CSTE

Wastewater Surveillance Toolkit

For state, local, and territorial (SLT) public health agencies

August 2026

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Acknowledgements

This toolkit was prepared by Eastern Research Group, Inc. (ERG) for the Council of State and Territorial Epidemiologists (CSTE) and was supported by the Centers for Disease Control and Prevention (CDC) of the U.S. Department of Health and Human Services (HHS) as a part or component of a financial assistance award totaling $2,4000,000 with 100 percent funded by CDC/HHS. The contents are those of the author(s) and do not necessarily represent the official views of, nor an endorsement by, CDC/HHS or the U.S. Government.

CSTE acknowledges the contributions of the following people and agencies to this project:

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  - Megan Gerdes, ASRT, Inc., contractor for CDC
  - Abby Hoffman, CSTE
  - Gabriela Iglesias-Mendoza, Colorado Department of Public Health and Environment
  - Sarah Koenigsberg, North Carolina Department of Health and Human Services
  - David Larsen, Syracuse University
  - Brian Lefferts, Yukon-Kuskokwim Health Corporation
  - Anilkumar Mangla, Southern Nevada Health District
  - Breanna McArdle, Washington State Department of Health
  - Jennifer Myers, Houston Health Department
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  - Angela Rabe, California Department of Public Health
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  - Hannah Turner, Goldbelt, Inc., contractor for CDC
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- Additional contributors from CDC's National Wastewater Monitoring Program, National Center for Emerging and Zoonotic Infectious Diseases, Division of Infectious Disease Readiness and Innovation
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How to Use CSTE's Wastewater Surveillance Toolkit

This toolkit is designed to support state, local, and territorial (SLT) public health agencies in using wastewater data as part of public health surveillance and response. It is intended for epidemiologists, surveillance staff, laboratories, and public health decision-makers.

This toolkit may also be used and adapted by Tribal communities. Implementation of wastewater surveillance by Tribal public health agencies warrants further collaboration and individualized approaches that honor federal Indian trust responsibilities, Tribal sovereignty, and unique community needs.

Contents

This toolkit focuses on infectious-disease surveillance using PCR-based wastewater concentration data. Wastewater surveillance for substances, other chemical markers, and sequencing-based approaches is outside its primary scope. It provides guidance on how to integrate wastewater data with other public health surveillance systems (e.g., syndromic, laboratory reporting, and electronic case reporting) and how to interpret signals within the broader context to inform public health decision-making.

Wastewater surveillance for infectious disease is a valuable public health tool that enhances situational awareness and complements traditional public health surveillance by providing a population-level signal that does not depend on healthcare-seeking behavior or diagnostic testing. For some pathogens, it can also provide early warning of community transmission. It is most effective when used as a complement to other data sources. It provides practical guidance and tools to help public health practitioners:

- Understand what wastewater data can (and cannot) tell you
- Integrate wastewater data with other surveillance systems
- Interpret wastewater signals within the appropriate epidemiological and operational context
- Translate data into informed public health actions

Overall, this toolkit emphasizes wastewater data as one component of a broader, integrated public health surveillance system to support coordinated, data-informed decision-making.

How to Use This Toolkit

This toolkit can be used in several ways, depending on the needs of an SLT public health agency:

- New or developing programs may use the toolkit to understand how wastewater data fit into public health surveillance and to inform program design.
- Established programs can use specific sections to refine data integration, interpretation, or response strategies.

- Cross-functional teams (e.g., epidemiology, laboratory, communications) can use different sections to support their roles in the workflow.

Users may choose to:

- Read the toolkit sequentially for a comprehensive overview, or
- Refer to individual sections as needed for specific guidance or tools

Each section is designed to stand alone and provide practical guidance adaptable to different jurisdictions, data availability, and public health priorities.

How This Toolkit Is Organized

The toolkit is organized into six sections, each focused on a key aspect of using wastewater data. These sections can be used independently or together as a comprehensive resource.

Section 1. Understanding Wastewater Data as a Public Health Tool
A high-level overview of wastewater surveillance, including its role in public health monitoring, program objectives, and key considerations.

Section 2. Analyzing Wastewater Data
How to evaluate wastewater data quality, normalize and aggregate data across sites and time, and identify meaningful trends.

Section 3. Interpreting Signals with Complementary Data
How to interpret wastewater signals in context using complementary surveillance systems and distinguish meaningful signals from background variability.

Section 4. Translating Wastewater Signals into Public Health Action
Guidance on how to move from signal detection to decision-making and response, including decision frameworks and communication considerations.

Section 5. Pathogen-Specific Considerations
How interpretation and response may vary across different types of pathogens (e.g., respiratory, enteric, emerging threats).

Section 6. Wastewater Surveillance Case Studies
Real-world examples illustrating how wastewater data have been used in practice to support public health decision-making.

1. Understanding Wastewater Data as a Public Health Tool

This section provides a foundation for understanding wastewater surveillance as a public health tool. It describes how to define program objectives, sampling strategies, information on which pathogens to monitor, ethical and governance considerations, and strengths and limitations of wastewater surveillance. Practitioners who are newer to wastewater surveillance may find it useful to read this section first before moving into the more technical guidance in later sections. Those with established programs may use it as a reference for revisiting foundational decisions.

Introduction to Wastewater Surveillance

Wastewater systems are designed for waste removal, public health promotion, and environmental protection, but they can also serve as a continuous source of insight into community health. This toolkit focuses primarily on surveillance conducted through centralized sewer systems, including sampling within sewer collection systems and at wastewater treatment plants.

Wastewater contains biological and chemical markers from human and nonhuman sources. These markers include pathogens, metabolized pharmaceuticals, and indicators of chemical exposure. By sampling wastewater before it is treated, public health practitioners can analyze these markers as a non-invasive tool for tracking community health.

This approach is known as wastewater-based epidemiology. Unlike traditional clinical surveillance, which relies on individuals feeling sick, seeking care, and receiving diagnoses, wastewater monitoring captures data from an entire population at once, including people who are asymptomatic or who lack access to healthcare. By tracking pathogen levels, antimicrobial resistance genes, or other infectious-disease-related targets in wastewater, public health practitioners can track pathogen presence in parallel with clinical data. Incorporating wastewater surveillance into routine public health practice can support more tailored interventions and more effective use of public health resources. For a general overview of wastewater surveillance, see CDC's About CDC's Wastewater Monitoring Program page.

Defining Program Objectives

Wastewater surveillance can serve many different public health goals, but its value depends on deliberate program design. Before samples are collected, public health agencies should determine exactly what questions they are trying to answer. Clearly defined objectives are critical for effective application of wastewater surveillance data. Programs should establish what they aim to achieve, as these goals will shape how data are collected, interpreted, and used.

Common objectives include:

- Early detection of emerging or re-emerging pathogens
- Using wastewater data to monitor pathogen trends over time
- Supplementing surveillance in populations with limited healthcare access

- Supporting outbreak detection and investigation
- Informing public health response, planning, and communication
- Evaluating the impact of public health interventions

Program objectives shape decisions throughout the surveillance process, including:

- Scale of monitoring (e.g., community-level vs. facility-level)
- Sampling strategies and frequency
- Data analysis and interpretation approaches
- Pathogen selection and laboratory methods
- Thresholds for action and decision-making

Downstream and Upstream Sampling

One of the most consequential early decisions for a wastewater surveillance program is determining the appropriate scale and location of sampling, as these choices will shape sampling strategy, data interpretation, and the types of public health actions the program can support. Programs may use downstream sampling, upstream sampling, or a combination of both.

- **Downstream sampling** is typically conducted at a wastewater treatment plant (WWTP) that serves as the endpoint for a single sewershed. (A sewershed is the geographic area served by a single wastewater collection system, similar to how a watershed is the area drained by a river.) Sampling at a WWTP provides a broad population-level signal representing an aggregate of everyone contributing to that sewershed. This approach is well suited for tracking endemic pathogens, identifying emerging trends, and generating situational awareness across large geographic areas.
- **Upstream sampling** refers to sampling at sites upstream of a WWTP, such as manholes, lift stations, or building-level access points. It can serve two purposes. First, it can narrow in on a smaller area within a larger sewershed, such as a specific neighborhood, to better understand transmission patterns and characterize the geographic distribution of a signal. Second, it can target a specific institution or setting, such as a school, dormitory, correctional facility, hospital, long-term care facility, or international terminal. Because signals from upstream sites reflect more defined populations, this approach can improve understanding, increase sensitivity, and enable tailored interventions when a signal needs to be better characterized, though it is generally more resource intensive than downstream sampling and may raise ethical considerations.

Most programs sample downstream, at the WWTP, as a baseline for community-level monitoring because of existing sample collection processes and infrastructure. Some programs supplement this with upstream sampling when a signal warrants further investigation and geographic narrowing. The right approach will depend on program resources, surveillance goals, and the pathogens being monitored. Regardless of scale, it is important to keep in mind the population represented by a given sample and whether there are known factors that might disproportionately influence the signal at a given site – for example, a large institutional contributor such as a hospital, correctional facility, or airport. Close collaboration with the utility can help understand the populations served.

All wastewater surveillance activities require agreement and collaboration with the wastewater utility. For most utilities, participation is voluntary and conducted in addition to their routine operational and regulatory responsibilities. Establishing a strong utility partnership early can help programs select appropriate sampling locations, understand the population and infrastructure, interpret unusual results, and develop procedures for sample collection and data sharing. The Water Environment Federation's Utility Engagement Toolkit provides additional guidance for building and sustaining these partnerships.

Sampling Strategies and Frequency

Decisions about how, where, and how often to sample are among the most practically consequential choices a wastewater surveillance program will make. These decisions should be guided by close collaboration with the utility, program and surveillance objectives, the pathogens being monitored, available laboratory capacity, and the characteristics of the sewershed. There is no universal right answer, but consistency and intentionality matter more than any single methodological choice.

- **Sampling methodologies** – Most wastewater surveillance programs use one of three sampling methods. Programs should document which sampling method is used at each site, as this affects how results should be interpreted and compared over time.
    - 24-hour composite sampling is when automated samplers collect small aliquots of wastewater continuously over a full day, either at fixed time intervals or proportional to flow rate. This approach produces a sample that is representative of the full daily contribution of the sewershed population, smoothing out the natural variability that occurs across the day as sewer use patterns shift.
    - Grab sampling is when samples are collected at a single point in time. They are simpler to collect and may be appropriate in settings where automated samplers are unavailable or where a rapid, opportunistic sample is needed. However, because they capture only a single moment, grab samples are less representative, may miss a signal, and are generally less suitable for trend monitoring.
    - Passive sampling is done by devices left in the wastewater stream over a period to passively adsorb target analytes; they offer a low-cost alternative that does not require power or active mechanical sampling, though they may have different sensitivity and recovery characteristics depending on the analyte and device used.
- **Sampling frequency** – Sampling frequency determines how quickly a program can detect an emerging signal and how reliably it can characterize trends. More frequent sampling provides better temporal resolution and reduces the risk of missing a short-lived signal but comes with higher laboratory and operational costs and places additional burden on wastewater utility staff. Sampling to or three times per week is common across many programs and is often sufficient for tracking endemic pathogens with predictable seasonal patterns. Higher-consequence pathogens or rapidly evolving outbreaks may warrant sampling more often. Frequency decisions should also account for pathogen-specific shedding dynamics: a brief shedding window may necessitate more frequent sampling to ensure detection. Pathogen-specific guidance is provided in Section 5, Pathogen-Specific Considerations.
- **Sampling location and prioritization** – Choosing sites to include in a monitoring network is a significant decision that should weigh population coverage, public health priority, and available infrastructure. Depending on program goals and jurisdictional level, programs may prioritize broad geographic coverage or target high-priority settings and populations, such as long-term care facilities or communities with greater social vulnerability. Once a network of sites is established, they need not all be sampled at the same frequency. Programs often allocate sampling resources strategically, maintaining routine monitoring at community-level treatment plants while sampling upstream or facility-level sites less regularly. For more detailed guidance on site selection methodology, see the Colorado National Wastewater Surveillance System Center of Excellence's site selection toolkit.

Whatever sampling frequency and strategy a program adopts, consistency is essential. Unplanned changes in sampling can make it difficult to distinguish a true signal from an artifact of incomplete data. Planned increases in frequency – for example, in response to an emerging signal – are a deliberate and appropriate exception. Programs should establish standard operating procedures that support consistent sample collection across weekdays, weekends, and holidays. Programs should also have contingency plans for when interruptions occur and should document any deviations so that they can be accounted for during interpretation.

Pathogen Selection

Not all pathogens are equally suited for wastewater surveillance: for example, some are ubiquitous in the environment, shed poorly, or have a lack of validated assays. Pathogen selection should be driven by a combination of scientific, practical, and public health considerations, with the overarching question being whether detection of a given pathogen in wastewater would inform a meaningful public health response.

Key factors to weigh when prioritizing pathogens include:

- **Shedding characteristics** – the degree to which infected individuals shed the pathogen into wastewater.
- **Persistence and detectability** – how well the pathogen's genetic material persists during transport through the sewer system and whether it can be reliably detected.
- **Public health priority** – the severity, transmissibility, and population impact of the pathogen.
- **Availability of complementary data** – whether clinical data are available to contextualize a wastewater signal or whether wastewater fills a current data gap.
- **Actionability of results** – whether a meaningful public health response exists if the pathogen is detected.
- **Analytical capabilities** – whether validated detection assays are available and feasible within program resources.
- **Ethical considerations** – whether monitoring the pathogen could create privacy, stigmatization, equity, or community-engagement concerns, particularly for small or identifiable populations. See Ethical Considerations later in this section.

Wastewater surveillance programs often monitor some combination of:

- Respiratory pathogens (e.g., influenza A, influenza B, RSV, SARS-CoV-2)
- Enteric pathogens (e.g., norovirus, hepatitis A)
- High-consequence or non-endemic pathogens (e.g., measles, mpox)
- Antimicrobial resistance markers

Programs may also monitor additional targets depending on local priorities and risks, such as vector-borne pathogens. The priority pathogens will depend on the jurisdiction's disease burden, population characteristics, and program resources. Programs should also think ahead about how new pathogens would be added to their surveillance portfolio if an emerging threat warranted it, including how to engage with the community and participating utilities and how to build support for implementation.

Detailed guidance on pathogens is provided in Section 5, Pathogen-Specific Considerations.

Governance of Wastewater Surveillance Data

Effective data governance supports the appropriate and consistent use of wastewater data. Programs should establish clear expectations for how data are accessed, shared, interpreted, and communicated.

Governance structures do not need to be complex, but they should provide clear, consistent guidance to support responsible data use.

Governance checklist

Programs should consider the following when establishing governance for wastewater surveillance:

Context and partnerships

- ☐ What jurisdictional policies or regulations apply to your wastewater surveillance program, including those that apply to your utility partners and are relevant to your partnership?
- ☐ Who are your key partners (e.g., utilities; laboratories; academic institutions; local, state, and federal health agencies), and what are each partner's roles and responsibilities?
- ☐ If your program serves Tribal communities, how does your governance approach reflect Tribal data sovereignty? (See the "Tribal Governance and Data Sovereignty" call-out box.)
- ☐ Does the scale of your monitoring (downstream vs. upstream) require different governance approaches, particularly around privacy and communication?

Data ownership, access, and use

- ☐ Who owns or stewards the data (e.g., the state, Tribal Nation, local health department, utility, laboratory)?
- ☐ Who has access to raw, summarized, or interpreted results, both internally and externally?
- ☐ Are there clear boundaries on how data can and cannot be used, including protections against use for non-public-health purposes?
- ☐ If private laboratories or commercial entities are involved, are data ownership and use rights explicitly addressed in a written agreement?

