end solutions:** Integrated systems that automate multiple workflow steps can streamline operations.

Key considerations:

- Can improve efficiency, consistency, throughput and workforce sustainability.
- May introduce dependencies on specific platforms, consumables or vendors.
- May increase cost and require a larger footprint in laboratory.
- It is important to balance benefits and challenges related to program scale, intended use and available resources.

More Information

- APHL: National Trends in Wastewater Surveillance 2023 Survey Report
- APHL: National Trends in Wastewater Surveillance 2025 Survey Report
- APHL: SARS-CoV-2 Wastewater Surveillance Testing Guide for Public Health Laboratories
- Federal Select Agent Program
- SARS-CoV-2 wastewater genomic surveillance: approaches, challenges, and opportunities | *Genome Biology* | Springer Nature Link
- Water Research Foundation (WEF): Best Practices for Collection and Storage of Wastewater Samples to Support Wastewater Surveillance of the COVID-19 Signal in Watersheds
- WEF: Utility Engagement Toolkit
- US EPA: Procedures for Collecting Wastewater Samples
- US EPA: Region 4 Procedures for Collecting Wastewater Samples
- US EPA: National Pollutant Discharge Elimination System (NPDES) Compliance Inspection Manual – Chapter 5

Laboratory Infrastructure

A successful microbial wastewater surveillance program relies on laboratory infrastructure that supports safe handling of wastewater samples, consistent execution of analytical workflows, contamination controls and secure data management. Infrastructure needs vary based on program scale, wastewater surveillance targets, analytical platforms employed and whether laboratories conduct sample collection in addition to testing. This section outlines key infrastructure components that support reliable and sustainable microbial wastewater surveillance implementation across PHL settings.

Laboratory Personnel

Microbial wastewater surveillance benefits from laboratory personnel with experience working with complex environmental matrices such as surface waters, soils, sediments or stormwater discharges. Familiarity with molecular methods, environmental microbiology, quality assurance practices and strategies for managing substances that can bias measurements (e.g., amplification inhibition) supports effective troubleshooting and workflow optimization. Laboratory wastewater surveillance programs may include personnel with bachelor's, master's or doctoral-level training, gained through academic research, regulatory laboratories or wastewater utility experience.

Laboratory staff conducting wastewater testing should be overseen by a supervisor with expertise in both analytical methods and personnel management. The scale of operations influences staffing needs, with small-scale programs (<15 samples per week) typically requiring approximately 1.5 full-time equivalent (FTE) laboratory scientists and one PhD-level (or equivalent) lead. Medium-scale programs (15–150 samples per week) require approximately three laboratory FTE positions plus a PhD-level (or equivalent) lead, while large-scale (> 150 samples per week) require five or more laboratory FTE positions and a PhD-level (or equivalent) lead. Certain programs may need additional staff depending on workflow complexity and need for on-going method development. Additional laboratory or field staff may be necessary when laboratories manage utility coordination or sample collection logistics. Cross-training personnel across workflows can strengthen operational resilience. Automation may reduce the demands on and for personnel.

Laboratory Space and Equipment Needs

Dedicated laboratory space is required for receiving wastewater samples, performing concentration and nucleic acid extraction steps and executing PCR- and sequencing-based workflows. Space for archiving (e.g., biobanking) both wastewater samples and nucleic acid extracts may also be needed. Space and equipment requirements depend on sample type and quantity (volume or mass), workflow design, microbial wastewater surveillance targets and contamination control practices. Laboratories should ensure adequate separation of activities to support unidirectional workflows and minimize cross-contamination risks.

Core Equipment

The following core equipment supports routine microbial wastewater surveillance for many workflows:

- Refrigerators (4°C) and freezers (–20°C and –80°C) for sample and reagent storage
- Biosafety cabinet and designated bench space for sample processing
- Autoclave or chemical inactivation supplies for decontamination of biological waste
- Homogenizer (e.g., bead mill, blender, shaker)
- Vortex mixer

- Microcentrifuge
- Heating and cooling block
- Nucleic acid quantification instruments (e.g., spectrophotometer, fluorometer)
- PCR hood for set-up of amplification reactions
- Micropipettes (with aerosol-resistant filter tips)
- Low-retention microtubes (certified DNase/RNase-free)
- Tube racks, consumables and other general laboratory supplies

Additional Needs

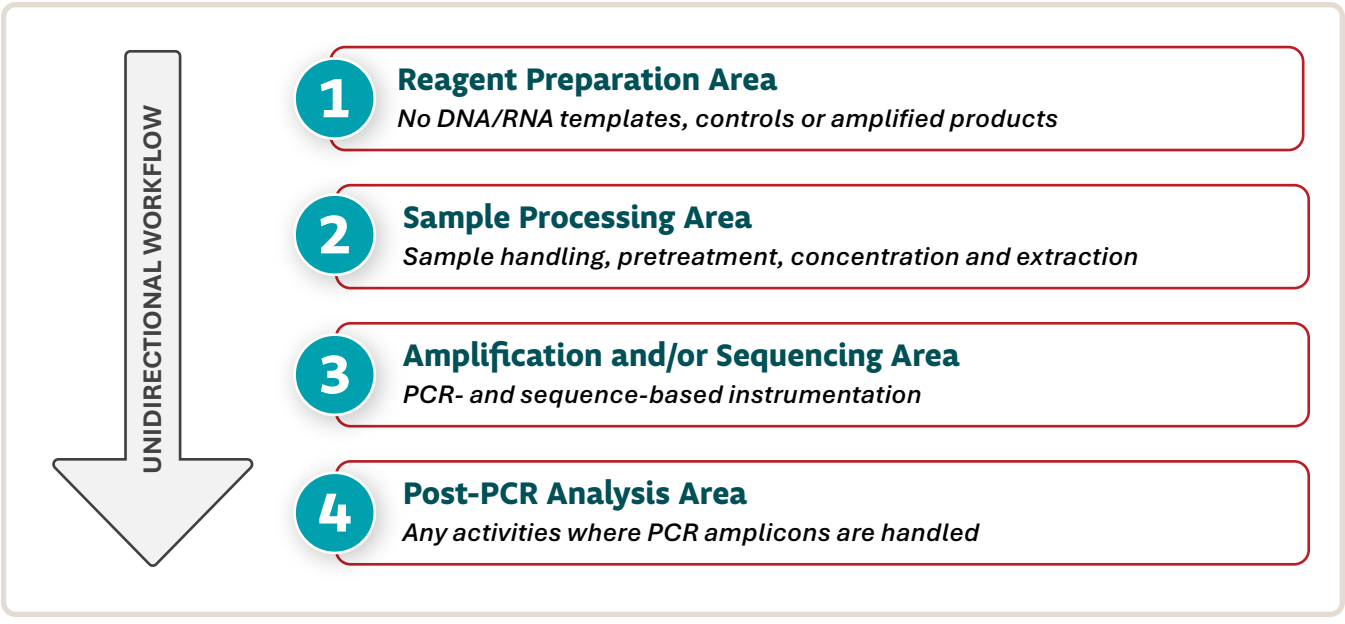
Additional equipment, such as automation systems, dPCR instruments, or sequencing platforms, may be required depending on testing methods. Laboratories are encouraged to consult validated protocols for wastewater surveillance applications or subject matter experts when selecting equipment for implementation.

Laboratory Layout for Contamination Control

Microbial wastewater surveillance involves detecting trace quantities of nucleic acids, making workflows particularly susceptible to contamination if laboratories are not properly organized. Contamination can arise from laboratory surfaces, equipment, positive control materials, or previously amplified PCR products (amplicons), potentially leading to false positives and data misinterpretation.

A unidirectional workflow should be implemented to minimize the risk of contamination, with laboratory activities progressing from clean areas to areas where amplified genetic material is present (**Figure 8**). This approach reduces the likelihood that amplified products or high-concentration control materials are inadvertently introduced into pre-amplification spaces where reagent preparation or sample processing occurs. When separate rooms are not feasible, clearly defined zones with physical barriers, dedicated equipment and restricted personnel movement can be used to maintain functional separation and support contamination control. Please refer to the **Laboratory Quality Management System (page 32)** section for information on controls to identify contamination.