Data sharing

- ☐ What is the process for sharing data internally across teams (e.g., public health laboratory, epidemiology, communications, leadership)?
- ☐ What is the process for sharing data externally with partner agencies and the public?
- ☐ What level of detail is shared, with whom, and at what frequency?
- ☐ Are required approvals in place before data are shared externally?
- ☐ Do formal data sharing agreements (e.g., memorandums of understanding, data use agreements) exist with key partners?

Interpretation and decision-making

- ☐ Who is responsible for reviewing wastewater data and interpreting signals?
- ☐ Who has authority to decide when a signal warrants a public health response?
- ☐ Is interpretation centralized within one team or distributed?

Communication and public reporting

- ☐ How are results communicated internally and externally (e.g., dashboards, reports, public messaging)?
- ☐ Who approves communications before they are released publicly?
- ☐ How is messaging coordinated across public health laboratories, epidemiology, communications, and leadership teams to ensure consistency?

Documentation and standard operating procedures

- ☐ Does your program have documented standard operating procedures covering not just data collection, but also signal interpretation, decision-making, and response?
- ☐ How are decisions, changes in approach, and deviations from standard procedures documented and communicated?

Tribal Governance and Data Sovereignty

The term "Tribal data sovereignty" refers to the inherent authority of Tribal Nations to govern the collection, ownership, access, use, interpretation, sharing, and stewardship of data about their citizens, communities, lands, and resources. Wastewater data may raise particular governance concerns because results can be associated with a geographically defined community even when no individuals are identified.

When wastewater surveillance includes Tribal lands, Tribal communities, or sampling sites that serve Tribal populations, governance should be developed in direct partnership with the contributing Tribal Nation or Nations. Programs should engage the appropriate Tribal government, Tribal public health authority, and, where relevant, Tribal Epidemiology Center before establishing sampling, data-sharing, interpretation, attribution, or public-reporting practices.

Governance agreements should clarify who has authority over the data, how results will be attributed, who may access and use the data, and what review or approval is required before findings are shared publicly. Programs should also identify and follow applicable Tribal laws, policies, review processes, and data-use agreements, as well as relevant federal data-sharing policies concerning Tribes and Tribal Epidemiology Centers.

Tribal Governance Checklist

- ☐ Does the governance approach respect the data sovereignty and decision-making authority of each contributing Tribal Nation?
- ☐ Have the appropriate Tribal government, Tribal health authority, and, where relevant, Tribal Epidemiology Center been engaged in program planning and governance?
- ☐ Do you have existing data use or data-sharing agreements with Tribal health authorities that define how wastewater data associated with Tribal communities may be accessed, used, interpreted, shared, and reported?
- ☐ Are sampling locations and results attributed accurately to the appropriate Tribal community rather than to a state, county, or local jurisdiction?
- ☐ Have Tribal partners identified the organizations or individuals they consider trusted data-sharing and communication partners?

Ethical Considerations

Although results of wastewater surveillance are inherently aggregated and do not identify individuals, their use still requires careful consideration to avoid unintended consequences. Past frameworks on ethical considerations in wastewater surveillance have focused on three areas: privacy, stigma, and data stewardship.

- **Privacy** extends beyond individual identity to include groups and communities. Because wastewater data reflect the pooled contributions of everyone in a sewershed, results can reveal information about a neighborhood, facility, or community without naming anyone. The risk to privacy increases as the scale of monitoring narrows. Facility-level or upstream sampling generates more granular signals that can be more directly linked to specific populations, and that tradeoff must be weighed against the public health benefit of finer spatial resolution. For example, out of concern for residents' privacy, CDC's Wastewater Monitoring Program does not process or publish data from sewersheds with less than 3,000 residents.
- **Stigma** can result when wastewater data link a community or group to a disease, particularly for populations that are already marginalized or have experienced historical harms that have contributed to mistrust of public health institutions. Clear, carefully framed public communication – and proactive engagement with affected communities before data are released – can help mitigate stigma-related harms. Prior data sharing agreements negotiated with other public health authorities, such as Tribal Nations, may also have specific guidelines on when communities may be named or how data are presented. These agreements should be reviewed and revised, as needed, to ensure agreements are respected and accurately followed.
- **Data stewardship** encompasses how wastewater data are collected, stored, shared, and used across the full data lifecycle. Programs should be attentive to who owns the samples and resulting data and for how long, how external partners or commercial entities may access those samples and data, and whether the use of samples and data remains aligned with the stated public health purpose. For data associated with Tribal Nations or communities, these practices should recognize Indigenous data sovereignty and the inherent authority of Tribal Nations to govern their data, regardless of which entity possesses or stores them. Governance structures should guard against expanding or changing use of wastewater data beyond the initial stated purpose.

Underlying all three of these areas is the importance of trust. Communities are more likely to support wastewater surveillance when they are confident that data will be handled responsibly, that findings will be communicated transparently, and that the program genuinely serves their interests. Engaging contributing communities is essential to running a wastewater surveillance program that is both effective and ethical.

For detailed guidance on applying an ethical framework to wastewater surveillance program decisions, including structured questions for analysis and case studies, refer to the Association of State and Territorial Health Officials' *Framework for Addressing Ethical Considerations in Infectious Diseases Public Health Wastewater Surveillance* (2025).

Strengths and Limitations of Wastewater Data

As discussed throughout this toolkit, wastewater surveillance provides a population-level signal of pathogen presence and trends, offering a valuable tool to support outbreak response and provide situational awareness of infectious disease risk. Unlike most traditional surveillance methods, wastewater monitoring does not depend on individuals seeking healthcare or being tested, and it can capture infections in people who never develop symptoms. It can help identify changes in pathogen activity – sometimes before clinical cases are widely detected – offering public health professionals a versatile tool to supplement traditional surveillance sources. Importantly, wastewater data are most effective when used as one component of a broader public health surveillance network, alongside data sources such as electronic laboratory reporting, syndromic surveillance, case investigations, and outbreak data.

Wastewater data also have important limitations. Signals reflect only the populations contributing to the monitored wastewater system and may underrepresent or exclude communities served by individual septic systems, particularly in rural areas. Wastewater data also cannot identify individual cases, provide clinical details, or always distinguish whether a signal originated from humans, animals, or other sources. These limitations make contextual information and complementary surveillance data essential for interpretation.

Understanding what wastewater data can and cannot tell you is fundamental to using those data well.

Benefits of Wastewater Data	Limitations of Wastewater Data
Reflect aggregate, community-level signals	Limited ability to determine the specific source of a wastewater signal
Detect pathogen presence and trends	Cannot provide precise estimates of case counts
Capture infections regardless of symptoms or healthcare-seeking behavior	Limited to populations using monitored wastewater systems (e.g., home, work)
Support early warning and situational awareness	Do not provide clinical information or healthcare utilization
Can be combined with other data sources to strengthen overall disease surveillance	Require contextual knowledge of the relevant pathogen and sewershed for meaningful interpretation

2. Analyzing Wastewater Data

This section provides a structured analytical workflow for evaluating and working with wastewater surveillance data. It covers how to assess data quality, when and how to apply normalization, approaches to aggregating data, spatial and temporal alignment considerations, and methods for identifying meaningful trends. Working through these steps in sequence helps programs move from raw laboratory results to signals they can act on with confidence.

Evaluating Data Quality

Before interpreting wastewater results, programs should examine the data for common quality issues and have documented procedures for addressing those issues. These procedures should cover the checks described below, which should be conducted alongside a review of the analytical laboratory's quality assurance/quality control (QA/QC) report.

Replicates

Replicate samples, including field replicates (samples collected at the same site) or lab replicates (samples split and analyzed multiple times), are used to assess precision and variability in sampling and laboratory analysis. When two replicates are collected or analyzed, they are commonly referred to as duplicates. Replicate checks help indicate and improve the reliability of results. Wastewater and environmental sampling can produce variable results, having replicates and being able to average replicates can help smooth some of this inherent variability. This QC step is typically performed by the laboratory according to pre-established protocols, often using a metric called relative percent difference. In many jurisdictions, replicate review and resolution occur entirely within the laboratory before results are reported to public health programs, though practices vary by jurisdiction; programs should confirm with their laboratory where and how this step is handled.

Epidemiologists should understand that this check exists and may affect the data they receive. If a program does receive flagged or unresolved duplicate discrepancies, results that cannot be easily explained should prompt a review of field notes, consultation with field samplers and treatment plant operators, and follow-up with the laboratory to identify and address underlying issues.

Outliers

Outliers are measurements that deviate substantially from recent results at the same site. They can reflect genuine spikes or dips in community pathogen levels, but they can also result from changes in sewer flow or system maintenance, non-homogeneous samples, errors in sample handling or transport, or laboratory error. Responding appropriately depends on distinguishing between these possibilities.

The first step to dealing with outliers is identifying them, which should be done separately for each site and pathogen given differences in baseline concentrations, flow, and shedding dynamics. Outlier checks should be applied not only to pathogen concentrations, but also to biomarkers and other variables used for normalization, since unusual values in these measures can affect the normalized result. Interquartile ranges and Z-scores offer two common methods for identifying

outliers. Either method should be applied to data after normalization and log transformation, and within the context of historical site data. Normalization can correct for certain issues, such as abnormal flow, that can cause the appearance of an outlier; log transformation is a technique commonly used for environmental data which is often right skewed. Other valid approaches exist. A program should choose and document its preferred method before applying it to active surveillance data.

Interquartile Range (IQR) Approach to Outliers	Z-Score Approach to Outliers
• Values more than 1.5 times the IQR above the third quartile are flagged as outliers. $Outlier \geq (1.5 \times IQR) + Third\ Quartile$ • For example, if the 25th percentile is 50 gene copies per liter and the 75th percentile is 200, the IQR is 150 and any value above 425 gene copies per liter would be considered an outlier.	• Values more than three standard deviations above the mean are commonly flagged as outliers (though programs may adjust this threshold). $Outlier \geq Mean + (3 \times Std\ Dev)$ • For example, if the historical mean concentration at a site is 100 gene copies per liter with a standard deviation of 50, any value above 250 gene copies per liter would be considered an outlier.

Once an outlier is identified, the next step is to investigate its possible cause by reviewing whether any unusual events occurred at the wastewater treatment plant on the sampling day and whether corresponding changes, such as a spike in flow, were observed in the operational data. This is one reason why close collaboration with utility operators is so important, as they will have details on sampling conditions that may impact measurements. Based on that investigation, the program should determine whether the value will be included as-is, included with a flag, or excluded from further analysis. CDC's Wastewater Viral Activity Level methodology, for example, identifies outliers before calculating trend metrics.

Missing or incomplete data

Gaps in wastewater data are common and can arise from equipment failures, missed sampling events, laboratory delays, or hold time exceedances. The reason that data are missing should always be flagged and documented in the dataset. This preserves transparency and allows anyone using the data to account for the gap during interpretation. Beyond documentation, programs have several options for how to handle missing data analytically, and the right choice depends on the nature and extent of the gap.

- **Leave the gap** – If missing data are rare and occur at random, acknowledge the missing point and continue interpreting the trend using the remaining data. A single missing observation will not usually prevent meaningful trend interpretation.
- **Imputation** – Missing values can be estimated from surrounding data using methods such as linear interpolation, last observation carried forward, or mean substitution using historical site data. Imputation is most appropriate when gaps are small and random. It becomes less reliable when gaps are large, frequent, or systematic, or when the available data surrounding the gap are sparse.
- **Increase sampling frequency** – If missing data are a recurring problem at a site, increasing sampling frequency provides redundancy that reduces the impact of any single missed sample on trend interpretation.

Whatever approach is chosen, programs should document which method was used and why. The right choice will depend on the amount of missing data, whether gaps are random or systematic, and whether the data feed into prediction models that are sensitive to incomplete inputs. These decisions affect how results should be interpreted and communicated, and undocumented handling of missing data can introduce inconsistencies that are difficult to identify or explain later.

Timeliness

A wastewater sample has a defined hold time – that is, the largest allowable interval between sample collection and laboratory analysis before sample integrity may be compromised. Reviewing collection and analysis dates against the sampling schedule is a routine but important quality check. If hold times were exceeded for a given analyte, the result will likely have been flagged by the laboratory and should be treated with caution or excluded depending on the degree of exceedance and the analyte's known stability. Programs should also track whether sample collection dates shifted from their intended schedule – particularly around holidays and weekends.

Approaches to Normalization

Raw pathogen concentrations in wastewater are generally reported as gene copies per liter (if using liquid influent) or gene copies per gram of dry weight (if using settled solids or primary sludge). This measurement is directly affected by how much water is flowing through the system at the time of sampling. Heavy rainfall, snowmelt, or industrial discharge can substantially dilute the sample, making a true increase in community transmission appear flat or even declining. Conversely, low-flow periods can concentrate the sample and suggest an apparent increase that does not reflect a real change in disease activity. The size of the population being monitored can also affect interpretation of results.

Normalization is the process of adjusting raw wastewater measurements so that changes in pathogen concentration reflect meaningful changes in community disease burden rather than artifacts of the sampling environment. Without normalization, apparent changes in concentration may not reflect true changes in transmission, but instead dilution from rainfall, fluctuations in daily flow, or differences in the size of the contributing population.

There is no universally agreed-upon normalization method for wastewater surveillance, and practice varies considerably across programs. This remains an active area of research and methodological discussion in the field. Notably, CDC's National Wastewater Surveillance System (NWSS) no longer normalizes wastewater data in its national reporting of the WVAL; however, normalization should be considered when interpreting concentration data. Programs should make a deliberate, documented decision about whether and how to normalize, guided by their surveillance objectives, the characteristics of their sewersheds, and the availability of supporting data.

Three normalization approaches are commonly used in wastewater surveillance programs, each with different data requirements and tradeoffs.

Flow normalization

Flow normalization adjusts results by multiplying the pathogen concentration by the daily flow rate, converting the result to a total daily load expressed as gene copies per day. This approach is particularly useful at a fixed monitoring site over time, where flow can fluctuate substantially while the contributing population remains relatively stable. By accounting for dilution, flow normalization makes it easier to distinguish genuine epidemiological trends from measurement artifacts driven by environmental conditions. Flow data are typically available from the treatment plant or through a separate flow measuring instrument deployed with the sampler. Flow is the most commonly

collected ancillary variable in wastewater surveillance programs, which is one reason flow normalization is the most widely used approach.

Flow and static population normalization

Comparing raw or flow-normalized concentrations across sites with very different population sizes can be misleading. A site serving 500,000 people and a site serving 10,000 people might show similar numbers of gene copies per liter, but the public health implications are entirely different: the same number at the larger site would represent a far greater absolute burden of disease. To make meaningful cross-site comparisons, the daily load can be further divided by the estimated population served, yielding a per capita load expressed as gene copies per person per day.

This approach is widely used because it produces a normalized concentration that can be more easily compared across sites regardless of their size. However, it relies on a population estimate, which is typically derived from census data or utility records. These estimates may not always reflect the actual population contributing to the sewershed at any given time. As discussed later in this section, dynamic populations such as tourists, commuters, and seasonal residents can cause the true contributing population to deviate substantially from static estimate.

Biomarker normalization

An alternative to using flow and population estimates is to normalize against a biological marker present in the wastewater sample itself. Biomarkers are intended to serve as a proxy for the amount of human fecal material in the sample, effectively accounting for both dilution and population size in a single measurement. Several biomarkers have been used for this purpose, including pepper mild mottle virus (PMMoV), crAssphage, RNaseP, creatinine, atenolol, caffeine, and nicotine. PMMoV is the most commonly used of these markers, as it is a plant virus that humans consume through food, is shed consistently in human feces, and is abundant and stable in wastewater. Normalizing the target pathogen concentration against PMMoV produces a unitless ratio that reflects the pathogen's relative abundance compared to the overall human fecal signal in the sample.

The advantage of biomarker normalization is that the normalized concentration responds dynamically to actual changes in the contributing population, rather than relying on a fixed estimate. If a large influx of tourists temporarily doubles the fecal load in a sewershed, the PMMoV concentration will rise accordingly, and a pathogen normalized against it will remain interpretable. However, biomarker normalization adds laboratory cost and analytical complexity, and the resulting unitless ratio can be less intuitive to communicate to public health audiences. Different biomarkers also have different properties and may perform differently across settings, adding another layer of methodological variability.

Normalization example

The figure below illustrates how each of these three approaches is applied to the same hypothetical sample, producing different numerical results that each answer a slightly different question about the data.

Visual Showing Examples of Normalization Calculations

Hypothetical Sample Data	
Pathogen Concentration (C)	1,000,000 copies/L
Daily Flow (Q)	6,600,000 gal/day
Service Population (P)	100,000 people
PMMoV Concentration (C_{PMMoV})	1×10^6 copies/L

1 Flow Normalization (Daily Load)

Load = C × Q

Calculation

1,000,000 copies/L × 6,600,000 gal/day × 3.785 L/gal

Result

2.4981×10^{13} copies/day

2 Flow and Static Population Normalization

Per Capita Load = $\frac{C \times Q}{P}$

Calculation

$$\frac{1{,}000{,}000 \text{ copies/L} \times 6{,}600{,}000 \text{ gal/day} \times 3.785 \text{ L/gal}}{1{,}000{,}000}$$

Result

2.4981×10^{8} copies/person/day

3 Biomarker Normalization (PMMoV)

Normalized Ratio = $\frac{C_{target}}{C_{PMMoV}}$

Calculation

$$\frac{1{,}000{,}000 \text{ copies/L}}{1{,}000{,}000 \text{ copies PMMoV/L}}$$

Result

1 copy of target pathogen/copy of PMMoV

Brief summary of wastewater normalization methods

Approach	Description	What's Needed	When It's Useful	Potential Pitfalls
Flow normalization	target concentration × flow = daily load (copies/day)	• Flow	• Tracking trends at a single site over time when flow fluctuates • Comparing sites with different flow capacities	• May not be sufficient when population size also varies substantially over time
Flow and population normalization	daily load ÷ population = per capita load (copies/person/day)	• Flow • Population estimate	• Comparing disease burden across sites of different sizes • Communicating in intuitive per capita units	• Relies on accurate population estimates
Biomarker normalization	target concentration ÷ biomarker concentration = unitless ratio	• Biomarker analysis (e.g., PMMoV, crAssphage, RNaseP)	• Accounts for dynamic population sizes • Can account for potential in-lab or sample effects (depending on biomarker) • Comparing times when flow rates have changed (e.g., from rain)	• Adds laboratory cost • Produces a unitless ratio that can be less intuitive to communicate • Biomarker behavior varies across settings

Averaging and Aggregating

Raw wastewater results represent pathogen concentrations for a specific sewershed area at a specific point in time. For many pathogens, before these measurements can be meaningfully compared with other surveillance data or used to inform public health decisions, they often need to be aggregated – either across sites or across time. CDC's Wastewater Viral Activity Level (WVAL) methodology, described in the box on the next page, illustrates how both types of aggregation are used together in practice. Before aggregating, all contributing data points must be in the same units and normalized consistently; otherwise, results will be misleading. Additionally, be careful aggregating data generated by different laboratory methods, as cross-method analysis can introduce bias.

Spatial aggregation

Wastewater sewershed boundaries rarely, if ever, align with the administrative or political jurisdictions used for clinical case reporting. Aggregating results from multiple sites into a single value for a city, zip code, county, or region can help bridge this gap and make wastewater data more directly comparable with case counts or syndromic data reported at the jurisdiction level. Public health responses are also typically made at a jurisdiction level rather than for individual treatment plant sewersheds, so aggregated estimates are often more actionable.

Three approaches are commonly used for spatial aggregation, each making different assumptions about how to weight the contributing sites. This applies whether you are aggregating raw concentrations (e.g., copies/liter), flow-normalized loads (e.g., copies/day), or population-normalized values (e.g., copies/person/day).

- **An unweighted mean** treats all sites equally regardless of how many people they serve, which can over-represent small sites and mask high burden at large urban sewersheds.
- **A population-weighted mean** scales each site's contribution by the population it serves, producing a result that better reflects the average exposure across the full population being monitored.
- **The median** offers a middle ground that is more robust to outliers and skewed data, making it a useful alternative when results are highly variable or when a small number of extreme values might otherwise distort the mean.

Temporal aggregation

Wastewater data can be averaged across time as well as across sites. This is particularly useful when aligning wastewater results with clinical data sources that are reported cumulatively. For example, case data may be available as weekly or monthly case counts or emergency department visit totals. A daily wastewater measurement is not always the most appropriate comparison point for a clinical metric that represents an entire week or month of activity. Averaging wastewater measurements over the same time window as the clinical data being compared creates a more valid basis for integration.

Temporal averaging also helps smooth day-to-day variability driven by factors such as differences in weekend versus weekday sewer use patterns, which can create artificial fluctuations unrelated to true changes in community transmission. Both the mean and the median are appropriate temporal aggregating approaches. More sophisticated smoothing approaches such as moving averages are discussed in the following section on trend analysis.

CDC's Approach to Aggregating Wastewater Viral Activity Levels

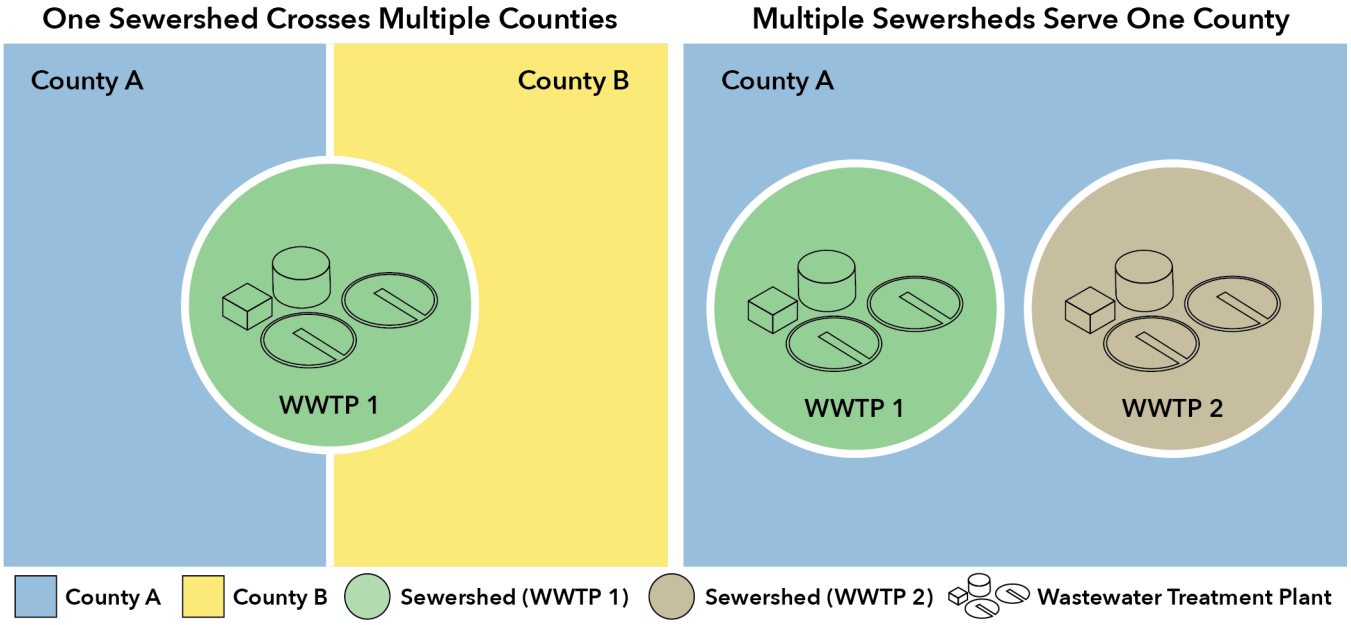
The WVAL is a standardized metric CDC uses to compare current pathogen activity in wastewater to a site's historical baseline, helping communicate whether levels are low, moderate, high, or very high relative to that site's own history. CDC's WVAL methodology applies both temporal and spatial aggregation in sequence. First, all measurements collected at a site within a given week are averaged to produce a single weekly value. Then, to represent a state or region, the median is taken across all contributing sites. The median is used at this step because it is more robust to extreme values from individual sites.

Source: CDC, 2026

Spatial Alignment

Health jurisdictions are typically defined by administrative and political boundaries (e.g., cities, zip codes, counties), while sewersheds are defined by subsurface wastewater infrastructure. These two geographies rarely, if ever, align neatly. For example, a single wastewater treatment plant may serve populations across multiple counties, or a single county may be served by several treatment plants. If clinical case data are only reported at the county level, this mismatch complicates the direct comparison of wastewater signals with clinical data, because the populations contributing to each data source may not be the same. The figure below illustrates this problem by showing a sewershed crossing county lines, which means the wastewater signal cannot be cleanly attributed to either jurisdiction's case counts.

Visual of Spatial Misalignment Between Wastewater and Clinical Data



This boundary mismatch is an example of the modifiable areal unit problem (MAUP) – a well-documented statistical phenomenon in which analytical results shift depending on how geographic boundaries are drawn. The MAUP manifests in two ways relevant to wastewater surveillance: moving between levels of aggregation (for example, from a county to a state) can change results, and indeed simply redrawing the boundary (for example, shifting from a sewershed boundary to a county boundary) can change results at the same scale. Practitioners should be aware that apparent

correlations or discordances between wastewater and clinical data can sometimes reflect these boundary effects rather than true epidemiological patterns.

Understanding the spatial resolution of both your wastewater and your clinical data is an important first step. Clinical data vary in the geographic detail they carry. In the best-case scenario, individual address-level data are the most precise, but these data are often only available at the zip code or county level, reducing the geographic resolution of comparisons. Even when address-level data are available, it is important to determine whether the address reflects the patient's residence or the location of the diagnosing facility: these can differ substantially, particularly when patients seek care outside their home sewersheds. On the wastewater side, wastewater infrastructure data, which include sewer pipes, manhole locations, and sewershed polygons, are typically maintained by the wastewater utilities. Wastewater operators will also have a good sense of the size and distribution of the population they serve. The U.S. EPA maintains polygon shapefiles for some publicly owned wastewater treatment works through its National Sewershed Dataset, which can be a useful starting point for programs working to map their sewershed areas.

Key spatial considerations

In addition to boundary mismatches, three other spatial factors routinely affect how wastewater data should be interpreted and compared with clinical data.

- **Sewered vs. unsewered populations** – Wastewater surveillance is limited to populations connected to municipal sewer systems. Approximately 75–80% of all residents in the United States are served by municipal sewers. In rural and peri-urban areas, residents are often on private septic systems and may not contribute to treatment plant influent where they live. However, these populations may contribute to municipal sewer systems through travel into sewersheds (e.g., for work, school). When interpreting wastewater data alongside aggregate case data, it is important to have a rough sense of what proportion of the jurisdiction is sewered and whether the unsewered population is likely to differ in ways that are epidemiologically relevant.
- **Dynamic populations** – Clinical data are typically tied to a patient's residential address or zip code or the location of the healthcare facility. Wastewater, by contrast, reflects whoever is physically present in the sewershed area at the time of sampling, which can include commuters, tourists, university students, seasonal workers, and attendees of large events. In sewershed areas with significant daytime population influx, wastewater signals can vary from cases residing in the sewershed, because cases may seek care and be counted in a different jurisdiction. The baseline population dynamics of a sewershed are an important context for interpreting signals accurately. Practitioners should understand periods when those dynamics shift, such as during tourist season or a major event.
- **Cross-border flow** – Pathogens do not adhere to administrative boundaries (e.g., state, county, zip code), and neither does wastewater infrastructure. A treatment plant may receive inflow from neighboring jurisdictions, meaning a signal detected in one area may originate from residents of another. For some Tribal communities, a utility may be owned by a neighboring state despite it serving Tribal lands or community members. Programs that share sewershed areas with neighboring jurisdictions should consider establishing data-sharing relationships so that cross-border signals can be investigated collaboratively rather than in isolation.

Wastewater Surveillance Across State Lines: Candidozyma auris in Utah

When three patients with confirmed *C. auris* infections were transferred from Nevada to a hospital in St. George, Utah, the Utah Department of Health and Human Services detected corresponding *C. auris* signals in the local wastewater treatment plant. The signal reflected a transmission event originating in another state. Interpreting it required clinical context from Nevada, underscoring why data-sharing relationships with neighboring jurisdictions are valuable.

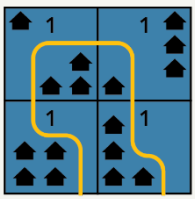
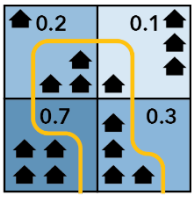
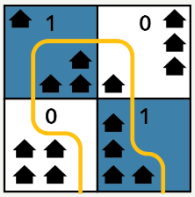
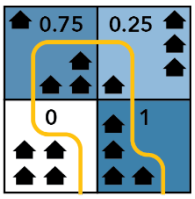
Source: *Chavez et al., 2024*

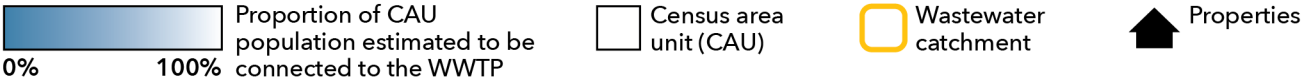
Estimating sewershed populations

Accurately estimating the population contributing to a sewershed is important for normalizing wastewater data and for comparing signals across sites. Partnering closely with utilities will help ensure accuracy of these measurements. Because sewershed boundaries rarely align with census boundaries, population estimates typically require areal interpolation – a statistical technique for transferring data from one set of geographic boundaries to another. Price et al. (2024) compared four approaches, summarized below. Other data sources and methods may also be useful, including gridded population datasets such as EPA dasymetric population estimates or WorldPop, as well as mobile-device mobility data where appropriate and available.

In practice, wastewater treatment plant operators are also a valuable resource: an operator is likely to have a working knowledge of their service area and a customer base that can serve as a practical cross-check on population estimates.

Which Way to Estimate Sewershed Population Is Right for Your Data?

Methods	Approach, Key Assumptions, Strengths, and Limitations
Touching 	• Includes the entire population of any census tract that intersects the sewershed area • Assumes all properties within the census tract are connected to the wastewater system • Simple to calculate • Consistently overestimates the population
Area Proportional 	• Uses the proportion of physical area shared between the census tract and sewershed area to calculate the population • Assumes the population and properties are uniformly distributed across the entire area • Common and straightforward for dense urban areas • Inaccurate in rural areas with sparse housing
50/50 	• Includes 100% of a census tract's population if more than half its properties fall within the sewershed; otherwise includes none • Assumes all properties are residential and have an identical number of inhabitants • Straightforward and intuitive • Can be skewed by large, low-occupancy properties
Properties 	• Uses the proportion of property tiles within the sewershed to determine the proportion of the census tract population while considering land use to better account for where people live • Ancillary spatial data are used to assume where people live • Most refined, but requires additional data and processing • Reduces "empty space" errors by focusing on specific land use



Temporal Alignment

Wastewater and clinical surveillance do not observe disease activity at the same point in time, and understanding this temporal offset is essential for interpreting the two data streams together.