Figure 8: Unidirectional workflow schematic for typical microbial wastewater surveillance laboratory

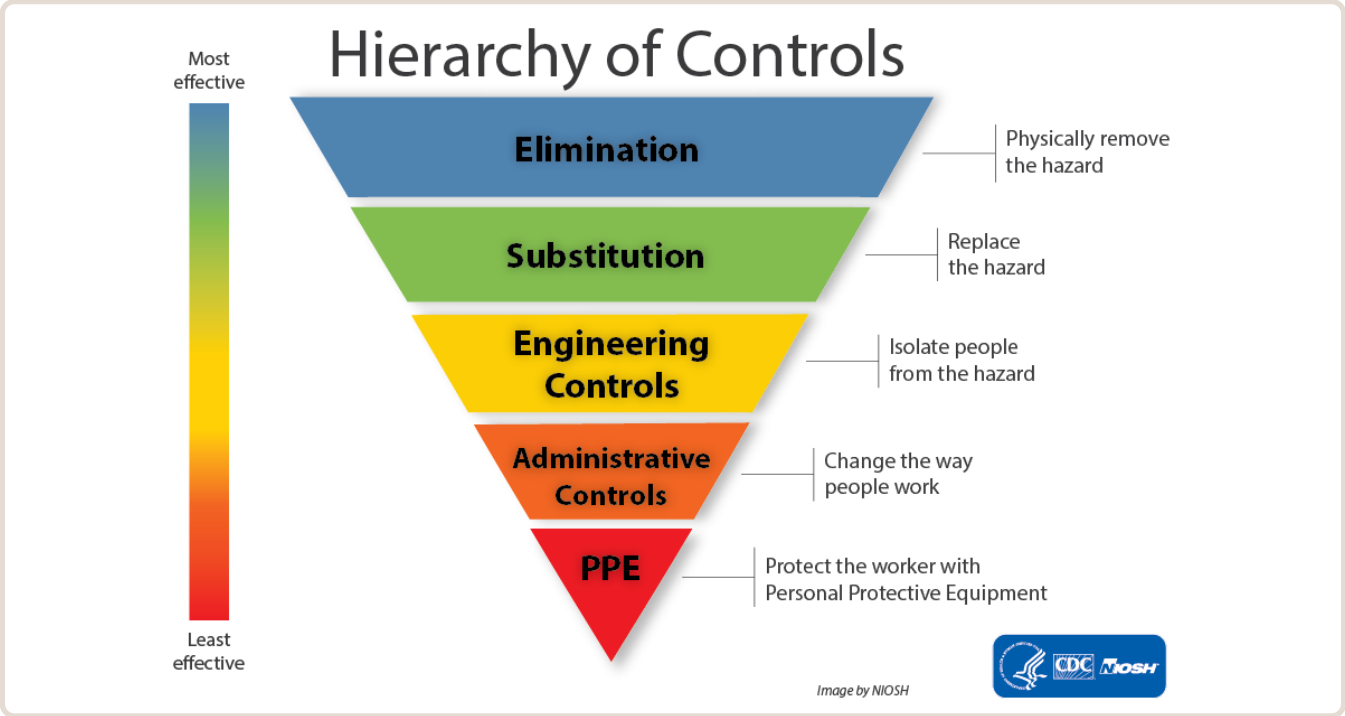


Biosafety

Biosafety requirements should be determined through site- and activity-specific risk assessments and may vary by target and wastewater sample type. Laboratories testing multiple targets should follow the requirements associated with the highest-risk target. Minimum recommendations for handling untreated wastewater include engineering, administrative and personal protective controls. Practices should align with the National Institute for Occupational Safety and Health (NIOSH) Hierarchy of Controls (**Figure 9**).

For example, engineering controls may include biosafety cabinets, sealed centrifuge rotors, closed extraction systems, or automated systems to minimize aerosol exposure. Administrative controls could include target-specific risk assessments, standard operating procedures and routine competency assessments. Personal protective equipment typically includes gloves, laboratory coats and eye or face protection. Additional mitigation measures may be required when infectious agents are anticipated. Laboratories should consult local Offices of Biological Safety or equivalent authorities and comply with all applicable regulations.

Figure 9: NIOSH Hierarchy of Controls*



Risk Assessment

Laboratories should conduct risk assessments tailored to workflows, sample types and facility design. Key considerations include the target(s) under surveillance, number and type of tests performed, aerosol generation potential, biosafety level, staff training and availability of appropriate equipment. Risk assessments support identification of mitigation strategies and ensure safe handling of wastewater and associated materials. Coordination with institutional Offices of Biological Safety or equivalent is recommended to review and approve risk minimization approaches. Consultation with CDC NWSS CoEs and other jurisdictions performing target monitoring can also be valuable.

* Source: CDC Hierarchy of Controls

Waste Disposal

Waste disposal procedures should align with biosafety level assignments and local, state and federal regulations. Considerations include autoclaving or chemically inactivating unused wastewater prior to disposal, following manufacturer guidance for reagent and consumable disposal and avoiding autoclaving PCR plates, which may release amplified material into the laboratory environment and contribute to contamination. Laboratories should also consider wastewater sample volume needs for analysis, re-testing and archiving to minimize waste generation. Clearly documented waste management plans support regulatory compliance and contamination prevention.

Information Technology, Security and Data Coordination

Microbial wastewater surveillance generates large datasets that are often shared across laboratories, utilities and public health agencies. Laboratories should ensure that information technology systems support secure data entry, sample tracking, quality control documentation and controlled data access. Key functions include secure storage, data transfer mechanisms, reporting and tracking of metadata and quality control results and ability to format and transfer data to national repositories such as the One CDC Data Platform (1CDP).

Quality control practices, data retention policies and re-analysis schema should be thoroughly documented. Laboratories new to wastewater surveillance reporting may require substantial investment in information technology infrastructure and may benefit from consultation with laboratories that have established data management systems.

More Information

- APHL: Risk Assessment Best Practices
- APHL: Risk Assessment for Ebola Testing
- CDC: About Hierarchy of Controls
- CDC: Biosafety in Microbiological and Biomedical Laboratories (BMBL), 6th Edition
- CDC: One CDC Data Platform
- Vanderbilt University: Example Laboratory Risk Assessment Process: A Laboratory Risk Assessment during the Coronavirus Pandemic

Laboratory Quality Management System

A robust laboratory quality management system is essential for producing reliable, interpretable and defensible microbial wastewater surveillance data. This section describes core laboratory quality management system principles that support consistent execution and documentation of microbial wastewater surveillance testing across diverse targets, matrices and analytical workflows. Many of the practices described here also apply during method validation and verification; however, detailed, method-specific validation guidance is beyond the scope of this document and will be addressed through future publications and resources.

Personnel Competency and Training

Personnel competency is a foundational component of laboratory quality management systems for wastewater surveillance. Effective programs rely on staff with training in molecular environmental microbiology or closely related disciplines, which supports appropriate method selection, troubleshooting and interpretation of wastewater data. Competency should be demonstrated and documented through structured training, supervised practice and routine performance evaluation. Training records should reflect competence across relevant workflows and be updated annually and/or as methods evolve or new targets are introduced.

Proficiency Testing

Proficiency testing is a critical element of laboratory quality management systems and supports confidence in data quality and comparability. Formal accreditation of methods in both clinical and environmental laboratories typically requires enrollment in proficiency testing activities (e.g., Clinical Laboratory Improvement Amendments, National Environmental Laboratory Accreditation Center). Participation in interlaboratory comparison or proficiency test exercises that reflect routine wastewater workflows is strongly encouraged. Effective proficiency test programs typically include distribution of characterized wastewater samples, incorporation of routine methods and platforms, collection of workflow metadata and evaluation of inter- and intra-laboratory variability. Structured feedback supports corrective actions, identification of underperforming protocols and continuous improvement.

Highlight: Wisconsin CoE Proficiency Testing Program

- Operates an active wastewater Proficiency Testing program at no cost to participants.
- The program distributes wastewater samples spiked with characterized levels of endogenous pathogens and/or spiked inactivated viral targets and instructs laboratories to analyze samples using normal workflows.
- Results are evaluated alongside detailed workflow metadata, providing insight into interlaboratory comparisons, identification of underperforming methods and drivers of performance.
- Participants reported workflow refinements, increased confidence in results and clearer interpretation of low-level detections.
- The Proficiency Test program represents a valuable resource for PHLs seeking to strengthen quality management system practices and improve comparability of data.

General Quality Control Metrics for All Method Workflows

This subsection describes quality control elements that apply broadly across PCR- and sequencing-based microbial wastewater surveillance workflows. Specific implementation details may vary by method, instrumentation and target.

Matrix Recovery Controls

Matrix recovery controls are exogenous materials introduced into wastewater samples to evaluate target loss during processing. These controls may be added directly to raw wastewater (process recovery controls) or after concentration (extraction recovery controls). Wastewater matrices may contain substances that can interfere with nucleic acid recovery (e.g., by sequestration), reverse transcription (i.e., conversion of RNA to cDNA) and/or amplification (e.g., humic acids), resulting in complete or partial target signal loss. Because recovery can vary substantially between samples, it is ideal to include a matrix recovery control with each wastewater sample to identify potential result bias and reduce the risk of false negatives.