- **Wastewater timing** – People may start to shed pathogens in their waste during the incubation period of a disease, which often begins before symptoms appear or before they seek care and receive testing. As a result, wastewater concentrations may increase before a corresponding rise is apparent in clinical surveillance. The timing and strength of this potential lead vary by pathogen and may be affected by shedding patterns, incubation period, the population contributing to the wastewater system, sampling frequency, and laboratory turnaround time.
- **Clinical timing** – Clinical signals depend on factors such as symptom severity, healthcare-seeking behavior, testing availability, laboratory reporting, and the strength of existing surveillance systems. For pathogens with robust clinical surveillance or limited and inconsistent fecal shedding, clinical data may provide a timelier or clearer signal than wastewater data.

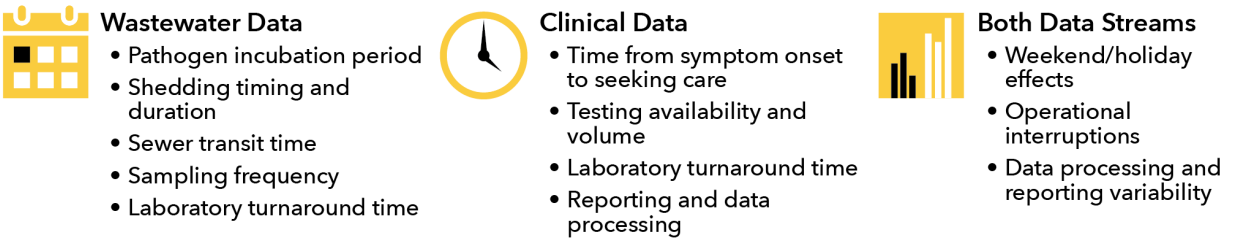
The length of the lead or lag time between wastewater and clinical data varies considerably by pathogen and depends on shedding profiles, incubation periods, and how quickly people seek care once symptomatic. Wastewater is best interpreted as a complement to clinical surveillance rather than as an inherently leading indicator. Further factors for different pathogens are further discussed in Section 5, Pathogen-Specific Considerations.

Both data streams also experience variability in how consistently and quickly data become available. For example, clinical testing volume may decrease on weekends and holidays when clinics may be closed. This can create artificial decreases in case counts that can be mistaken for true declines in transmission. For this reason, case counts are commonly aggregated by standardized epidemiological week (e.g., the *Morbidity and Mortality Weekly Report* [MMWR] week) rather than examined daily. Wastewater sampling frequency introduces a different kind of variability. Many programs batch samples once or twice a week to manage costs, meaning multiple samples arrive in batches. Once samples are processed, wastewater results may also require manual steps to reach public health staff, whereas clinical data flow through automated electronic laboratory reporting systems once results are logged. Data dashboards that display wastewater signals alongside clinical case counts in a shared view can help programs monitor both streams in near real time and identify emerging discordances more quickly.

The practical implication is that, despite the additional laboratory processing steps involved, the total time from infection to data availability often varies and may be shorter for wastewater than for clinical systems. When integrating the two data streams, programs should account for both the biological lead time and the operational latency of each system and should be cautious about interpreting short-term discordance between the two as a true epidemiological signal before ruling out differences in data cadence.

The figure below summarizes the various factors that can influence when wastewater and clinical surveillance data become available and how their timing compares.

Factors Affecting Data Availability and Timing of Wastewater and Clinical Data



Identification of Signals and Trends

Once data quality, normalization, and aggregation decisions have been made, trend analysis is the primary tool for translating wastewater measurements into public health response. Trend analyses reveal increases or decreases in pathogen concentrations over time, helping to identify the onset or resolution of elevated community transmission and prompting further investigation into complementary data sources.

The appropriate approach to trend analysis depends on the pathogen being monitored:

- **Endemic pathogens** – those expected to circulate continuously, such as influenza or norovirus – have an established baseline, and methods like percent change, benchmark comparisons, and time series modeling can detect meaningful deviations from expected levels.
- **Emerging or rarely detected pathogens** – such as measles or poliovirus – have no established baseline, and trend analysis should focus on presence and persistence rather than magnitude. For these pathogens, a single detection may be sufficient for public health action, and the key questions are whether the signal is real, whether it recurs and is persistent, and whether it is strengthening or fading.

The specific methods used to characterize a signal will differ depending on whether the pathogen is endemic or rarely detected. For endemic pathogens, the emphasis is generally on quantifying change relative to an expected baseline; for emerging or rarely detected pathogens, the emphasis is on whether the pathogen is present in the sample and evaluating whether detections persist over time. The methods described below can be applied individually or in combination depending on the pathogen, program objectives, and available data.

Transforming Data Before Trend Analysis

Before trend analysis methods are applied, wastewater concentrations are typically log-transformed regardless of whether they have been normalized. Because wastewater data span several orders of magnitude and are right-skewed, working in log units produces more stable trend estimates and more interpretable visualizations. Percent change and benchmark comparisons are often calculated on log-transformed values, and results should note whether they are reported in original or log units.

Smoothing methods

Moving averages smooth out day-to-day noise in wastewater data, making underlying trends easier to identify and communicate, and are among the most commonly used tools for public-facing

dashboards and reports. A moving average is calculated from a rolling window of recent measurements and updated as each new data point is added. A seven-day window is the most common choice, as it captures a full week of data and smooths out variability driven by differences in weekday versus weekend patterns. The tradeoff is a lag: the moving average continues to reflect past values for several days after the true signal has changed direction.

Other smoothing methods, such as locally estimated scatterplot smoothing (LOESS) or spline-based approaches, are also used in wastewater surveillance. These methods can offer a more flexible fit to the underlying trend than a simple moving average, but they are more analytically complex to implement and communicate, and they may require statistical expertise that not all programs have readily available.

Percent change

Percent change is a common tool for communicating wastewater trends to public health audiences. It expresses how much a current measurement differs from an earlier reference value, typically over a defined time window such as one or two weeks.

$$percent\ change = \frac{new\ value - old\ value}{old\ value} \times 100$$

A positive percent change indicates increasing pathogen concentrations over the period, a negative value indicates a decline, and a value near zero indicates stability. Percent change is straightforward to calculate and easy to communicate, but it can be sensitive to the choice of reference point. A low baseline value can produce a large percent change from even a modest absolute increase, and vice versa. Programs should define and document their reference periods consistently.

Baseline values

A baseline value provides a fixed reference point to compare current results against (rather than the recent prior measurements used to calculate percent change). Baselines can be set in a variety of ways — some programs use historical site lows, others use a percentile from the site's full historical record. CDC's WVAL uses the 10th percentile of historical data from the most recent two years as its baseline, then calculates how far current results deviate from that baseline in standard deviation units. This approach anchors interpretation to the site's own historical distribution rather than an absolute concentration, making it more comparable across sites with different baseline levels. Well-defined baselines established before an outbreak or surge are more defensible and consistent than thresholds set reactively.

Time series modeling

Time series modeling uses statistical methods to separate meaningful epidemiological signal from background noise, account for longitudinal trends and seasonal patterns, and identify statistically significant deviations from expected concentrations. Unlike the tools above, time series models can incorporate multiple variables simultaneously, such as flow, temperature, day-of-week effects, and socioeconomic factors. Incorporating these additional factors can generate more precise estimates of underlying transmission trends.

Time series approaches have also been used to integrate wastewater signals with clinical data such as case counts and hospital admissions, producing models that can forecast outbreak trajectories earlier than either data source alone. These models can be powerful but are also more complex to implement and require sufficient historical data to train reliably. Programs considering time series modeling should assess whether their data volume, analytical capacity, and program objectives justify the additional complexity.

Additional Resources for Analyzing Wastewater Data

Data quality

- Houston Wastewater Epidemiology – Statistical Analysis and Reporting
  GitHub repository and best practices guidance for wastewater data quality, statistical analysis, and reporting, developed by a CDC NWSS Center of Excellence.

- Colorado NWSS Center of Excellence – On-Demand Learning
  Video training modules covering wastewater data analytics and data quality for wastewater surveillance dashboards.

- Association of Public Health Laboratories – Public Health Laboratory Guide to Microbial Wastewater Surveillance
  Guidance on new target selection considerations, laboratory methods, quality controls, and data reporting for wastewater surveillance programs.

Normalization

- Biobot Analytics – Normalizing Wastewater Data
  White paper with an overview of normalization approaches for wastewater surveillance data, including flow, population, and biomarker methods.

- Houston Wastewater Epidemiology – Wastewater Epidemiology for SARS-CoV-2: Houston Wastewater Epidemiology (HWE) Best Practices
  Document with practical guidance on normalization approaches used in operational wastewater surveillance programs.

Trend analysis

- CDC – Wastewater Monitoring Data Methodology
  Describes CDC's Wastewater Viral Activity Level (WVAL) methodology, including how benchmark values, percent change, and aggregation are applied in national wastewater surveillance reporting.

- CDC – National Wastewater Surveillance System (NWSS) Data Repository
  CDC-maintained GitHub repository of analytical code, shared tools, and resources for wastewater surveillance data analysis.

3. Interpreting Signals with Complementary Data

This section describes the core surveillance systems most frequently paired with wastewater data, evaluates their respective strengths and limitations, and outlines key considerations for integrating them with wastewater signals to support interpretation.

Overview of Public Health Surveillance Systems

Wastewater surveillance is most effective as a complement to existing systems, not as a standalone signal. Understanding how it fits alongside other data sources is foundational to using it well. The core distinction between wastewater and clinical surveillance lies in how each captures disease activity.

- **Clinical surveillance** operates through an individual-level diagnostic framework where an infection must progress to symptomatic illness, prompt a healthcare visit, and result in a test or diagnosis before it is recorded. This usually happens when someone seeks care for symptoms but can also happen through screening programs that detect infection before symptoms appear. Clinical surveillance produces high-resolution data on specific individuals but is inherently constrained by healthcare access and testing behavior, missing those who never seek care or who never receive a clinical test.
- **Wastewater surveillance** operates at the population level, monitoring pathogen shedding across an entire sewershed, or sampling area, without requiring individuals to interact with the healthcare system. In other words, it yields a broader signal of community-level transmission that includes people who may or may not appear in clinical records. However, it is limited by infrastructure and individual needs, as those on private septic systems or diapered populations, for the most part, are excluded from these estimates.

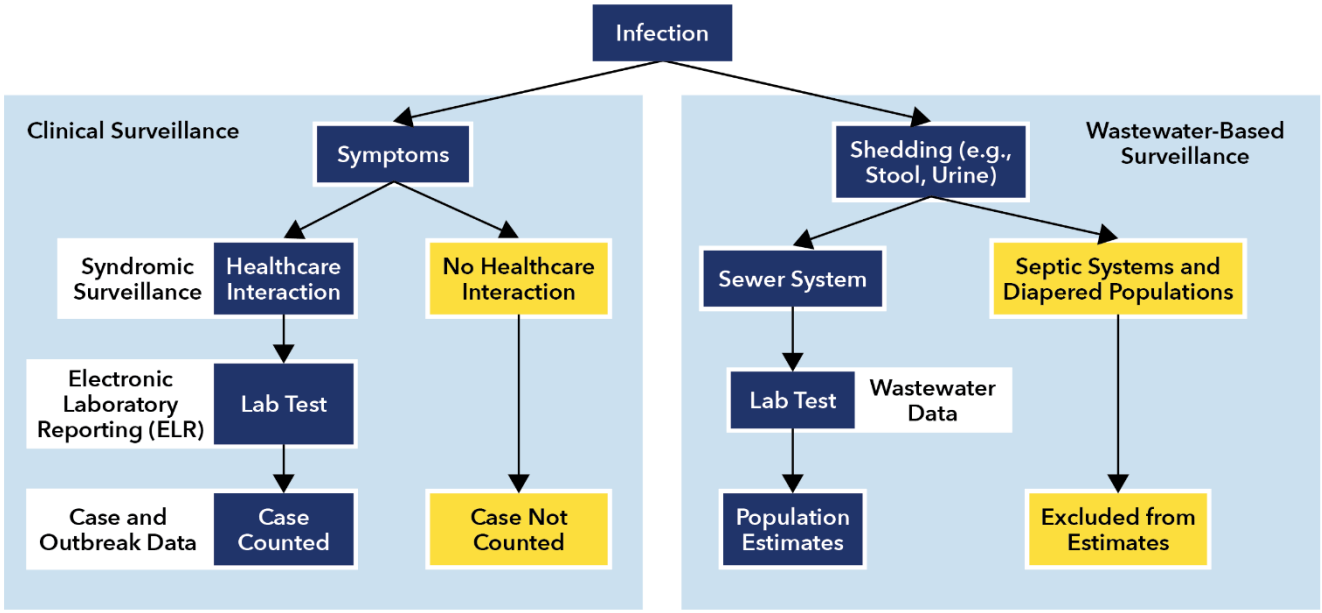
Combining these data streams requires navigating real differences in spatial scale, temporal resolution, and reporting structures. The sections that follow describe the core surveillance systems most commonly used alongside wastewater data, their strengths and limitations, and practical approaches for integrating them effectively.

Wastewater Surveillance	Clinical Surveillance
• Population-level • No healthcare interaction needed • Populations contributing to sewer systems only • Can capture otherwise missed infections	• Individual-level • Requires care-seeking • Population accessing healthcare only • Provides clinical details

Core Surveillance Systems

This section describes the surveillance systems most commonly used alongside wastewater data in public health. Understanding how each system works, what population it captures, and where its limitations lie is essential for interpreting wastewater signals in context and knowing what complementary data to look for when a signal warrants further investigation.

Visual Overview of Core Public Health Surveillance Systems



Syndromic surveillance

Syndromic surveillance tracks patients' primary complaints or the reasons the patients say they're seeking care (e.g., flu-like symptoms, cough and fever, severe diarrhea) as well as the patient's diagnosis determined upon discharge. Many syndromic surveillance systems, like the National Syndromic Surveillance Program (NSSP), pull de-identified data from emergency departments and urgent care centers. Other syndromic systems, such as the U.S. Outpatient Influenza-like Illness Surveillance Network (ILINet), instead draw from outpatient providers reporting influenza-like illness based on symptoms alone, without chief complaint or discharge diagnosis data.

Computer algorithms group these chief complaints and discharge diagnoses into syndromes (collections of medical signs, symptoms, and medical diagnosis codes [e.g., ICD-10 codes] that tend to occur together) rather than specific diseases. For example, complaints of fever and cough may be grouped as influenza-like-illness because these symptoms are associated with influenza, though confirmatory testing would be needed to distinguish influenza from other respiratory infections. CDC's ESSENCE platform monitors the daily volumes of these syndromes and triggers alerts when a syndrome spikes above expected levels in a given area (e.g., zip code). Because some syndromic data are near-real-time and do not require a confirmed diagnosis, syndromic surveillance can detect increases in healthcare-seeking behavior earlier than laboratory-based systems.

What Is CDC's ESSENCE Platform?

ESSENCE (Electronic Surveillance System for the Early Notification of Community-based Epidemics) is the primary analytical platform used to query and visualize syndromic surveillance data from CDC's NSSP. Access is available to state and local public health practitioners through their health department. Users can query data by syndrome category, geography, and time period – making it a useful tool for cross-referencing with wastewater data.

Source: CDC, 2017

Electronic laboratory reporting (ELR)

ELR is the automated digital transmission of laboratory results from laboratories to public health agencies. When a laboratory identifies a positive result for a reportable or notifiable disease, the result is formatted to a set of national data standards and transmitted automatically to state or local health departments, where it often triggers an alert for follow-up investigation. States, Tribal Nations, and territories determine which diseases are reportable within their jurisdiction. Nationally notifiable conditions are determined through a process driven by CSTE members, who develop and vote on standardized case definitions and national notifiability; CDC maintains the resulting National Notifiable Conditions list. A disease can be both reportable and notifiable. ELR provides confirmed, lab-based diagnoses relatively quickly, but only captures individuals who were tested and is subject to reporting lags.