Positive Controls

Positive controls contain purified target nucleic acid at a known concentration and serve as a benchmark for evaluating the target characterization step (e.g., PCR-based or sequence-based). While genomic material from the target microbe is ideal, synthetic DNA or RNA materials are commonly used when genomic material is unavailable or impractical.

Sources for Microbial Wastewater Surveillance Control Materials

Control materials are critical for the success of wastewater surveillance laboratory quality management systems.

- **National programs:**
  - National Institute of Allergy and Infectious Diseases BEI Resources Repository (BEI Resources)
  - National Institute of Standards and Technology (NIST) - Research Grade Test Material 10223 (Monkeypox Virus)
  - American Type Culture Collection (ATCC)
- **Ready-to-use commercial materials**
- **In-house options:**
  - Heat inactivated microorganisms
  - Commercial services can generate custom nucleic acid targets but will require knowledge of target sequence and ability to design functional construct

Negative Controls

Negative controls should be included at all stages of the wastewater testing workflow to identify potential contamination and support interpretation of low-level detections. Two control types are commonly used across characterization platforms, representing different steps in a molecular workflow.

- **Full process blank control (method blank):** Control that represents the entire workflow, including concentration, nucleic acid extraction and amplification and/or sequencing steps, with either molecular grade water or buffer substituted for a wastewater sample. Positive results may indicate contamination at any stage of the workflow.
- **Extraction blank control:** During the nucleic acid extraction step, a blank sample (e.g., molecular grade water or buffer) should be processed in parallel to wastewater samples. Extraction blank controls monitor for potential contamination introduced by reagents and/or cross-contamination during processing. If a false positive is observed, contamination could originate from the process of nucleic acid extraction and/or PCR amplification or sequencing steps.

When contamination is observed, laboratories should evaluate signal magnitude, reproducibility and patterns across control types. Single sporadic detections may reflect random events, whereas repeated detections across instrument runs and/or control types may indicate persistent contamination. In such cases, laboratories should pause sample processing and data reporting as appropriate, review recent workflow changes, reagents and changes in staff to implement appropriate decontamination procedures before resuming testing.

Endogenous Wastewater Controls

Wastewater is a complex and dynamic mixture composed primarily of water, suspended and dissolved organic and inorganic solids and pooled human waste (i.e., feces and urine). The relative contribution of human waste within wastewater systems fluctuates continuously due to factors such as stormwater runoff in combined sewer systems, groundwater infiltration in aging infrastructure and variations in household water use and industrial discharges. Human waste contributions can also vary across temporal and spatial scales, influenced by seasonal patterns, population mobility and occurrence of special events in the respective sewershed. Evidence indicates that typically less than 30 percent of the bacterial population in a wastewater sample originates from human waste, underscoring the potential importance of contextual indicators when interpreting microbial wastewater surveillance data.

Endogenous wastewater controls provide a means to account for this variability by serving as internal positive indicators of human fecal input within a given sample. These controls target genetic material from microorganisms or anthropogenic chemicals (e.g., caffeine, creatine) that are strongly associated with human fecal matter and are present at sufficiently high abundance to be routinely quantified across wastewater surveillance workflows. Common genetic material examples include pepper mild mottle virus (PMMoV), crAssphage and the human-associated bacterial marker HF183. While these genetic markers can help distinguish true changes in target signal from fluctuations in human waste concentration (i.e., normalization), they should not be used as substitutes for matrix recovery controls or PCR-based amplification inhibition assessments.

Additional Quality Assurance Metrics for PCR-based Methods

PCR-based platforms (qPCR and dPCR) are widely used in microbial wastewater surveillance because of exceptional sensitivity, specificity and adaptability across a range of targets. However, amplification-based methods require distinct quality assurance considerations that extend beyond general workflow controls. This subsection describes additional PCR-specific metrics that support reliable detection, quantification and interpretation of wastewater surveillance data.

qPCR Standard Curve Controls

For qPCR-based workflows, high-quality, reproducible standard curves are central to accurate target quantification. Standard curves establish the relationship between a control material with known target concentrations and observed quantification cycle (Cq) values and provide key performance indicators such as amplification efficiency ($E = 10^{(-1/\text{slope})} - 1$) and linearity ($R^2$). E is typically calculated from the slope of the standard curve and reflects how effectively the assay doubles target material during each cycle. Standard curves should demonstrate acceptable linearity ($R^2 \geq 0.98$) and E (0.90 to 1.10) across a defined range of quantification. Best practices include generating control material dilution series that span at least five orders of magnitude and incorporate a minimum of three technical replicate measurements at each dilution level.

No Template Controls

No template controls serve as a contamination control, where a PCR-based reaction includes all assay components except the nucleic acid template, which is typically replaced with molecular-grade water. Positive detections indicate potential reagent or laboratory environment contamination and require investigation before sample results are interpreted and reported. No template controls should be included in each PCR-based instrument run.

Amplification Inhibition

Amplification inhibition occurs when substances co-extracted from wastewater interfere with amplification, reducing or preventing detection or accurate quantification of the target. Amplification inhibition may be complete or partial and can vary from one sample to another due to differences in wastewater composition. Although dPCR platforms are generally more tolerant of amplification inhibition compared to qPCR, inhibition can still influence performance and should be routinely assessed for all PCR-based workflows.

- **Spike control approach:** The spike control approach involves adding a known quantity of target nucleic acid or surrogate control to PCR-based test reactions. Performance of the spike in wastewater extract-containing reactions is then compared to performance in reactions containing molecular-grade water (substituted for wastewater extract) to estimate the extent of amplification inhibition.
- **Extract dilution approach:** Dilution of nucleic acid extracts can reduce the concentration of inhibitory substances and restore unbiased amplification. Diluted extracts are re-analyzed and compared to undiluted results to assess the presence of amplification inhibition. While dilution can mitigate amplification inhibition, it also decreases the target concentration and can result in a false negative result

  Note: The dilution factor must be accounted for to compare with original sample concentration.

Acceptance threshold determination for amplification inhibition testing varies by PCR platform (qPCR or dPCR). Thresholds should account for normal analytical variability using replicate measurements and statistical evaluation to distinguish amplification inhibition from expected variation. Because PCR-based measurement variability is concentration dependent, amplification inhibition assessments should be conducted within a concentration range representative of the expected surveillance target. Laboratories should define, document and apply amplification inhibition criteria consistently within a surveillance program.

Multiplex Performance Confirmation

Multiplex PCR-based assays combine multiple molecular tests within a single reaction to increase throughput and reduce costs. However, it is important during method development to confirm that each assay performs in a comparable fashion when used individually (singleplex). Performance confirmation typically involves parallel testing of each assay in both singleplex and multiplex formats across a range of target concentrations using shared control materials and representative wastewater extracts. If multiplexing results in substantial loss of sensitivity or quantitative disagreement for a given target, the multiplex assay may require further optimization, or the respective assay may need to be implemented in a singleplex format for optimal performance.

Limit of Detection

The limit of detection (LOD) represents the lowest target concentration that can be reliably distinguished from background signal with a stated level of confidence. Conceptually, LOD is defined relative to a limit of blank (LOB), which characterizes any potential background in the absence of target. The LOD is therefore the lowest concentration at which target signal can be differentiated from background and detected with acceptable confidence. Three LOD definitions are commonly used in microbial wastewater surveillance PCR-based workflows (PCR platform/instrument LOD, PCR assay LOD and Process LOD), each accounting for different sources of variability across analytical scales.

- **LOD for qPCR:** For qPCR, LOD is typically determined empirically by repeated testing of a low concentration control material. A commonly applied approach defines the LOD as the lowest concentration detected in a specified proportion of replicates (e.g., ≥ 95%, LOD^{95}). Alternatively, LOD can be estimated using statistical approaches that explicitly incorporate LOB and low-concentration control measurements to define the minimum detectable signal above background. Detections at or below the LOB are considered indistinguishable from background and are not evidence of target detection. Background signals can arise from factors such as rare false positives and/or low-level contamination and/or non-specific probe fluorescence. qPCR LOD should be established empirically for each assay.
- **LOD for dPCR:** Establishing LOD for dPCR typically involves a two-step process. First, the LOB is determined as the highest number of positive partitions or droplets expected from replicate blank samples (e.g., no template controls, extraction blanks, or confirmed target-free wastewater), often reported as the 95th percentile of blank responses. Second, the LOD is defined as the lowest target concentration that can be detected above the LOB with a specified confidence level, commonly 95%. This process should be established empirically for each dPCR assay.

It is important to note that low-level detections near the LOD should be interpreted cautiously. Results should be supported by quality controls, replicate measurements and temporal trends. For example, the presence of low-level contamination in negative controls (e.g., process blanks, extraction blanks or no template controls) can influence LOD estimates. In addition to contamination, assay cross-reactivity and, in some instances, non-specific amplification can also compromise the integrity of trace detections in a wastewater sample, potentially leading to incorrect conclusions about the presence of the target.

Common Limit of Detection Types

- **PCR platform/instrument LOD:** The lowest number of target copies that an instrument itself can reliably detect under ideal conditions (e.g., purified nucleic acid target and optimized reaction chemistry with no sample, nucleic-acid extract or assay level variability); typically reported in instrument specifications from manufacturer validation.
- **PCR assay LOD:** The lowest number of target copies that an assay (i.e., specified reaction chemistry, primer/probe set and platform/instrument) can reliably detect with a defined confidence level under ideal conditions (does not include nucleic acid extraction variability); typically determined by repeated testing of a target standard control material.
- **Process LOD:** The lowest sample level concentration (e.g., copies/mL, copies/gram) that can be detected after all steps of the method workflow (sample collection, concentration, nucleic acid extraction, characterization); typically orders of magnitude higher than PCR platform/instrument and PCR assay LOD.

Limit of Quantification

The limit of quantification (LOQ) is the lowest target concentration that can be measured with acceptable precision and reported as a reliable quantitative result. Like LOD, LOQ determination practices also differ between qPCR and dPCR platforms due to differences in measurement principles.

- **qPCR LOQ:** For qPCR, LOQ is typically evaluated based on standard curve performance, including *E*, R^2 and replicate variability in Cq values. A common precision-based criteria for qPCR is the lowest target concentration on the linear portion of a standard curve where the standard deviation from replicate Cq measurements (≥ 6 technical replicates) is ≤ 0.5 and the standard curve exhibits an $R^2 \geq 0.98$ and *E* is between 0.90 to 1.10.
- **dPCR LOQ:** In contrast, dPCR LOQ is derived from direct counting of positive and negative partitions/droplets and is evaluated based on statistical precision. A common practice for dPCR LOQ is the lowest target concentration that consistently achieves a percent coefficient of variation (%CV) ≤ 30 based on repeated measurements (≥ 6 technical replicates).

Additional Quality Assurance Metrics for Sequencing-based Methods

Sequencing-based wastewater surveillance requires robust bioinformatic quality controls due to potentially degraded nucleic acids, complex microbial backgrounds and low-abundance targets. Quality controls should address nucleic acid integrity, library preparation success, sequencing run performance and bioinformatic processing (**Table 7**).

Table 7: Common controls for sequencing-based platform workflows

Workflow	Type	Description
Nucleic Acid Integrity	Purity	Ensures nucleic acid extract is of sufficient quality (e.g., A260/A280 > 1.8)
	Concentration	Confirms that nucleic acid extract has adequate concentration (e.g., > LOD)
	Fragment Length	Check to confirm nucleic acid extract contains fragments of appropriate length
Library Preparation	Positive Control	Known spike material to demonstrate successful library preparation
	Negative Control	Nucleic acid extract substituted with molecular-grade water to demonstrate absence of contamination and support interpretation of low-level target detection
Sequencing Run	Spike Control	Well characterized genome control that is spiked into sequence library preparation that provides an internal error rate benchmark, identifies sequence run failures and serves as an internal positive control
Bioinformatic Pipeline	Quality Trimming	Ensure sequence reads are free from poor quality bases, adapter sequences and technical artifacts
	Contamination Detection	Ensures that data represents true wastewater sequences and not artifacts from human DNA, laboratory carryover, kit contaminants, or cross-sample leakage
	Coverage and Depth	Ensure sequencing depth and coverage are sufficient to detect target and variants with confidence

Quality Management Considerations for Commercial and External Testing

PHLs may implement microbial wastewater surveillance testing using commercialized kits, proprietary analytical pipelines and/or external service laboratories. These approaches can support rapid scale-up, reduce method development time, promote workflow standardization and provide access to specialized technologies or expertise that may not be readily available within individual laboratories. When appropriately integrated, commercial and external testing approaches can strengthen surveillance capacity and sustainability. At the same time, use of external products or services also presents several key quality management systems and transparency considerations (**Table 8**). Ultimately, PHLs remain responsible for ensuring that results generated through these approaches meet program needs and ethical guidelines, support appropriate interpretation and maintain confidence among partners, decision-makers and the public.

Table 8: Key quality management system considerations when using commercial or external testing approaches

Considerations	Description
Performance Reliance	Use of external products or services shifts reliance to vendors or contractors to maintain method performance and quality control practices.
Transparency	Access to information on protocols, reagents, analytic logic and quality control practices should be provided to support confidence in results and enable PHLs to communicate findings, limitations and uncertainty to partners.
Quality System Alignment	Vendor and service laboratories should operate under a documented quality management system suitable for wastewater surveillance testing.
Change Communication	Clear mechanisms should exist to notify PHLs of changes to reagents, workflows, or analytical pipelines.
Data Stewardship	Roles and responsibilities for data access, ownership, retention, reanalysis and reuse of samples should be clearly defined.

More Information

- The Environmental Microbiology Minimum Information Guidelines: qPCR and dPCR Quality and Reporting for Environmental Microbiology
- Minimizing errors in RT-PCR detection and quantification of SARS-CoV-2 RNA for wastewater surveillance
- MIQE Guidelines: Minimum Information for Publication of Quantitative Real-Time PCR Experiments
- MIQE 2.0: Revision of the Minimum Information for Publication of Quantitative Real-Time PCR Experiments Guidelines
- Digital MIQE Guidelines: Minimum Information for Publication of Quantitative Digital PCR Experiments
- Digital MIQE Guidelines Update: Minimum Information for Publication of Quantitative Digital PCR Experiments for 2020
- National Center for Biotechnology Information: Wastewater Surveillance Submission Portal
- CDC: The Next Generation Sequencing Quality Initiative
- NIST: Mpox (MPXV) Synthetic DNA PCR Standards
- Comparison of the Microbial Community Structures of Untreated Wastewaters from Different Geographic Locales | *Applied and Environmental Microbiology*
- Utility Engagement Toolkit
- Clinical Laboratory Improvement Amendments (CLIA)
- The NELAC Institute (TNI)
- WSLH Proficiency Testing

Data Reporting and Documentation

Microbial wastewater surveillance results are used by public health agencies, wastewater utilities and other partners including the public to inform time-sensitive decisions across preparedness, response and routine monitoring contexts. Clear, consistent data reporting and supporting documentation strengthen trust, support appropriate interpretation and improve comparability across laboratories and jurisdictions. While laboratory methods and quality management practices are addressed elsewhere in this guide, this section focuses on how results, metadata and contextual information are communicated and documented to end users in a public health setting. Established frameworks such as Minimum Information for Publication of Quantitative Real-time PCR Experiments (MIQE) and Environmental Microbiology Minimum Information (EMMI) guidelines provide useful reference points for transparency and reproducibility; selected principles from these guidelines are adapted here to support routine surveillance reporting needs rather than scientific publication.

Reporting Audiences and Intended Use

Microbial wastewater surveillance results are commonly reported to multiple audiences, including federal, state, tribal, local and territorial public health agencies; wastewater utilities and facility operators; and other partners such as healthcare systems, emergency response teams, academic collaborators and the public through online dashboards. Reporting formats, level of detail and frequency should be tailored to the intended use of wastewater monitoring data (e.g., early detection, trend monitoring) and audience. At the same time, laboratories and partners involved in data analytics and interpretation should maintain a consistent internal record of methods, metadata and quality management system information to support longitudinal analysis, retrospective review and coordination across reporting pathways.

Core Elements of Microbial Wastewater Surveillance Data Reporting

Routine microbial wastewater surveillance reports are not expected to include full method descriptions; however, laboratories should maintain concise, accessible documentation that supports appropriate interpretation and comparability of results. Across reporting formats, such as spreadsheets, dashboards and written summaries, key elements should be consistently captured and communicated, as summarized in Table 9 (page 40). These elements include what was measured, when and where samples were collected, sample handling and properties, how results were generated, if methodologies changed, how results were expressed and contextual information relevant to interpretation. Programs may benefit from developing standardized data dictionaries and reporting templates under version control, enabling efficient updates as methods, targets or reporting requirements evolve.