Electronic case reporting (eCR)

eCR is the automated exchange of case reports directly from electronic health records (EHRs) to public health agencies. While ELR transmits only laboratory results, eCR provides a fuller clinical picture including patient demographics, symptoms, travel history, comorbidities, medications, and laboratory findings. eCR is automatically triggered when a provider enters a diagnosis into a patient's EHR that matches a notifiable condition; the system — often within seconds — verifies that the case report meets the reporting requirements for its jurisdiction without manual reporting steps, then delivers it to the public health agency. eCR is one of the richest individual-level data sources available to public health agencies, and like ELR it is limited to those who seek and receive clinical care.

Tribal Nations and Tribal Epidemiology Centers may face distinct legal, technical, and operational considerations when establishing direct access to eCR data. The National Indian Health Board's Electronic Case Reporting Roadmap for Tribes and Tribal Epidemiology Centers provides a step-by-step framework for assessing readiness, planning implementation, establishing connections, and advancing Tribal access to public health data.

Case Study: eCR During a Measles Outbreak in Colorado

Measles is highly contagious and requires rapid response. While eCR is the gold standard for measles due to the need to immediately isolate infected people, wastewater data have been successfully used as a complementary data source. In August 2025, measles was detected in wastewater samples from a wastewater treatment plant in Mesa County, Colorado. Within one week, two clinical cases were confirmed within that service area. This proactive detection enabled health officials to coordinate rapidly, alerting healthcare providers and launching vaccination campaigns before a wider outbreak could take hold.

Source: *Jensen et al., 2026*

Case surveillance

Case surveillance is the systematic collection of demographic and epidemiological information on individuals who meet the case definition for a reportable disease. State, territorial, and local health departments collect these data and report cases of nationally notifiable conditions to CDC. These data include how a person became ill, their contacts, and relevant risk factors. Case surveillance provides the richest individual-level detail of any surveillance system and is particularly valuable for outbreak investigation and contact tracing, but it is resource-intensive, has significant lag time, and captures only confirmed and investigated cases. Case surveillance structures, reporting authorities, and data-access arrangements may differ for Tribal Nations and Tribal Epidemiology Centers and may require additional adaptation and coordination.

Hospitalization surveillance

Hospitalization surveillance tracks the number and characteristics of patients admitted to the hospital for a given condition, providing a measure of disease severity and healthcare system burden that case counts alone do not capture. Some hospitalization data come from surveillance networks, such as CDC's Respiratory Virus Hospitalization Surveillance Network (RESP-NET). Other hospitalization data come from broader administrative sources, such as hospital discharge records. Hospitalization trends often lag behind both wastewater and case-based signals, because patients typically progress through symptom onset, care-seeking, and worsening illness before requiring admission, but they provide a measure of clinical severity and can help anticipate strain on healthcare capacity.

Outbreak data

Clusters of case reports may trigger formal outbreak investigations. CDC collects outbreak reports from state, local, and territorial health departments for some pathogens through the National Outbreak Reporting System (NORS). Outbreak tracking is useful because for some conditions, individual cases may not be reportable but outbreaks are, making NORS an important supplementary source for understanding disease burden that may not be fully visible in routine case surveillance.

Many states also maintain their own outbreak tracking systems, particularly for outbreaks in high-risk congregate settings such as long-term care facilities and healthcare facilities. Because these facilities are often required to report outbreaks promptly for infection control and resource allocation purposes, this data can be relatively timely, and provides a useful, facility-specific signal.

Strengths and limitations of common surveillance data sources

System	Strengths	Limitations
Wastewater	Captures asymptomatic and symptomatic cases; population-level signal; does not require healthcare interaction	Cannot identify who is sick, or if they live in the sewershed or are passing through; signal influenced by flow, weather, and infrastructure; limited to populations contributing to sewer systems
Syndromic	Near-real-time; captures healthcare-seeking trends before diagnoses are confirmed	Chief complaint data are symptom-based, non-specific, and cannot distinguish between conditions without confirmatory testing; discharge diagnosis data can be more specific but less timely
ELR	Lab-confirmed diagnoses; standardized and automated reporting	Misses those who do not seek care or get tested; subject to being a reportable disease
eCR	Confirmed diagnoses; provides demographic details and case history on patient	Misses those who do not seek care; requires provider to document a notifiable diagnosis in an EHR
Case reports	Richest individual-level detail including age, race, and location	Resource-intensive; significant lag; captures only confirmed and investigated cases
Hospitalization data	Capture disease severity and healthcare system burden; useful for capacity planning	Lagged relative to other indicators; captures the most severe cases; coverage and completeness vary by source
Outbreak data	Captures clusters that may not be visible in individual case reporting; a reporting mechanism for some diseases where cases are not notifiable	Significant lag between outbreak onset and reporting; dependent on local capacity to investigate; substantial underreporting

Summary of common public health surveillance data sources

Dimension	Wastewater	Syndromic Surveillance	ELR	eCR	Case Surveillance	Hospitalization	Outbreak
Detection timing	Pre-symptomatic to early symptomatic (shedding period)	Symptom onset (care-seeking)	Post-testing (following symptoms)	Post-diagnosis (during clinical encounter)	Post-confirmation and investigation (by health department)	Post-admission (after illness has progressed to require inpatient care)	Cluster or unusual pattern identified (investigation initiated)
Data availability	Moderate (sampling and lab processing, daily to weekly)	Very fast (near-real-time following encounter)	Moderate (lab and reporting lag)	Moderate (diagnosis of notifiable condition)	Slow (days–weeks)	Slow to moderate; varies by source	Slow (investigation, confirmation, and reporting lag)
Early detection sensitivity	High (population signal often precedes clinical detection)	Moderate (detects case-seeking increases quickly)	Moderate	Moderate	Low	Low	Low (requires a recognized cluster before triggering)
Level of case confirmation and context	Population-level pathogen signal; no individual case or clinical context	Symptom- or syndrome-based; generally not diagnosis-confirmed	Laboratory-confirmed result with limited clinical context	Case-level report with clinical, demographic, and laboratory context	Confirmed or probable case with detailed epidemiologic investigation	Individual-level clinical encounter with diagnosis and severity information	Confirmed cluster with epidemiologic context and, in some cases, an identified source
Population coverage	Entire sewershed population	Care-seeking population	Tested individuals	Care-seeking and diagnosed	Confirmed and investigated cases	Hospitalized patients only	Confirmed outbreak-affected individuals and settings
Representative-ness	Influenced by sewer coverage, population mixing	Skewed toward healthcare users	Skewed toward tested individuals	Skewed toward healthcare access	Skewed toward reported/ responding cases	Skewed toward the most severe illness	Skewed toward large outbreaks that are detected
Demographics	None (aggregate only)	Limited (basic demograph-ics)	Limited–moderate	Moderate–rich	Rich (detailed individual data)	Rich (detailed individual data)	Moderate–rich
Spatial resolution	Sewershed- or facility-level	Facility- or zip-level	Address- or facility-level	Address-level	Address-level	Facility- or address-level	Varies

Supplementary Public Health Data Sources

Genomic surveillance

Genomic surveillance uses sequencing-based methods to characterize pathogens in greater detail than PCR alone. These methods include whole-genome sequencing to identify specific strains or variants, as well as metagenomic approaches that analyze all genetic material in a sample without targeting a single organism in advance. These data enable public health practitioners to determine whether infections are part of the same transmission cluster or represent distinct introductions, and can identify characteristics like transmissibility, severity, or antimicrobial resistance.

Immunization data

State public health agencies collect immunization records through immunization information systems (IIS), which can be used to identify areas of low vaccine coverage and prioritize outreach during an outbreak. When a wastewater signal is detected for a vaccine-preventable pathogen, immunization data can help contextualize the likely severity of community impact, identify potentially vulnerable populations, and target response efforts geographically. Federal immunization coverage data are available through ChildVaxView and additional datasets are accessible at data.gov.

Animal and vector surveillance

State and federal public health agencies conduct surveillance for zoonotic diseases, including tracking animal health, animal bite incidents, and vector populations such as mosquitoes and ticks. Animal and vector surveillance can provide insight into pathogens like *Borrelia burgdorferi* (the cause of Lyme disease), West Nile virus, and influenza A(H5). These data can help distinguish human from animal sources and inform risk assessment and public health response.

Demographic data

Demographic and census data are used to estimate the populations contributing to a sewershed and to characterize the risk profile of a community. Understanding age distribution, comorbidity burden, socioeconomic factors, and housing density can help public health practitioners interpret wastewater signals in context and tailor communication and intervention strategies accordingly.

School and workplace data

Schools and workplaces track absenteeism that may be attributable to illness, providing a signal of community health burden that does not depend on healthcare-seeking behavior. This data can serve as an early corroborating indicator when wastewater signals suggest rising transmission, particularly for respiratory or gastrointestinal illnesses that commonly circulate in congregate settings.

Healthcare capacity data

Healthcare capacity data include occupied and available inpatient beds, emergency department volumes, and ICU utilization. These data provide real-time insight into where disease burden is straining the health system and can help contextualize wastewater signals by showing whether rising pathogen concentrations are translating into increased healthcare demand.

Death certificate data

Death certificate data provide information on cause of death that can help track the severity and spread of an outbreak over time. However, because death records are often finalized weeks after the event, these data lag significantly behind other indicators and are most useful for retrospective analysis rather than real-time situational awareness.

Key Questions to Guide Data Integration

Data availability

- ☐ What datasets do you currently have access to, and what are the key gaps?
- ☐ For each data source, what can it tell you – and what can it not tell you?
- ☐ Are there populations or geographies that are not captured by any of your current data sources?
- ☐ Are data sharing agreements in place that allow you to access and integrate the datasets you need?
- ☐ What is the typical lag between when data are generated and when they reach you for each source?

Spatial alignment

- ☐ How well do your sewershed boundaries map to the health jurisdictions used for clinical case reporting?
- ☐ What proportion of the jurisdiction's population is connected to the municipal sewer system, and how might the unsewered population differ epidemiologically?
- ☐ Are there dynamic population factors, such as commuters, tourists, seasonal residents, that could decouple the wastewater signal from locally reported cases?
- ☐ What approach will you use to estimate the sewershed population, and is that approach documented and consistent across sites?
- ☐ Do your clinical data carry sufficient geographic detail (e.g., addresses or zip codes) to permit meaningful comparison with sewershed boundaries?

Temporal alignment

- ☐ For the pathogens you are monitoring, does wastewater shedding begin before symptoms appear, and by how much?
- ☐ What is the total duration of the shedding window, including how long shedding may continue after symptoms resolve?
- ☐ What is the expected lead or lag between a wastewater signal and a corresponding rise in clinical cases for the pathogens you are monitoring?
- ☐ Is your sampling frequency sufficient to detect a trend, or could meaningful signals fall between sampling events?
- ☐ How are dates recorded? For example, are you comparing the wastewater sample collection date to the symptom onset date, specimen collection date, test date, or report date?
- ☐ How are weekend, holiday, and operational gaps handled in each data stream?

Interpreting Signals in Context

To transform pathogen concentrations into actionable public health responses, practitioners must contextualize and interpret results. The same numerical result can mean very different things depending on the characteristics of the sewershed, the pathogen being monitored, and what was happening in the community at the time of sampling. Developing a habit of asking *"What else was going on?"* before acting on a signal is one of the most important practices a wastewater surveillance program can build.

Wastewater system and site characteristics

The physical and operational characteristics of a sewershed directly shape what wastewater data can and cannot tell you, and understanding them is a prerequisite for accurate signal interpretation. A large combined sewer system serving a dense urban population may vary from a smaller system serving a rural or mixed-use community, and the same numerical result can have very different implications depending on the system it came from.

- **Sample collection method** matters for interpretation. Composite samples that are collected continuously over a 24-hour period and weighted by flow are more representative of the full daily contribution of the sewershed population than grab samples, which capture only a single moment in time. Samples collected at the treatment plant represent the aggregate of the entire sewershed, while samples from upstream manholes or localized collection points reflect smaller, more defined populations. Both the method and the location of collection should be documented and considered when interpreting results. For more details on sample collection methods, see Section 1, Understanding Wastewater Data as a Public Health Tool.
- **Sewershed area composition** also influences what the wastewater signal represents. Industrial facilities, hospitals, nursing homes, correctional facilities, and university dormitories can all contribute to pathogen concentrations in ways that are unrelated to general community transmission. A signal that appears to reflect widespread community spread may in fact originate from a single institutional source. Understanding the sewershed area composition in each sewershed is important context for interpretation.
- **Wastewater treatment plant staff** are an essential and often underutilized resource. They are typically the first to know about infrastructure changes, maintenance events, unusual influent conditions, or operational anomalies that could affect a sample. Programs should maintain regular communication with plant operators and consult them when a signal is unexpected or difficult to explain. Any operationally driven changes, such as sewer rehabilitation, flow rerouting, new service connections, or shifts in sampling location, should be documented so they can be distinguished from true epidemiological shifts during interpretation.

Pathogen-specific disease dynamics

Understanding how a pathogen behaves biologically is essential for interpreting when and why it appears in wastewater, how its signal relates to clinical cases, and whether a signal reflects local transmission or other external contributors such as visitors or travelers. The concepts below are the most relevant for wastewater interpretation. Further pathogen-specific details are provided in Section 5, Pathogen-Specific Considerations.

- **Shedding rate and shedding profile** – Shedding rate is the quantity of pathogen or its genetic material that an infected person excretes over time, which affects the strength, duration, and interpretability of a wastewater signal. Shedding rates vary by individual and by

pathogen. A pathogen's shedding profile describes how that rate changes over the course of an infection. Some pathogens are shed most intensively before symptoms appear, while others peak during the symptomatic period or persist into recovery. Understanding these patterns can help analysts distinguish persistent low-level detections due to prolonged shedding from signals more consistent with ongoing community transmission.

- **Incubation period** – The incubation period is the time between exposure and the appearance of symptoms. Incubation periods range from hours (norovirus, salmonella) to weeks (measles, mononucleosis) and affect how far in advance of clinical detection a wastewater signal might appear. During this window, most people do not know they are infected but may already be shedding pathogen into the wastewater system.
- **Temporal lead or lag time** – Temporal lead or lag is the time difference between pathogen detection in wastewater and detection through clinical surveillance. Because wastewater can capture shedding before people seek care, it can provide earlier warning than clinical data alone. However, the lead or lag time that wastewater provides can vary by geography, pathogen, and season. The magnitude of this lead time depends on pathogen-specific factors such as shedding profile and incubation period, as well as the timeliness of wastewater testing and clinical reporting.
- **Asymptomatic infections** – Some people carry and shed pathogens without ever developing symptoms. Wastewater surveillance captures shedding from these people, while clinical surveillance does not. For pathogens with high rates of asymptomatic carriage, wastewater signals may substantially exceed what clinical case counts would suggest.
- **Environmental and animal sources** – Some pathogens that cause human disease may also occur in the environment or be carried and shed by animals. When interpreting a wastewater signal, particularly for zoonotic pathogens, programs should consider whether the detected target could reflect environmental reservoirs, animal populations, or human infections.
- **Endemic vs. emerging pathogens** – Certain pathogens circulate continuously in the United States (norovirus and influenza are common examples) and will almost always be detectable in wastewater. For these endemic pathogens, any single detection is unremarkable and trend analysis is the primary interpretive tool. Other pathogens are rare or absent from most communities (measles and poliovirus are current examples). A single detection of one of these warrants immediate investigation regardless of concentration; the key questions are whether the signal is confirmed, whether it persists across consecutive samples, and whether clinical or epidemiological data corroborate it.
- **Degradation rate and persistence of genetic materials** – Pathogen genetic material degrades in wastewater at rates that vary by pathogen, temperature, pH, and microbial activity. Deviations from standard sampling protocols, such as shipping delays or temperature excursions, should be evaluated with these factors in mind, as degradation can cause concentrations to appear lower than they truly were at the time of collection.