Table 9: Core elements for microbial wastewater surveillance data reporting

Element	Description
What was Measured?	• Target name(s), general description (e.g., viral, bacterial, fungal) with lineage or variant (if applicable) and nucleic acid sequence • Sample type (e.g., influent, primary sludge) and format (e.g., grab, composite, passive) • Target characterization platform (e.g., qPCR, dPCR, NGS)
When and Where Samples Were Collected?	• Sample collection date, time and quantity • Site identifier and location type (e.g., wastewater utility, sub-sewershed, facility-level) • Approximate community size and catchment size (when available) • System characteristics that may influence interpretation (e.g., average flow rate, treatment capacity, combined or separate sewer system)
Sample Handling and Properties	• Analysis date, reporting date, holding time, storage conditions and pretreatment (if used) • Key sample properties if available (e.g., temperature, total suspended solids, pH) • Sample archiving and long-term storage conditions
How Were Results Generated?	• Analytical workflow description (e.g., concentration, nucleic acid extraction, PCR primer/probe sequences and platform characterization) • Volume or mass of sample tested and wastewater equivalent volume analyzed • Types of quality controls used (e.g., negative controls, recovery controls), frequency of use, acceptance thresholds and results • Description of endogenous control assay (if used) • Description of amplification inhibition approach, assessment and mitigation • Definitions and values for LOB, LOD and LOQ • Documentation of method validation and/or verification
How Were Results Expressed?	• Description of all calculation steps from raw measurements to reported values • Reporting units (e.g., raw data, copies per mL or gram, normalized ratios, qualitative detection) • Any normalization data and transformation approaches applied (e.g., flow, human fecal markers, solids mass) • Notes on uncertainty or analytical limitations that may influence interpretation
Context for Interpretation	• Sampling frequency and reporting cadence • Indication of preliminary results, data revisions, or atypical conditions when applicable • Reference to program specific data dictionaries or submission requirements when reporting to national systems

Reporting Timelines and Delivery Mechanisms

Reporting timelines should align with the intended use and laboratory capacity. Key factors to consider include the turnaround time required to generate the data and inform public health action, reporting frequency and delivery mechanisms such as secure file transfer, shared databases, dashboards, or written reports. Expectations regarding reporting cadence, data revision and correction should be established in advance with data recipients and documented where appropriate.

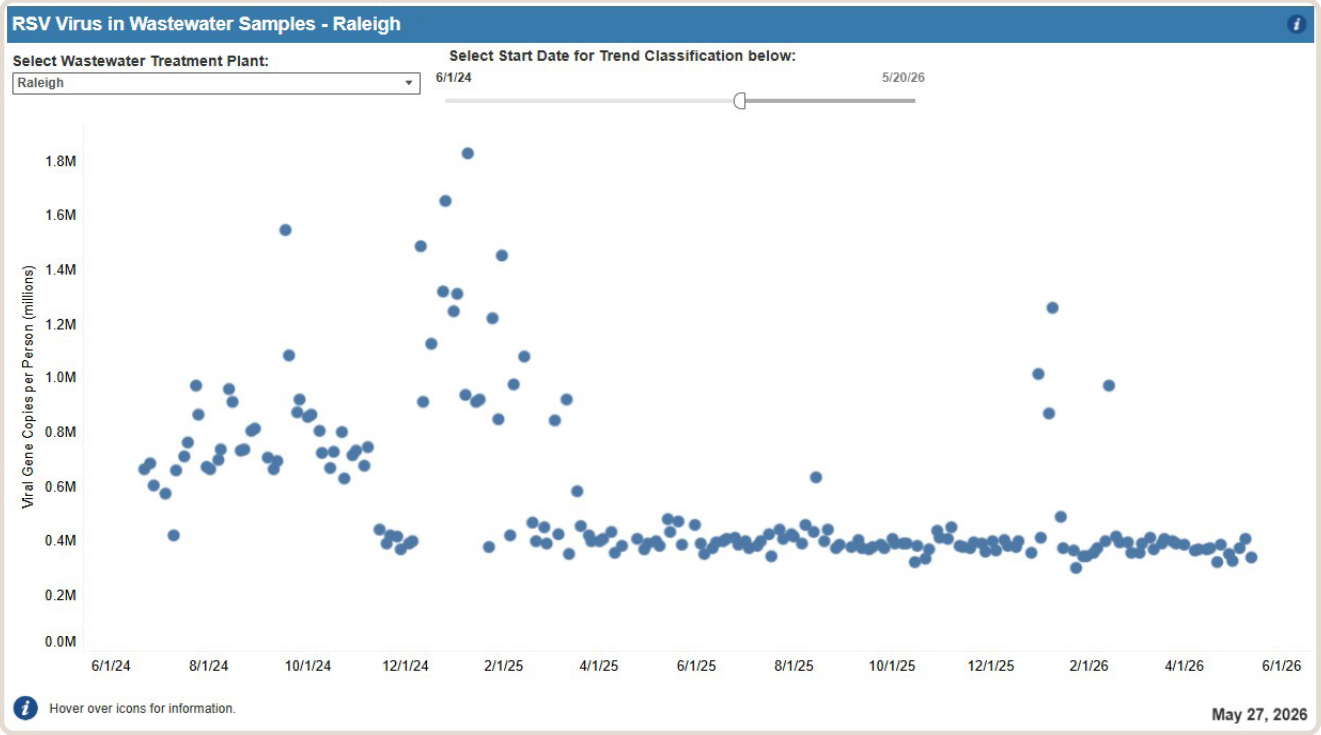
Public-facing data dashboards are a common mechanism for communicating microbial wastewater surveillance results to decision-makers and the public (**Figure 10, page 42**). Effective dashboards are interactive, web-based visualization tools that present summarized information in a timely, consistent and interpretable format, typically incorporating key elements described in **Table 10**. Common challenges include over-interpretation of preliminary data, insufficient context to understand uncertainties and misalignment between dashboard complexity and audience needs. In some instances, dashboards may present both clinical and wastewater monitoring data.

Table 10: Key elements of an effective public-facing microbial wastewater surveillance dashboard

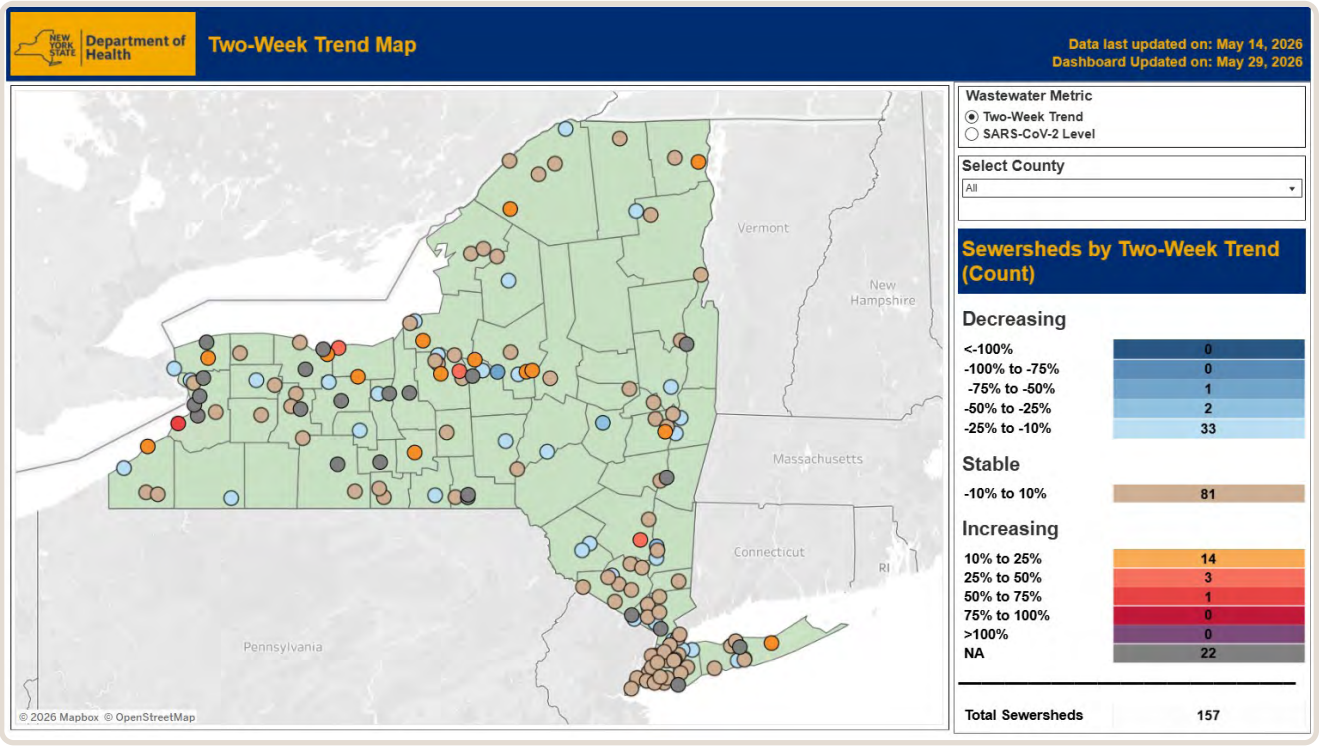
Element	Description
Clear Purpose and Audience Alignment	Focused on specific intended use (e.g., early detection, trend monitoring) and alert flagging with visual complexity scaled appropriately for technical and non-technical audiences.
Temporal and Geographical Context	Time-series displays and mapped views that help users understand how target signals change over time and vary across locations.
Transparent Data	Explanatory text, legends and annotations provide context on what is being measured, how results should be interpreted and known limitations or sources of uncertainty.
Consistent Update Cadence	Clearly communicated reporting frequency and data refresh schedules.
Accessibility and Clarity	Use of plain language, intuitive visuals and consistent color schemes to enhance interpretation.

Figure 10: Example PHL dashboards used to communicate microbial wastewater surveillance results to the public, demonstrating clarity of purpose, temporal and geographic context, transparent interpretation guidance and alignment with public health reporting needs

A. North Carolina Wastewater Monitoring Network Dashboard for RSV*



B. New York State Department of Health COVID-19 Wastewater Surveillance Dashboard for SARS-CoV-2**



* Source: NCDHHS Wastewater Testing Dashboard, depicting RSV data from Raleigh, NC from June 1, 2024 - May 20, 2026.

** Source: NYSDH COVID-19 Wastewater Concentration Dashboard, depicting two-week trends for SARS-CoV-2 levels, as of May 14, 2026.

Coordination and Communication with Data Users

Effective microbial wastewater surveillance reporting relies on continuous coordination between laboratories, health departments and other partners. Programs are encouraged to collaboratively define preferred metrics and visualization approaches that align with decision-making needs. In addition, it is important to establish a shared understanding of data flags and limitations, so any quality issues are communicated clearly and proportionately. It is also key to periodically revisit reporting practices as new targets, methods or analytical approaches are introduced. Clearly documented communication pathways help ensure that wastewater surveillance data are interpreted appropriately and used consistently alongside other public health information.

More Information

- Environmental Microbiology Minimum Information Guidelines: qPCR and dPCR Quality and Reporting for Environmental Microbiology
- Minimizing errors in RT-PCR detection and quantification of SARS-CoV-2 RNA for wastewater surveillance
- MIQE Guidelines: Minimum Information for Publication of Quantitative Real-Time PCR Experiments
- MIQE 2.0: Revision of the Minimum Information for Publication of Quantitative Real-Time PCR Experiments Guidelines
- Digital MIQE Guidelines: Minimum Information for Publication of Quantitative Digital PCR Experiments
- Digital MIQE Guidelines Update: Minimum Information for Publication of Quantitative Digital PCR Experiments for 2020
- Wastewater Monitoring Dashboard | NC COVID-19
- COVID-19 Wastewater Surveillance | NY Department of Health

Ethical Considerations

Microbial wastewater surveillance generates community-level public health insights and does not identify individuals or support clinical diagnosis. Ethical considerations should be integrated across program design, laboratory practices, sample and data archiving and data reporting to protect privacy, maintain public trust and support responsible use of wastewater surveillance data. Because wastewater data represents pooled contributions from many individuals, surveillance inherently provides an important level of anonymity, but the scale of wastewater surveillance can vary. Smaller, more granular scale efforts may necessitate additional careful ethical consideration and safeguards. It is important to note that human genetic material, while present in wastewater, should not be tested or analyzed as part of wastewater surveillance for public health. Programs are encouraged to evaluate whether data collection, analysis, or reporting practices could reasonably contribute to identification, stigmatization or misuse of information. In addition, the purpose of the program should be clearly defined and articulated to understand the public health value and ethical considerations of the wastewater surveillance effort. All partners should be informed of ethical practices.

Privacy Considerations for Microbial Wastewater Surveillance

- **Avoid reporting of data that could be combined with clinical or other datasets to identify individuals, directly or indirectly.**
- **Consider additional privacy protections for routine surveillance in sewersheds serving small communities (<3,000 individuals).**
- **Avoid facility-level sampling locations (e.g., schools, correctional facilities, workplaces) for public reporting, unless explicitly justified and governed by appropriate agreements.**
- **Respect tribal sovereignty and actively collaborate with tribal public health officials (explicit authorization/consent is necessary before collection or reporting of wastewater data from tribal communities).**
- **Withhold or flag data from sewersheds with known data quality limitations, where results may be misleading or inappropriate for interpretation.**

Accuracy, Interpretation and Responsible Reporting

PHLs and surveillance programs have an ethical responsibility to ensure that analytical methods and sample management are appropriate for the intended use and are supported by robust laboratory quality management system practices. Responsible reporting includes clearly communicating uncertainty, limitations and contextual information so that results are not misinterpreted or over-interpreted. Reporting practices should avoid implying individual infection status or clinical diagnosis and should emphasize the community-level nature of wastewater surveillance data. When applied consistently, responsible interpretation and communication support appropriate public health action while minimizing the risk of misunderstanding or misuse.

Alignment with National Ethical Frameworks

National ethical frameworks provide an important foundation for practice and decision-making related to wastewater surveillance. Guidance from organizations such as the Association of State and Territorial Health Officials (ASTHO) and CDC NWSS emphasizes principles related to privacy protection and data stewardship, equity and avoidance of stigma and community trust and engagement. These frameworks are intended to inform local implementation while recognizing that ethical considerations may vary by jurisdiction, defined community and surveillance use case. Alignment with established national guidance helps preserve the public health value of wastewater surveillance while promoting ethical consistency across programs.

More Information

- ASTHO: Framework for Addressing Ethical Considerations in Infectious Diseases Public Health Wastewater Surveillance
- CDC: Security, Privacy and Protection in 1CDP | Data Modernization
- CDC: About Wastewater Data | National Wastewater Surveillance System

Key Challenges and Strategic Priorities

Microbial wastewater surveillance has matured rapidly, moving from emergency response applications to a sustained component of public health infrastructure. As programs expand in scope, scale and target diversity, laboratories and partners face technical, operational and interpretive challenges that must be addressed to maintain data quality, comparability and long-term sustainability. At the same time, emerging applications create opportunities for innovation, coordination and integration across public health systems. The following priorities highlight areas where targeted investment and collaborative action can strengthen the future impact of microbial wastewater surveillance.

Method Evolution Management

Microbial methods continue to advance as new targets and technologies are introduced and workflows improve. Changes such as new reagents, updated instrumentation, or workflow refinements can complicate data comparability in the absence of formal equivalency frameworks. Development of structured method change management approaches, including bridging studies, documentation standards and cross-platform comparability assessments, will enable laboratories to adopt improvements while preserving data integrity and longitudinal comparability.

Control Material Availability

Availability of appropriate control materials remains a major obstacle, particularly for newly emerging targets. Although national repositories (e.g., BEI Resources and ATCC) and commercial vendors provide critical support, ready-to-use materials are often unavailable during early target development and deployment. As a result, laboratories may rely on interim or in-house controls, increasing variability and complicating cross-laboratory comparisons. The development of coordinated wastewater surveillance biobanking resources composed of samples containing endogenous pathogens would support future method validation and verification activities as well as proficiency testing programs.

Workflow Harmonization and Interlaboratory Comparability

Variation in wastewater surveillance workflows and quality control practices across laboratories can contribute to differences in measured target concentrations, complicating data comparison. At the same time, overly rigid standardization limits innovation and may introduce vulnerabilities, including supply chain dependencies and reduced adaptability to diverse wastewater matrices. Future efforts should focus on fit-for-purpose harmonization strategies that promote data comparability while preserving carefully defined methodological flexibility. Tiered guidance frameworks, shared performance benchmarks and expanded interlaboratory studies may help identify the appropriate balance across state, tribal, local, regional and national scales.