Wastewater Can See What Clinical Surveillance Can Miss: Poliovirus in New York

In 2022, a case of vaccine-derived poliovirus was confirmed in an unvaccinated adult in New York. This was the first case of paralytic polio in the United States in nearly a decade. Subsequent wastewater testing across the index case county and five surrounding jurisdictions detected poliovirus in nearly 9% of samples, indicating that the virus had been circulating silently in the community long before the single clinical case came to light.

This is a direct consequence of poliovirus biology: only about 1 in 200 infections causes paralysis, while the vast majority of infected people shed the virus in their feces without ever developing symptoms. Clinical surveillance, which depends on people becoming ill enough to seek care, is nearly blind to this silent majority. Wastewater surveillance is not.

For details on this case, see the poliovirus case study in Section 6, Wastewater Surveillance Case Studies.

External factors that influence wastewater signals

Changes in pathogen concentrations do not always reflect true changes in disease transmission. Population dynamics, environmental conditions, infrastructure changes, and public health interventions can all shift wastewater signals in ways that are unrelated to underlying community burden. Routinely considering these factors alongside wastewater data reduces the risk of over- or under-reacting to changes in signals.

- **Population dynamics** – Tourism, seasonal residents, commuter populations, and large events can all shift the number and composition of contributors to a sewershed, sometimes substantially. Travel hubs present a particular challenge: the total number of contributors may be relatively stable while the specific people cycling through are constantly changing, making it difficult to link a wastewater signal to a defined population. To accurately interpret data, the practitioner should understand baseline population patterns for each monitored site and flag periods when those patterns are likely to deviate, such as during tourist season or around a major event.

  #### Example: Seasonal Tourism

  A beach community may observe a sharp increase in wastewater pathogen concentrations during summer months. This rise might be driven by tourists, rather than the local population. In addition, if wastewater data are reported with population-based normalization, consider how the underlying population changed between summer and winter months. As a result, public health interventions during the tourist season may look different from interventions during the winter months: communication channels to reach tourists are often different from those used to reach residents.

- **Weather and environmental conditions** – In combined sewer systems, heavy rainfall and snowmelt may dilute analyte concentrations, and large precipitation events may trigger combined sewer overflows that bypass treatment plants entirely, potentially changing the population and flow represented by the sample. Flow-based or biomarker normalization, discussed in the normalization section, can help mitigate dilution effects. Temperature also affects pathogen stability and degradation rates in wastewater, with the degree of impact

varying by pathogen, deviations from standard sample handling protocols are particularly important to flag during periods of extreme heat.

- **Animal and other nonhuman sources –** Some pathogens detected in wastewater may originate partly or entirely from animal populations, agricultural operations, or other nonhuman sources. For example, influenza A(H5) signals may reflect contributions from infected dairy cattle, poultry, or dairy-processing waste rather than human infections. These potential sources should be considered when interpreting wastewater detections for certain pathogens and comparing them with clinical data.
- **Wastewater infrastructure changes** – As noted in the "Wastewater system and site characteristics" section above, infrastructure changes can also introduce discontinuities in trend data over time. Documenting and communicating these changes as they occur is essential for distinguishing operational shifts from true epidemiological signals.
- **Vaccination coverage and public health interventions** – In well-vaccinated populations, wastewater signals and clinical case counts may reflect different aspects of pathogen activity. High vaccine coverage can reduce the likelihood of symptomatic or severe illness, which may result in fewer healthcare visits, tests, and reported cases even when transmission and shedding continue. Wastewater measurements of vaccine-preventable diseases should always be interpreted alongside current information on vaccination coverage and recent public health interventions, which may affect both pathogen circulation and patterns of clinical detection.

Contextual interpretation checklist

Answering the following questions will help add context to your wastewater data and can guide choices related to data processing and interpretation.

Sample collection

- ☐ Were samples collected as 24-hour flow-weighted composites or grab samples?
- ☐ Were samples collected at the treatment facility or at an upstream manhole or localized site?
- ☐ Were standard collection protocols followed, including temperature maintenance and transport time?
- ☐ Were any hold times exceeded between collection and laboratory analysis?

Sewershed area characteristics

- ☐ How large is the sewershed area, and is it primarily residential, commercial, or mixed use?
- ☐ Are there institutional contributors – hospitals, nursing homes, correctional facilities, dormitories, or large industrial users – that could influence concentrations independently of community transmission?
- ☐ What proportion of the jurisdiction's population is connected to the municipal sewer system?
- ☐ Are there known cross-border flows from neighboring jurisdictions?
- ☐ Does the sampling area include any Tribal lands or Tribal communities? If so, how are you collaborating with their public health authority to understand the sewershed area?

Population dynamics

- ☐ Is the contributing population stable, or are there known temporary changes that may have affected the wastewater signal, such as tourism, seasonal residents, commuters, or large events?
- ☐ Has the contributing population changed since the baseline for this site was established?

Environmental and weather-related factors

- ☐ How much rainfall has the region received in the period of interest? Could dilution from inflow or infiltration explain a lower-than-expected signal?
- ☐ Were there any combined sewer overflow events that may have bypassed the treatment plant?
- ☐ What is the ambient temperature, and could it have affected pathogen stability before sample collection?
- ☐ Could precipitation have introduced animal-derived pathogens into the sewer system?

Wastewater infrastructure

- ☐ Have there been any recent changes to sewer infrastructure, flow routing, sampling locations, or treatment processes that could affect results?
- ☐ Have any new service connections been added to the sewershed?
- ☐ Has the wastewater monitoring team been informed of any planned or unplanned maintenance events?

Pathogen biology

- ☐ When does shedding begin relative to symptom onset for this pathogen, and what does that imply about the lead time between the wastewater signal and clinical detection?
- ☐ Is this pathogen endemic or rarely detected in this jurisdiction? Does the signal warrant immediate investigation or trend monitoring?

- Is asymptomatic infection common for this pathogen and could that contribute to a stronger wastewater signal than would be suggested by reported clinical cases?
- What is the expected degradation rate for this pathogen under current sample conditions?

Public health context

- What is the current vaccination coverage in the sewershed population for this pathogen?
- Have there been recent vaccination campaigns, changes in testing availability, or other public health interventions?
- Are complementary data sources — clinical case counts, syndromic surveillance, ELR — consistent with the wastewater signal, or is there discordance that warrants further investigation?

When Wastewater and Clinical Data Disagree

Discordance between wastewater and clinical data is expected because these sources capture different aspects of disease activity. Differences between these signals can therefore provide useful context rather than indicate a failure of either system. Wastewater is most informative when interpreted alongside clinical and other surveillance data to build a more complete picture of community pathogen activity. Understanding the reasons behind a mismatch is often as informative as the signals themselves. The sections below describe the most common explanations for two distinct patterns of disagreement and the key questions to ask when each is encountered.

Wastewater signal rising, cases flat or absent

A rising wastewater signal without a corresponding increase in clinical cases is one of the most valuable patterns wastewater surveillance can produce. Before drawing conclusions, consider the following possible explanations:

- **Early warning of emerging transmission** – Cases may be in the incubation period and not yet symptomatic, or symptomatic but not yet tested or reported.
- **Underdetection in clinical surveillance** – People with asymptomatic or mild cases may not seek care, people using home tests may not report positive results, and infections in populations with limited healthcare access may not appear in reporting.
- **Spatial mismatch and transient contributors** – Cases may be assigned to a home address, healthcare facility, or jurisdiction that does not align with the wastewater sewershed area. Travelers, commuters, hauled waste from portable toilets, or other temporary contributors may also affect the signal without being counted in local clinical data.

When this pattern is observed, the most important questions to ask are: how long has the signal been elevated, and is it sustained and increasing or transient and declining? A persistent and rising signal warrants more active investigation than a single elevated sample.

Cases rising, wastewater signal flat or absent

Increasing case counts without a corresponding wastewater signal might be explained by:

- **Insufficient shedding for detection** – In large sewersheds with very few cases, pathogen concentrations may be too diluted to detect.
- **Spatial mismatch** – Cases may occur among individuals on private septic systems not connected to the municipal sewer, those that do not travel into sewersheds monitored through wastewater surveillance, diapered populations, or cases diagnosed immediately upon return from travel who may not yet have contributed to the local wastewater system.
- **Pathogen degradation** – High temperatures, shipping delays, or pathogens with low environmental stability may result in genetic material degrading before analysis.
- **Infrequent sampling** – For pathogens with short incubation periods, weekly or less frequent sampling may miss the peak shedding window entirely, particularly when cases are few and shedding is brief.
- **Outbreak coming under control** – If cases were diagnosed with a delay relative to peak shedding, the wastewater signal may already be declining by the time case counts appear to be rising.

When this pattern is observed, key questions to ask are: how many cases have been diagnosed, and how recently? Have the cases been traveling? Have there been recent disruptions to wastewater sample collection or weather events that could have affected results?

Additional Resources for Interpreting Signals

- CDC – About Wastewater Data
  Overview of how NWSS collects, processes, and shares wastewater surveillance data, including data standards and access.

- Rockefeller Foundation – Integrating Wastewater and Public Health Data
  Practical integration guide covering decision frameworks, data sharing agreements, and approaches for combining wastewater and clinical data streams.

- European Centre for Disease Prevention and Control (ECDC) – ECDC Framework to Guide the Integration of Wastewater-Based Surveillance into Infectious Disease Surveillance at the EU/EEA Level
  Framework for integrating wastewater surveillance into national public health monitoring systems, with applicability to multi-pathogen programs.

- World Health Organization (WHO) – Wastewater and Environmental Surveillance for One or More Pathogens: Guidance on Prioritization, Implementation and Integration
  WHO's current guidance on implementing wastewater surveillance as part of multi-modal public health surveillance, covering pathogen prioritization and integration with existing systems.

4. Translating Wastewater Signals into Public Health Action

This section provides guidance on how to move from detection of a wastewater signal to informed public health action. It covers how to define action thresholds, how to communicate findings to different stakeholder audiences, and how to build decision frameworks that support consistent, defensible public health responses. Because the appropriate response depends on the pathogen, the signal, and the broader public health context, this section emphasizes structured decision-making over prescriptive rules.

Using Wastewater to Inform Action

Wastewater signals vary widely in their public health implications, and no single factor determines whether or how to respond. The following should be considered together:

- **The signal characteristic** – Its magnitude, whether it exceeds baseline expectations, and whether it is sustained across consecutive samples.
- **The pathogen** – Its public health importance, its shedding characteristics, whether it is endemic or rarely detected, and whether environmental, animal, or vector sources could contribute to the signal.
- **Clinical and syndromic correlation** – Whether other surveillance data support or contradict what the wastewater is showing.
- **Epidemiologic context** – Whether external factors such as the time of year, local immunity, vaccination coverage, and recent changes in population behavior can explain the signal.

These factors should be evaluated together within a pre-established decision framework. A large signal from a rarely detected pathogen calls for a very different response than a modest but sustained signal from an endemic pathogen trending alongside rising emergency department visits. Even when a signal does not trigger an immediate intervention, awareness of pathogen presence or changing community risk can support situational awareness, particularly when clinical data are limited or absent.

Programs best positioned to act are those that have worked through these questions before a signal appears. The subsections that follow walk through a decision tree for translating these factors into specific actions and provide guidance for establishing clear action thresholds, communication plans, and decision frameworks in advance of an emerging situation.

Defining Action Thresholds

Action thresholds define the point at which a wastewater signal moves from observation to response. These thresholds should be established before a signal occurs – not in the middle of one. Pre-defined thresholds make responses faster, more consistent, and more defensible, and they reduce the risk that decisions are driven by urgency or uncertainty rather than evidence. Thresholds are also best when anchored to a specific site, because wastewater concentrations reflect the unique characteristics of each sewershed and laboratory method. Action levels should be anchored to a

site's own historical data rather than absolute concentrations and are generally not directly comparable across different treatment plants or programs. In practice, however, many programs need to define and communicate thresholds at the level of a local health department or jurisdiction, particularly when a single jurisdiction is served by multiple sewersheds.

The appropriate analytical approach to a wastewater signal depends on whether a pathogen is endemic or rarely detected. This distinction carries through directly to response.

- **For emerging or rarely detected pathogens**, such as measles, poliovirus, or mpox, any confirmed detection warrants immediate investigation. Programs, in collaboration with subject matter experts, should establish confirmatory criteria to gain confidence in the signal before response escalation. This could collecting and testing an additional wastewater sample to determine whether the detection persists. For these pathogens, a non-detect should not be interpreted as confirmation of absence – the pathogen may be circulating below the detection threshold, particularly in large sewersheds with small numbers of cases. For these pathogens, programs should err toward rapid response given the public health consequences of delayed action.
- **For endemic pathogens**, such as influenza, SARS-CoV-2, or norovirus, presence in wastewater is expected, particularly during the peak season. Data should be evaluated to determine whether the rate of change represents a meaningful departure from baseline which warrants a response. Questions and considerations for choosing thresholds are included below, under "Creating a Decision Tree for Your Agency." Programs should select from the statistical methods described in Section 2, which include percent change, benchmark comparison, Z-scores, or rolling averages. These thresholds should be applied to site-specific historical data, because wastewater concentrations reflect the unique characteristics of each sewershed and are not directly comparable across sites. For these pathogens, a non-detect can also serve as a meaningful signal that significant community circulation is not occurring, and that an elevated response is not warranted.

Thresholds should also be designed to trigger graduated responses: internal notification at one level, provider alerts at another, public communication at another – rather than a single binary decision. The decision tree section that follows provides a framework for structuring these tiers.

Public Health Responses

Wastewater signals can prompt a range of public health responses, from enhanced internal monitoring to active community intervention. The appropriate response will depend on the pathogen, the strength and persistence of the signal, and whether clinical or epidemiological data corroborate what the wastewater is showing. Most situations will involve several of the following public health responses in combination, distributed across functional teams and partner organizations. Public health response will also depend on health department resource availability. Smaller agencies may not have dedicated departments for every function, but will have staff who carry out those roles. Programs should establish clear lines of responsibility for each response activity in advance.

Notification and communication

Timely notification of the right partners is often the first and most time-sensitive response to a wastewater signal. Depending on the jurisdiction and the nature of the signal, this may include:

- **Internal notification** – Alerting data and epidemiologic teams, program leadership, and other relevant staff within the health department, so that follow-up activities can be initiated promptly.

- **Utility partner notification** – Informing wastewater utility partners when a signal may warrant follow-up, changes in sampling, review of operational conditions, or other coordination.
- **Healthcare provider alerts** – Notifying hospitals, clinics, emergency departments, and relevant specialists to increase clinical vigilance, lower their threshold for testing, and ensure appropriate supplies and treatment are available. Health Alert Networks (HANs) are used in some jurisdictions to facilitate rapid provider notification.
- **Interagency notification** – Informing partner agencies such as Tribal Nations, local health departments, neighboring health departments, state-level authorities, or federal partners when the signal has implications beyond the local jurisdiction.
- **Institutional notification** – Alerting schools, daycares, long-term care facilities, correctional institutions, or other congregate settings when the signal suggests elevated risk in those environments.
- **Public communication** – Press releases, health advisories, social media, and community messaging to inform the public of elevated risk and recommended protective actions. Public communication should be coordinated with communications staff and leadership before release, and messaging should be tailored to the audience and proportionate to the level of concern. If describing Tribal communities, public communications should follow the procedures and guidelines agreed upon with the Tribal Nation, if such agreements exist.

Internal and public-facing data dashboards that display wastewater trends and/or detections alongside clinical data in near real time are increasingly common. When designed to be clear and easily interpretable, they can serve as a proactive communication tool that reduces the need for reactive messaging when signals rise.