Sustaining Workforce, Infrastructure and Funding

Microbial wastewater surveillance programs require sustained investment in trained personnel, laboratory infrastructure and information technology systems. Workforce instability can limit innovation, disrupt institutional knowledge and increase burnout risk among experienced personnel. Long-term stability will depend on predictable funding mechanisms, cross-training strategies and career pathways that support workforce retention. Continued integration of wastewater surveillance into core public health infrastructure may help transition programs from emergency-supported activities to durable surveillance capacity.

Expanding Access to Sequencing Capacity

Broader access to sequencing technologies and bioinformatic support will enhance variant detection, genomic characterization and detection of unexpected or new targets. Investments in shared infrastructure, regional sequencing hubs and workforce training can help reduce barriers for laboratories with limited in-house capacity and improve national coverage. Strengthening pilot programs, analytic, bioinformatic and data-sharing support will further increase the public health value of targeted and metagenomic sequencing-enabled microbial wastewater surveillance.

Broadening the Microbial Methods Toolbox

Continued development of complementary analytical approaches, including improved concentration methods, expanded multiplexing capacity and agnostic detection workflows, will enhance microbial wastewater surveillance capabilities. In some contexts, expanding beyond nucleic acid detection alone may provide additional public health insight. For example, integration of culture-based approaches could help address questions related to infectivity or phenotypic characteristics for select targets. Ongoing method innovation, coupled with rigorous validation, will be important to ensure that new tools meaningfully advance public health decision-making.

Collectively, addressing these priorities will help ensure that microbial wastewater surveillance continues to mature as a robust, adaptable and sustainable component of modern public health systems.

More Information

- BEI Resources
- ATCC: The Global Bioresource Center

Appendices

List of Acronyms

1CDP: One CDC Data Platform

APHL: Association of Public Health Laboratories

ASTHO: Association of State and Territorial Health Officials

ATCC: American Type Culture Collection

BMBL: Biosafety in Microbiological and Biomedical Laboratories

CDC: Centers for Disease Control and Prevention

cDNA: Complementary deoxyribonucleic acid

CoE: Centers of Excellence

COVID-19: Coronavirus Disease 2019

Cq: Quantification cycle

CSTE: Council of State and Territorial Epidemiologists

CV: Coefficient of variation

DNA: Deoxyribonucleic acid

dPCR: Digital polymerase chain reaction

EMMI: Environmental Microbiology Minimum Information

FTE: Full-time employee

H1 / H3 / H5: Influenza A hemagglutinin subtypes

IFU: Instructions for use

LOB: Limit of blank

LOD: Limit of detection

LOQ: Limit of quantification

LIMS: Laboratory information management systems

MIQE: Minimum information for publication of quantitative real-time PCR experiments

mL: Milliliter

MPXV: Monkeypox virus

NACCHO: National Association of County and City Health Officials

NCBI: National Center for Biotechnology Information

NGS: Next Generation Sequencing

NIOSH: National Institute for Occupational Safety and Health

NIST: National Institute of Standards and Technology

NPDES: National Pollutant Discharge Elimination System

NTC: No template control

PCR: Polymerase chain reaction

PMMoV: Pepper mild mottle virus

PEG: Polyethylene glycol

PHL: Public health laboratory

qPCR: Quantitative polymerase chain reaction

R^2: Coefficient of determination

RNA: Ribonucleic acid

RSV: Respiratory syncytial virus

SARS-CoV-2: Severe Acute Respiratory Syndrome Coronavirus 2

TGS: Third Generation Sequencing

WEF: Water Environment Federation

Glossary of Terms

Amplicon: A specific DNA or cDNA fragment that is generated by amplification during PCR-based testing.

Amplification efficiency: A measure of how effectively a qPCR assay doubles the target nucleic acid during each amplification cycle, typically derived from the slope of a standard curve.

Amplification inhibition: Inhibition of PCR-based amplification caused by interfering substances that may be present in samples or reagents.

Antimicrobial resistance gene: A genetic sequence that confers reduced susceptibility or resistance to one or more antimicrobial agents when present in a microorganism.

Bioinformatics: The application of computational tools and statistical methods to store, process, analyze and interpret nucleic acid sequence information and related data.

Bioinformatic pipeline: Software algorithms executed in a predefined sequence to process and analyze raw sequencing data.

cDNA: Complementary DNA synthesized from a single-stranded RNA template produced by reverse transcriptase.

Cq value: The number of cycles taken to detect a signal from the sample when using real-time quantitative PCR (qPCR). Also referred to as the Ct value.

Combined sewer system: A wastewater collection system that conveys sanitary sewage and stormwater runoff in the same pipe network.

Composite sample: A sample created by combining multiple subsamples collected over time or proportional to flow to provide a time-integrated representation of wastewater.

Control material: A characterized nucleic acid biological or synthetic material used to evaluate performance of laboratory methods, instruments or workflows.

Digital PCR (dPCR): This technique detects and quantifies nucleic acids after partitioning (dividing) the sample, resulting in absolute quantification using statistical analysis.

Direct extraction: A workflow in which nucleic acids are extracted directly from wastewater or solids.

Endogenous wastewater control: A naturally occurring genetic marker in wastewater, typically human fecal-associated, used to contextualize target measurements.

Extraction blank control: A negative control consisting of molecular-grade water or buffer processed through nucleic acid extraction to monitor for potential contamination introduced during extraction.

Extraction recovery control: An exogenous material added after concentration but before nucleic acid extraction to evaluate extraction efficiency.

False negative: A test result indicating that a target is absent when it is actually present in the sample.

False positive: A test result indicating that a target is present when it is actually absent in the sample.

Fecal normalization: Adjustment of target concentrations using a human fecal-associated endogenous wastewater control or other variable (i.e., flow) to account for variability in human waste contribution in a wastewater sample.

Full process blank control: A negative control that substitutes molecular-grade water or buffer for a sample and is carried through the entire workflow to detect potential contamination.

Grab sample: A single wastewater sample collected at one point in time.

Instructions for Use (IFU): CDC-provided documentation describing procedures, materials and conditions for method implementation.

Laboratory information management system (LIMS): Software used to track samples, manage laboratory workflows, store results and support data quality and reporting.

Limit of Blank (LOB): The highest detection expected when replicate blank samples are tested with digital PCR (dPCR), used to distinguish background from true target detection.

Limit of Detection (LOD): The lowest target concentration that can be reliably distinguished from a blank with a stated level of confidence.

Limit of Quantification (LOQ): The lowest target concentration that can be measured with an acceptable precision and accuracy.

Lineage (viruses): Group of closely related viruses with a common ancestor.

Linearity: The ability of a qPCR assay standard curve to produce results that are directly proportional to target concentration across a defined range, often reported as a correlation coefficient (R^2).

Matrix recovery control: Process control used to assess target concentration lost during sample processing.

Membrane filtration: A concentration method that captures microorganisms or particles by passing wastewater through a porous membrane.

Metadata: Data that are used to describe or provide more information about target measurements.

Metagenomic sequencing: An untargeted sequencing approach that characterizes the collective genetic material from all organisms in a sample.

Method validation: Process used to confirm that the analytical procedure employed for a specific test is suitable for its intended use.

Method verification: Confirmation that a validated or established method performs as expected in a specific laboratory setting, typically through pilot testing with representative samples to demonstrate reliable performance for the intended use.

Microbial wastewater surveillance: Strategic sampling and testing of wastewater to detect pathogens or other microbial health targets to better understand disease burden and spread within a community.

Multiplex: PCR-based test that simultaneously amplifies multiple targets in a single reaction.

Next generation sequencing (NGS): High-throughput sequencing technologies that simultaneously determine the sequences of thousands to millions of nucleic acid fragments.

No template control: A PCR-based reaction containing all assay components except nucleic acid template, used to detect potential contamination.

Nucleic acid extraction: Process of separating nucleic acids from other microbial components (i.e., lipids, proteins) and concentrating.

Nucleic acid sequestration: The binding or partitioning of nucleic acids or microorganisms to solids or other substances that reduce recovery.

Passive sampling: A sampling approach in which an absorbent material is deployed in wastewater for a defined period to accumulate targets over time.

Pasteurization: Heating a wastewater sample to reduce potential pathogen exposure from bioaerosol-generating procedures during sample processing.

PCR assay LOD: The lowest number of target copies that a specific PCR-based assay can reliably detect with a stated level of confidence.

PCR platform/instrument LOD: The lowest number of target copies an instrument can reliably detect under optimized conditions with a stated level of confidence independent of assay or sample variability.

Percent coefficient of variation (%CV): A measure of relative variability calculated as the standard deviation divided by the mean and expressed as a percentage.

Positive control: A control containing known target nucleic acid, often at a known concentration, used to confirm that an assay or workflow is functioning properly.

Pretreatment: Optional processing steps applied to wastewater prior to concentration or nucleic acid extraction to improve consistency, safety, or downstream performance.

Primary sludge: Settled solids collected during primary sedimentation at a wastewater treatment facility.

Process LOD: The lowest target concentration detectable with a stated level of confidence after the entire workflow, including sampling, concentration, extraction and analysis.

Process recovery control: An exogenous material added to raw wastewater prior to processing to assess overall method recovery.

Proficiency testing: Programs established to independently assess laboratory performance in clinical and environmental tests, where the participating laboratories are typically directed to analyze specific samples using prescribed protocols and receive reports on performance to improve overall data quality and comparability.

Protease: An enzyme that degrades proteins and is sometimes used during pretreatment to improve nucleic acid recovery.

qPCR (quantitative PCR): A real-time PCR method that measures amplified DNA during each cycle using fluorescence to enable quantification.

Reverse transcription: The enzymatic conversion of RNA into complementary DNA (cDNA) prior to PCR-based amplification.

Sanger sequencing: A first-generation sequencing method that determines the nucleotide sequence of a specific nucleic acid molecule with high accuracy.

Separate sewer system: A wastewater infrastructure in which sanitary sewage and stormwater runoff are conveyed in separate pipe networks.

Sewershed: The geographic area from which wastewater drains to a defined sampling point or wastewater facility.

Singleplex: PCR-based assay that detects one target sequence.

Standard curve: A tool used to estimate target concentration in an unknown sample by comparing concentrations to standards with known concentrations; also used to determine amplification efficiency, linear range and reproducibility of a qPCR assay.

Sub-sewershed: A smaller, defined drainage area within a larger sewershed.

Subtyping: A classification approach that distinguishes subgroups within a defined microbial type based on defined characteristics. In wastewater surveillance, subtyping is commonly applied to identify distinct variants withing a broader category (e.g., influenza A subtypes H1, H3 and H5) to support more refined public health interpretations.

Surfactant: Chemical agents that reduces surface tension and may be used in microbe lysis or sample processing.

Target: An RNA or DNA sequence of interest.

Third generation sequencing (TGS): Long-read sequencing technologies that analyze individual DNA or RNA molecules in real-time.

Total suspended solids: Total organic and inorganic particles suspended in a wastewater sample that can be captured by a filter, typically greater than two microns in size.

Typing: The process of categorizing a microbial organism into a defined group based on shared biological or genetic characteristics. Typing typically occurs at a broader level than subtyping and may include classification by species, serotype, genotype or lineage.

Ultrafiltration: A concentration method that uses size exclusion membranes to retain microorganisms and nucleic acids from large wastewater volumes.

Unidirectional workflow: A laboratory layout and practice in which work progresses from clean to dirty areas to minimize contamination risk.

Untreated wastewater influent: Wastewater sampled entering the headworks of a wastewater utility and excluding stream returns from inside the plant, consisting of pooled liquid waste generated from household and building use. May include fecal, urine and other human waste contributions, as well as potential non-household inputs such as stormwater, agricultural and industrial discharges.

Variant: A version of a microbial organism that differs from a reference strain due to one or more genetic mutations. Variants may exhibit differences in transmissibility, pathogenicity, or detection characteristics. In wastewater surveillance, variant identification supports monitoring of emerging strains and assessment of potential public health risk.

Variant of concern: A variant for which there is evidence of an increase in transmissibility, more severe disease (e.g., increased hospitalizations or deaths), significant reduction in neutralization by antibodies generated during previous infection or vaccination, reduced effectiveness of treatments or vaccines, or diagnostic detection failures.

Additional Resources

APHL

- ColLABborate Community of Practice
- Metagenomics May Offer Value Across Many Areas of Public Health | *Lab Matters* Summer 2025
- National Trends in Wastewater Surveillance 2023 Survey Report
- National Trends in Wastewater Surveillance 2025 Survey Report
- Risk Assessment Best Practices
- Risk Assessment for Ebola Testing
- SARS-CoV-2 Wastewater Surveillance Testing Guide for Public Health Laboratories

US Federal Resources

- CDC:
  - About Hierarchy of Controls
  - About Wastewater Data
  - Biosafety in Microbiological and Biomedical Laboratories (BMBL), 6th Edition
  - National Wastewater Surveillance System (NWSS)
  - CDC NWSS Centers of Excellence
  - Next Generation Sequencing Quality Initiative | Laboratory Quality
  - One CDC Data Platform | Data Modernization
  - Security, Privacy and Protection in 1CDP | Data Modernization
  - Wastewater Monitoring Fact Sheet
- Clinical Laboratory Improvement Amendments (CLIA)
- Environmental Protection Agency:
  - National Pollutant Discharge Elimination System (NPDES) Compliance Inspection Manual – Chapter 5
  - Procedures for Collecting Wastewater Samples
  - Region 4 Procedures for Collecting Wastewater Samples
- Federal Select Agent Program
- National Center for Biotechnology Information:
  - Wastewater Surveillance Submission Portal
  - Human Read Removal Tool
- National Institute of Standards and Technology: Mpox (MPXV) Synthetic DNA PCR Standards

Wastewater Testing Programs

- California Wastewater Surveillance Program
- Colorado Center of Excellence
- Houston, TX:
  - Houston Wastewater Epidemiology Center of Excellence
  - Wastewater Monitoring Training Modules
- New York State:
  - New York Center of Excellence
  - COVID-19 Wastewater Surveillance Dashboard
- North Carolina: Center of Excellence COVID-19 Wastewater Monitoring Dashboard
- Wisconsin:
  - Wisconsin Center of Excellence
  - WSLH Proficiency Testing

Partners

- ASM: Wastewater Surveillance for Bacterial Targets—Current Challenges and Future Goals
- Association of State and Territorial Health Officials
  - Framework for Addressing Ethical Considerations in Infectious Diseases Public Health Wastewater Surveillance
- American Type Culture Collection:
  - The Global Bioresource Center
  - BEI Resources
- Council of State and Territorial Epidemiologists
- National Academy of Sciences: Community Wastewater-based Infectious Disease Surveillance
- NACCHO: Wastewater Surveillance Resource Library
- NELAC Institute (TNI)
- Water Environment Federation
  - Utility Engagement Toolkit
- WastewaterSCAN Public Wastewater Monitoring Project
- Water Research Foundation: Best Practices for Collection and Storage of Wastewater Samples to Support Wastewater Surveillance of the COVID-19 Signal in Watersheds

International Guidance

- European Union: Wastewater Observatory for Public Health
- WHO: Wastewater and environmental surveillance for one or more pathogens: guidance on prioritization, implementation, and integration

References and Journal Articles

- Comparison of the Microbial Community Structures of Untreated Wastewaters from Different Geographic Locales | *Applied and Environmental Microbiology*
- Early Evidence of the SARS-CoV-2 B.1.1.529 (Omicron) Variant in Community Wastewater — United States, November–December 2021 | Notes from the Field, *Morbidity and Mortality Weekly Report (MMWR)*
- Environmental Microbiology Minimum Information (EMMI) Guidelines: qPCR and dPCR Quality and Reporting for Environmental Microbiology | *Environmental Science & Technology*
- Example Laboratory Risk Assessment Process: A Laboratory Risk Assessment during the Coronavirus Pandemic | Vanderbilt University
- Genomic and Wastewater Surveillance Data to Guide a Hepatitis A Outbreak Response — Los Angeles County, March 2024–June 2024 | Notes from the Field, *MMWR*
- Minimizing errors in RT-PCR detection and quantification of SARS-CoV-2 RNA for wastewater surveillance | *Science of The Total Environment*
- Minimum Information for Publication of Quantitative Real-time PCR Experiments (MIQE):
  - MIQE Guidelines | *Clinical Chemistry*
  - MIQE Guidelines 2.0 | *Clinical Chemistry*
  - Digital MIQE Guidelines (Quantitative Digital PCR) | *Clinical Chemistry*
  - Digital MIQE Guidelines Update | *Clinical Chemistry*
- SARS-CoV-2 wastewater genomic surveillance: approaches, challenges, and opportunities | Genome Biology | *Genome Biology*
- Wastewater Surveillance Data as a Complement to Emergency Department Visit Data for Tracking Incidence of Influenza A and Respiratory Syncytial Virus — Wisconsin, August 2022–March 2023 | MMWR
- Wastewater Surveillance for Measles Virus During a Measles Outbreak — Colorado, August 2025 | Notes from the Field, *MMWR*
- Wastewater Target Pathogens of Public Health Importance for Expanded Sampling, Houston, Texas, USA | *Emerging Infectious Diseases*

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