Wastewater surveillance enhancement

A wastewater signal may prompt programs to strengthen or expand their wastewater monitoring to better characterize its scope and trajectory. Before expanding monitoring, programs should have a clearly defined public health rationale for doing so and consider logistical planning (e.g., time, funding, personnel) and ethical implications (e.g., privacy concerns for small sewersheds) of the actions to determine what scope of expansion would be beneficial. Actions to strengthen or expand wastewater monitoring can include:

- **Increased sampling frequency** – Moving from weekly to more frequent sampling at existing sites to improve temporal resolution and reduce the risk of missing signal changes between samples.
- **Upstream or facility-level sampling** – Adding sampling points at manholes or specific facilities to narrow the geographic source of a signal to a specific neighborhood, institution, or population. Ethical considerations of sub-sampling smaller populations should be considered.
- **Genomic sequencing** – Sequencing wastewater-derived material. For high-priority or rarely detected pathogens such as measles or poliovirus, this can confirm the presence of a specific strain, distinguish outbreak from vaccine-derived sources, and possibly help link detections across sites or jurisdictions.

Clinical and syndromic surveillance review

In parallel, programs should review complementary surveillance data to assess whether the wastewater signal is reflected in clinical systems:

- **Syndromic surveillance review** – Examining emergency department visit data and chief complaint trends in ESSENCE or similar platforms to identify corroborating signals in healthcare-seeking behavior.
- **Clinical case data monitoring** – Tracking case counts, laboratory results, and hospitalization data for the pathogen of interest to assess concordance with the wastewater signal.

- **Cross-jurisdictional data sharing** – Consulting with neighboring health departments or state partners to determine whether clinical signals are being detected elsewhere. This can help distinguish local transmission from broader regional trends.

Epidemiological investigation

When a signal suggests active transmission, epidemiological follow-up may be warranted to identify cases, characterize the outbreak, and inform a tailored response:

- **Active case finding** –Epidemiology teams may proactively reach out to healthcare providers, emergency departments, and high-risk settings to identify individuals with symptoms consistent with the pathogen of interest, inform them of the potential increased pathogen activity, or temporarily modify criteria for identifying suspected cases.
- **Case investigation** – When cases are identified that align with the wastewater signal, standard case investigation collects demographic, exposure, and contact information to characterize transmission and identify potential sources. Findings can also help validate and contextualize the wastewater signal– confirming that cases fall within the sewershed or revealing discordance that warrants further investigation of both data sources. Wastewater data alone should not be used for contact tracing or identification of individuals.
- **Source investigation** – For a pathogen with a plausible specific exposure source, such as a food source for hepatitis A or a specific healthcare facility for *C. auris*, the wastewater signal can help geographically focus the investigation.
- **Clinical specimen sequencing** – When cases are identified that align with a wastewater signal, sequencing of clinical isolates can confirm the circulating strain and link individual cases to each other or to the wastewater-derived sequence data. For high-consequence pathogens such as measles or poliovirus, sequencing clinical specimens can confirm outbreak strain identity and distinguish imported from locally acquired transmission.

Clinical and community interventions

Depending on the pathogen and the scale of the signal, active interventions may be initiated to reduce transmission or mitigate harm:

- **Vaccination** – Deploying vaccination clinics or outreach in the affected sewershed or among high-risk populations. Immunization teams should be engaged early when a signal involves a vaccine-preventable pathogen.
- **Community health education** – Outreach to inform the public or specific communities about risk, symptoms, and protective behaviors, tailored to the transmission route of the pathogen.
- **Healthcare capacity preparation** – Alerting hospitals and health systems to anticipate potential increases in patient volume, ensure availability of treatments or antivirals, and consider staffing adjustments.
- **Expanded clinical screening and/or testing** – Offering clinical screening or testing to identify and treat cases in the community. This can also include increased communications to educate communities on the benefits of testing.

Wastewater Data for Single Detections

In August 2025, the Colorado Department of Public Health and Environment detected a low-level measles signal in a wastewater sample. Two days later, a second sample returned a high-concentration detection of wild-type measles RNA. Rather than waiting for a clinical case to confirm circulation, the local public health agency acted immediately:

- **Provider alerts** – A comprehensive alert was issued to local healthcare providers warning that measles was circulating, urging them to stock vaccine inventories and lower their threshold for testing.
- **Staffing readiness** – Increased staffing was planned in anticipation of an imminent outbreak response.
- **Vaccination guidance** – Providers were directed to prioritize vaccination recommendations for patients who were unvaccinated or incompletely vaccinated.

The early action proved consequential. Just two days after the high-concentration detection, a suspected measles case was reported in an unvaccinated teenager living within the same sewershed. Because the health department was already mobilized, investigators could rapidly identify five additional cases among 225 contacts – containing the outbreak far more quickly than would have been possible had they waited for the first clinical report.

Source: *Jensen et al., 2026*

Communicating Findings to Stakeholders

Effective communication is essential for translating a wastewater signal into a coordinated public health response. As noted in the preceding section, notification and communication are often the first actions taken when a signal warrants attention.

Tailoring messaging to the audience

Different audiences have different information needs and different capacities to act on wastewater data. Communication should be tailored accordingly.

- **Healthcare providers and hospitals** are often the most immediate and action-oriented audience for a wastewater signal. Provider communication should focus on clinical implications including what to watch for, when to test, how to report, and what treatment or prevention resources are available. For hospitals, rising wastewater signals can serve as an early warning to prepare for potential increases in patient volume, allowing them to adjust staffing, treatment availability, and capacity. Provider alerts should be specific about the pathogen, the nature of the signal, and what action is being requested.
- **Public health leadership** will make decisions based on how a wastewater signal is presented to them. Messaging should contextualize and interpret the wastewater results and, include potential public health implications of the signals. It is helpful to provide a summary of recommended response actions by established response frameworks and/or decision trees. Uncertainty should be communicated to public health leadership, along with possible steps to reduce uncertainty, if warranted (e.g., increased sampling). If working with a Tribe or Tribal Epidemiology Center, ensure that the proper leadership is included and in practices that are acceptable to the community.
- **Utility partners** are key to wastewater surveillance efforts, so keeping them informed and engaged is important for a successful program. If an unusual result or trend is seen, utility partners should be consulted first for any information on the samples that could help provide

context and change how the data are interpreted (e.g., differences in flow or sampling at different times than usual). Utilities should also receive advance notice of significant or unusual detections, particularly when public communication or press coverage is anticipated, so their communications staff can prepare for questions. For rare or high-concern pathogens, programs should also coordinate with utilities on any implications for worker safety and appropriate precautions. Clear and open communications with utility partners will also facilitate realistic response actions, such as increased sampling frequency.

- **The public** needs messaging that translates technical wastewater data into personal risk and protective behavior. Metrics such as gene copies per liter are not meaningful to a general audience and should be converted into accessible risk communication. A tiered framework can help communicate both the current situation and what people should do in response. This approach is sometimes described as a stoplight approach, with low, moderate, and elevated risk levels corresponding to increasing protective recommendations. Messaging should be proportionate to the signal, pathogen-specific in its recommended actions, and calibrated to avoid both alarm fatigue and complacency.

EXAMPLE FIGURE: STOPLIGHT APPROACH FOR COMMUNICATING TO THE PUBLIC*

Green – routine activity

Pathogen levels are at or below baseline. No unusual activity detected.

- Maintain routine hygiene practices such as handwashing
- Stay up to date on recommended vaccinations

Yellow – increasing activity

Signal indicates an upward trend or emerging presence above baseline.

- Follow current public health recommendations for the pathogen
- Monitor for early symptoms and seek care promptly if they appear
- Ensure vaccinations are current

Red – elevated activity

Elevated levels (or multiple, high-risk pathogen detections) indicate significant community circulation of the pathogen.

- Follow enhanced public health recommendations issued for the pathogen
- Reduce avoidable exposure by avoiding settings where transmission risk is highest
- Follow guidance from local public health authorities

** This simplified framework is an illustrative example. It should be adapted to the pathogen, local epidemiology, available clinical data, and recommended public health actions.*

- **Communication for high-density environments**, such as schools, universities, homeless shelters, and congregate care facilities, should focus on objective triggers for intervention. Establishing specific wastewater data thresholds allows these institutions to implement tiered safety protocols, such as enhancing ventilation and air filtration systems; promoting personal protective equipment (PPE) use, including temporary mask mandates (if appropriate for the pathogen's primary mode of transmission); and increased frequency of onsite diagnostic testing to identify cases before they spread.

Transparency and uncertainty

Maintaining public and partner trust requires honest communication about what wastewater data can and cannot tell you. Wastewater signals are subject to variability. For example, a single elevated result may reflect a true increase in community transmission, a sampling artifact, or a transient contributor. Programs should communicate the limitations of individual results clearly, particularly when acting on early or single detections, and should explain what confirmatory steps are being taken before escalating to broader public action.

The meaning of a non-detect also requires careful communication and varies by both pathogen and context. For a pathogen with lower or intermittent shedding, such as poliovirus or mpox, a non-detect should not be communicated as confirmation of absence: the pathogen may be circulating below the detection threshold. For a pathogen with high and consistent shedding, such as SARS-CoV-2 or norovirus, a non-detect carries considerably more reassurance. The size and nature of the sewershed also matters. A non-detect from a large community treatment plant can mean something different than one from a sampling site that services a small facility. Programs should document what a non-detect means for each monitored pathogen and site context and communicate this clearly to partners and the public.

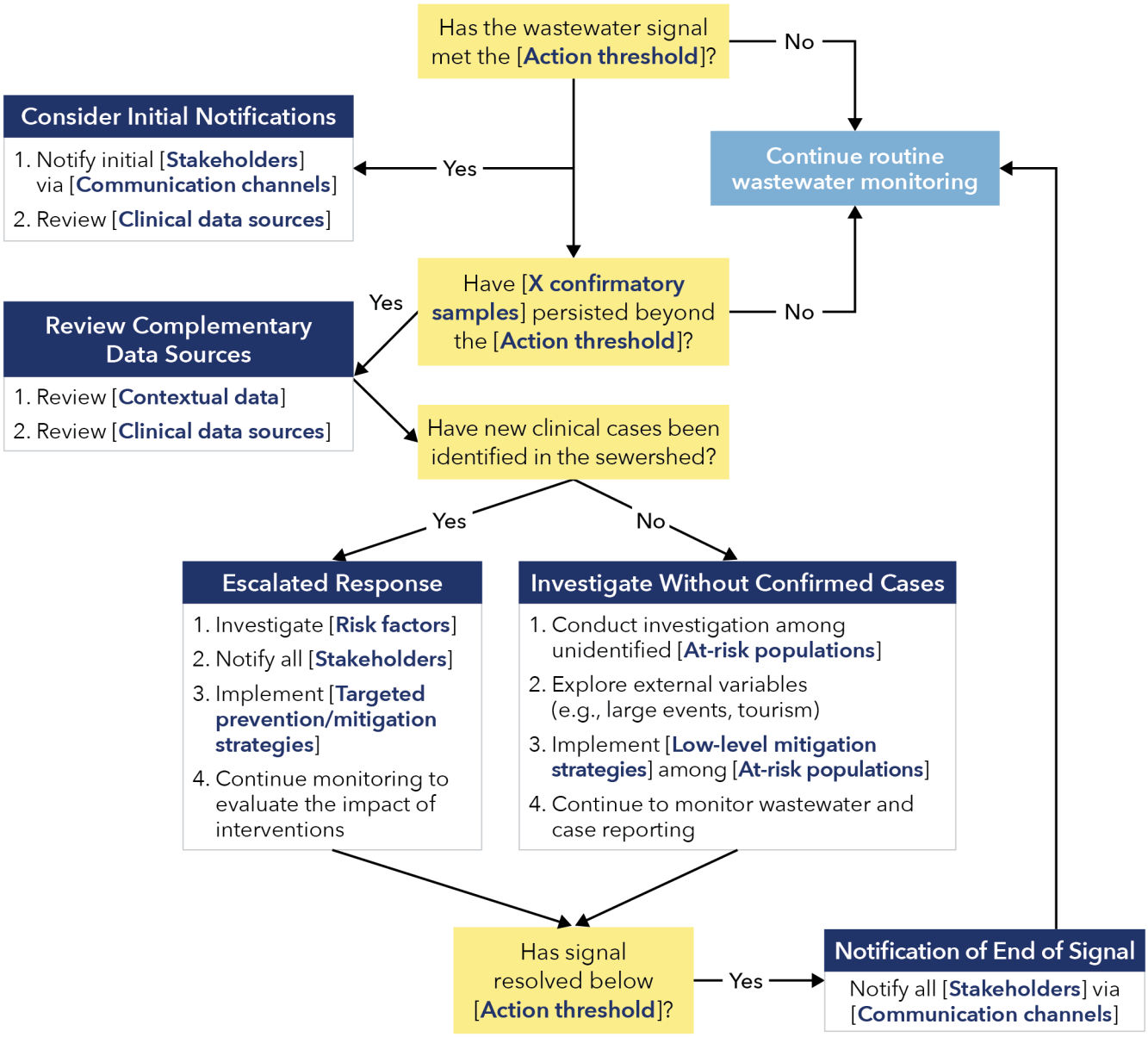
Decision Trees and Standardizing Responses

To help avoid making decisions reactively or inconsistently when a wastewater signal appears, programs should document standardized response protocols through decision trees. A well-designed decision tree turns a signal into a defined sequence of actions by specifying what action is triggered at each signal level, who is responsible for each step, and what criteria determine when to escalate or stand down.

The template below illustrates a simplified decision framework that programs can adapt for their jurisdiction and pathogens. It begins with a threshold question; if the signal meets the threshold, it triggers initial notifications and a review of complementary data sources. Confirmatory samples check against reacting to a single anomalous result before escalating to broader response. From there, the framework branches depending on whether clinical cases have been identified in the sewershed — leading either to an escalated response with active investigation or to a more tailored investigation in the absence of confirmed cases. When the signal resolves, stakeholders are notified and routine monitoring continues.

The bracketed placeholders throughout the template — such as [Action threshold], [Stakeholders], and [Low-level mitigation strategies] — are intentionally left generic. Each should be defined in advance for each pathogen being monitored, using the guidance in the following section and in consultation with subject matter experts and partner jurisdictions. Decision trees will also look different depending on context: a community-level program monitoring an endemic pathogen will have different thresholds and response steps than a facility-level program responding to a single detection of a high-consequence pathogen.

Example Decision Tree for Public Health Response



Action threshold: Site-specific baseline value, percent change, or Z-score defined before a signal occurs

At-risk populations: Facilities, congregate settings, or subpopulations within the sewershed that are at risk or may not be represented in clinical data

Clinical data sources: For example, syndromic surveillance, electronic laboratory reports, cases

Communication channels: e.g., HAN alerts, dashboards, direct email, press release

Contextual data: Non-clinical information that helps explain or validate a wastewater signal, such as population data, weather, flow records, and vaccination rate

Low-level mitigation strategies: For example, provider messaging, public health advisories

Risk factors: Population vulnerability, vaccination coverage, exposure settings

Stakeholders: Initially might include internal teams, partnering health departments, or health care facilities; can expand to the public

Targeted strategies: For example, vaccination clinics, case investigation, community outreach

X confirmatory samples: Number of consecutive detections required before escalating; pathogen-specific

Creating a Decision Tree for Your Agency

The following questions are designed to help programs work through the key decisions required to complete a decision tree for each pathogen being monitored. These decisions should be made in advance in collaboration with subject matter experts, laboratory partners, and communications staff, and documented in writing so that response actions are consistent.

Pathogen-specific decisions

The first and most consequential decision is what type of threshold applies to this pathogen. As discussed in the action thresholds section, this depends on whether the pathogen is endemic or rarely detected.

- Is this pathogen expected to circulate continuously, or is any detection noteworthy?
- If endemic, what is the baseline level in this specific sewershed, and does it shift seasonally? A threshold appropriate for influenza in January may not be appropriate in July.
- If non-endemic or high-consequence, is a single confirmed detection sufficient to trigger the initial response actions, or are confirmatory samples required first?
- How many consecutive detections or sustained increases are required before you escalate from initial notification to a broader response?
- What counts as a confirmatory sample for this pathogen given its shedding window, shedding rate, and sampling frequency?
- How sensitive/specific is the assay for the pathogen?

Action thresholds

Once the threshold type is established, define the specific numerical or logical triggers that move the signal from one stage of the decision tree to the next.

- What specific value or criterion defines the action threshold — for example, a 20% increase over the prior week, a Z-score above a defined cutoff, or an exceedance of a historical baseline? If historical data are available, use the relationship between wastewater levels and past clinical outcomes such as hospitalizations or emergency department visits to inform where thresholds are set and when escalation to a broader response is warranted.
- How will the decision tree distinguish a genuine signal from a spike driven by a rain event, a holiday weekend, or a single anomalous sample? Document explicitly on how data quality exceptions are handled before escalating.

Assigning roles and defining feasible responses

A decision tree is only useful if every action in it has someone responsible for carrying it out and is actually achievable given program capacity.

- For each action in the decision tree — who is the responsible person or team? In smaller agencies, one person may carry multiple roles, but these responsibilities should still be explicitly assigned.
- What response options are actually available for this pathogen? Is it vaccine-preventable? Are antivirals or treatments available? If a high-alert threshold is reached for a pathogen with no specific countermeasure, what behavioral or environmental actions can be recommended?
- Should the response be population-wide or tailored to specific settings (e.g., long-term care facilities, correctional institutions, dialysis centers) or populations (e.g., farm workers, unhoused populations) at high risk for a given pathogen?
- Who has authority to approve external communications and public-facing actions?

Handling ambiguous or uncertain signals

Not every signal will be clean, and the decision tree should anticipate common sources of uncertainty.

- How will the tree handle a signal that meets the action threshold but coincides with a high-flow event such as heavy rainfall? Is there a data quality check built into the threshold step?
- Is a single large spike sufficient, or must the signal persist across multiple samples? How many consecutive detections or sustained increases in signal are required before moving from monitoring to action?

Adapting the framework over time

Public health surveillance evolves, pathogens change, and each response generates information that should improve the next one. Programs should build in a formal process for regularly reviewing and updating their decision trees, focused on three areas:

- **Post-signal calibration** – After each significant response, compare wastewater signal peaks to clinical outcomes such as hospitalizations and emergency department visits. Use that comparison to refine thresholds for the next event.
- **Seasonal and contextual adjustment** – Shifts in population immunity, vaccination coverage, and seasonal patterns mean that a previously established threshold may no longer reflect current conditions. Revisit baseline definitions and thresholds at least annually.
- **Methodological updates** – As laboratory methods evolve and new tools such as digital PCR or next-generation sequencing become more accessible, decision trees should be updated to reflect what the data can now tell you.

Additional Resources for Public Health Action

Communication guidance

- CDC – Health Alert Network (HAN)
  Information on using HAN for rapid notification of healthcare providers and public health partners when a significant signal is detected.

- CDC – Communication Resources for Wastewater Monitoring
  Guidance and templates for communicating wastewater surveillance findings to stakeholders and the public.

- Association of State and Territorial Health Officials – Framework for Addressing Ethical Considerations in Infectious Diseases Public Health Wastewater Surveillance
  A report on how to support decision-making through ethical frameworks that consider privacy, stigma, and data stewardship.

- National Academies of Sciences, Engineering, and Medicine – Increasing the Utility of Wastewater-Based Disease Surveillance for Public Health Action
  A consensus study report on how to increase the utility of wastewater-based disease surveillance for public health action.

- New York State Wastewater Surveillance CoE – Communications Library
  A library of example communications, messaging templates, and public-facing materials developed for wastewater surveillance programs.

Example public-facing wastewater dashboards

- CDC – National Wastewater Data for Respiratory Viruses
- New York State Wastewater Surveillance CoE – Dashboards
- Wisconsin Department of Health Services –Wastewater Monitoring: Statewide Wastewater Respiratory Summary
- City of Houston – Wastewater Monitoring Dashboard
- Boston Public Health Commission – Boston Wastewater Monitoring
- California Department of Public Health – California Wastewater Surveillance Dashboard
- Chicago Department of Public Health – Respiratory Illness Data
- Colorado Department of Public Health and Environment – Wastewater Surveillance for Infectious Disease Pathogens
- Indiana Department of Health – Indiana COVID-19 Wastewater Dashboard
- North Carolina Department of Health and Human Services – Wastewater Testing Dashboard
- Utah Department of Health and Human Services – Utah Wastewater Surveillance System

5. Pathogen-Specific Considerations

Pathogen Selection and Surveillance Profiles

Not all pathogens are equally suited for wastewater surveillance, and most programs will monitor only a subset of those detectable in wastewater. Choosing which pathogens to monitor requires balancing public health priority against practical constraints, such as laboratory capacity, detection methods, and the availability of complementary clinical data. Another important consideration is whether detecting a pathogen in wastewater could inform a meaningful public health response.

When prioritizing pathogens for a wastewater surveillance program, consider:

- **Shedding and detectability** – Whether and how infected people shed the pathogen into wastewater, whether validated wastewater or clinical laboratory methods exist for detection, and how signal loss due to issues such as viral degradation, impacts from biofilms, inhibition from pollutants, or cross-reactivity affects detectability.
- **Disease burden and public health importance** – The prevalence, severity, and transmissibility of the pathogen within the jurisdiction, and whether existing clinical surveillance is capturing it adequately and in a timely manner.
- **Actionability** – Whether a detection, in combination with other types of surveillance data, would support situational awareness or an actionable response, such as vaccination, audience-specific communication, or clinical guidance.
- **Lead time advantage** – Whether wastewater surveillance would provide meaningful early warning relative to existing clinical surveillance for this pathogen.
- **Assay availability and laboratory capacity** – The availability of assays, reagents, and analytical expertise, including turnaround time from sample collection to result.
- **Population characteristics** – The comorbidities, vaccination status, and travel patterns of the sewershed population, and how these factors influence vulnerability to specific pathogens.
- **Environmental and animal sources** – Whether environmental reservoirs or animals may contribute to the wastewater signal and limit the ability to attribute detection to human infections.
- **Clinical presentation** – The observability of infections in clinical data and the commonality of asymptomatic infections.

Programs should also establish in advance what conditions would prompt adding a new pathogen to the surveillance portfolio – for example, a national outbreak, a new detection method becoming available, or a change in local disease burden. As wastewater surveillance continues to evolve, the list of commonly monitored pathogens will expand, and programs that have defined that process ahead of time will be better positioned to adapt quickly.

The pathogen profiles that follow provide key information to support both selection decisions and ongoing interpretation of wastewater signals. Each profile covers transmission dynamics, shedding window, incubation period, clinical presentation, and wastewater-specific considerations. Federal case notifiability is included for each pathogen as a reference point for how wastewater signals can be integrated with other surveillance data streams, though state and local reporting mechanisms may also be relevant depending on the jurisdiction.

Wastewater Surveillance by Pathogen Category

Pathogens are grouped below by primary transmission route, which influences how they enter the wastewater stream, how consistently they are shed, and what their detection in wastewater implies for community transmission. For each category, detailed profiles are provided for the pathogens most commonly monitored in U.S. wastewater surveillance programs, followed by brief descriptions of additional pathogens that are emerging as candidates for wastewater surveillance as methods and program capacity continue to evolve. Programs should use these profiles alongside the pathogen selection guidance at the beginning of this section to determine which pathogens are the right fit for their surveillance objectives and laboratory capacity.

Respiratory pathogens

Pathogens commonly monitored in wastewater that are primarily transmitted through airborne or respiratory droplet pathways include:

- Influenza A and B
- Measles
- Respiratory syncytial virus
- SARS-CoV-2

A detailed pathogen profile for each is included below.

Several additional respiratory pathogens are emerging as candidates for wastewater surveillance. ***Bordetella pertussis***, a bacteria that causes pertussis, may be suitable for wastewater surveillance as methods and laboratory capacity evolve. **Human metapneumovirus (hMPV)** follows a seasonal winter pattern and is often undiagnosed due to its similarity to the common cold, suggesting that clinical surveillance substantially undercounts its burden — a gap wastewater surveillance could help fill. **Enterovirus D68 (EV-D68)** peaks in late summer and early fall. While most infections are mild, severe infections can result in acute flaccid myelitis (AFM), making early detection particularly valuable. **Human parvovirus B19** causes flu-like symptoms and a characteristic bright red facial rash, though many infections are asymptomatic. Because it is most contagious before symptoms appear, wastewater surveillance could provide earlier warning of increased community transmission than clinical reporting alone. As more information and laboratory testing for these pathogens becomes more common, they may be integrated into more wastewater monitoring programs.

Fecal-oral transmission pathogens

Pathogens primarily transmitted through fecal-oral pathways and frequently monitored in wastewater include:

- Hepatitis A
- Norovirus
- Poliovirus

A detailed profile for each of these pathogens is included below.

Several additional fecal-oral pathogens are emerging as candidates for wastewater surveillance. These include **rotavirus**, which causes acute gastroenteritis similar to norovirus. Rotavirus has declined substantially in the United States since the introduction of rotavirus vaccination in 2006. However, recent increases in rotavirus activity highlight the importance of continued surveillance. Wastewater surveillance can monitor community circulation, detect changes in transmission, and evaluate the impact of vaccination coverage and immunization programs over time. **Sapovirus** causes acute gastroenteritis and is often underdiagnosed clinically due to its similarity to norovirus. **Adenoviruses** can cause both respiratory illness and acute gastroenteritis. Their broad clinical spectrum and often mild asymptomatic presentation means a substantial proportion of infections may not be captured through routine clinical testing. As wastewater surveillance programs evolve, these pathogens may be incorporated into routine surveillance.

Close-contact transmission pathogens

Mpox is transmitted primarily through direct close contact with an infected person's bodily fluids, lesions, or contaminated materials. Wastewater surveillance for mpox gained significant traction following the 2022 global outbreak and has since been implemented in a number of U.S. jurisdictions. A detailed pathogen profile for mpox is included below.

Zoonotic pathogens

There are some pathogens with the potential for wastewater surveillance that are zoonotic with host species present within wastewater sewersheds. Wastewater surveillance of these pathogens requires additional scrutiny of the source of the pathogen in wastewater. Open sewer systems are more likely to be affected by host-species contribution of zoonotic pathogen signals. Some pathogens that fall into this category are **hantavirus** and **influenza A (H5N1)**. Testing of zoonotic pathogens with host species that are not present in the sewershed area will more clearly reflect infections among humans; some zoonotic pathogens that fall into this category in the United States would include ebola and mpox.

Healthcare-associated and antimicrobial resistance

Candidozyma auris (*C. auris*) is a multidrug-resistant fungal pathogen. It has been monitored through some routine wastewater surveillance programs in the United States since the early 2020s. A detailed pathogen profile for *C. auris* is included below.

Beyond *C. auris*, **antimicrobial resistance (AMR)** represents a growing application for wastewater surveillance. AMR pathogens are those resistant to antibiotics, antifungals, antivirals, or antiparasitics. These pathogens often develop and spread in settings with frequent antimicrobial use, such as hospitals and long-term care facilities. Researchers have developed methods to detect AMR genes directly from wastewater, providing population-level signals of resistance burden that do not depend on clinical testing or individual case identification. Pathogens and gene targets used in AMR wastewater surveillance have included **ESBL-producing Enterobacterales**, **carbapenemase-producing Enterobacterales (CPE)**, **vancomycin-resistant enterococci (VRE)**, and **sulfonamide-resistance genes**. AMR wastewater surveillance is an evolving area, and programs interested in adding AMR targets should consult current laboratory guidance and available benchmarking resources before implementation.

For more information on AMR, see WHO's Wastewater and Environmental Surveillance Summary for Antimicrobial Resistance.

Influenza A and B (Flu)

Influenza A and B are RNA viruses transmitted primarily through respiratory droplets, aerosols, direct contact, and surfaces or objects (fomites). Seasonal influenza epidemics occur annually in the United States, typically peaking in winter months. Some influenza A subtypes/variants – including avian influenza strains such as H5N1 – have pandemic potential through spillover from animal reservoirs to humans.

Clinical presentation: Common symptoms include sudden onset of fever, muscle aches, chills, cough, chest discomfort, fatigue, and headache. Illness is typically self-limiting but can cause severe disease and death, particularly in older adults, young children, and immunocompromised people. Influenza typically has a seasonal pattern, with cases rising in the winter months.

Incubation period: The incubation period is typically two days, but it ranges from one to four days from exposure to symptom onset.

Shedding: Shedding begins about one day before symptom onset and typically continues for five to seven days, with peak shedding in the first three to five days of illness. Viral particles may be present in feces, mucus, sputum, and saliva.

Wastewater detection: Influenza RNA is detected in wastewater through PCR testing that targets influenza A and B genetic sequences. Viral particles enter the waste stream primarily through feces, though not all infected people shed influenza in their stool, and shedding levels vary considerably across individuals. Despite this variability, wastewater influenza signals have shown strong correlation with clinical case trends at the population level.

Federal case notifiability: Influenza-associated pediatric mortality is routinely notifiable through the National Notifiable Diseases Surveillance System (NNDSS). Novel influenza A virus infections (e.g., avian influenza) are immediately notifiable. Additional federal surveillance systems track influenza-associate hospitalizations and emergency department visits through FluSurv-NET and the National Syndromic Surveillance Program (NSSP). Wastewater monitoring of influenza is conducted through National Wastewater Surveillance System (NWSS).

Wastewater surveillance considerations: Because fecal shedding of influenza occurs intermittently and varies from person to person, wastewater signals may underrepresent true case burden and may correlate more strongly with trend direction than with absolute case counts. Programs should interpret influenza wastewater signals primarily as a trend indicator rather than a direct proxy for prevalence, and a non-detect should not be interpreted as confirmation of absence in the community. Wastewater surveillance is particularly valuable for monitoring novel or zoonotic influenza strains that may not yet be captured through routine clinical surveillance. However, contribution from animals to wastewater should be considered in interpretation of the resulting data. Note that novel strains may have different shedding characteristics, and interpretation should account for this uncertainty.

Additional resources:

- CDC – National Wastewater Data for Respiratory Viruses
- Water Environment Federation – Influenza Information for Water Professionals
- WHO – Wastewater and Environmental Surveillance Summary for a Combined Suite of Respiratory Viruses

Measles

Measles is a highly contagious RNA virus transmitted primarily through respiratory droplets and aerosols. Measles was considered eliminated in the United States as of 2000; however, declining vaccination coverage has led to large outbreaks in recent years. Cases in the United States typically occur in in under-immunized communities.

Clinical presentation: Symptoms typically begin with high fever, cough, runny nose, and conjunctivitis, followed by a characteristic red rash appearing three to five days after symptoms begin. Measles can cause severe complications including pneumonia, encephalitis, and death, particularly in unvaccinated children and immunocompromised people.

Incubation period: Typically, the characteristic measles rash appears seven to 14 days from exposure to the virus. Prodromal symptoms (fever, cough, runny nose, and conjunctivitis) appear two to four days before rash onset.

Shedding: Infectious viral shedding typically begins three to five days before the appearance of the measles rash and continues for about four days after rash onset. RNA is shed primarily through respiratory secretions and may also be detectable in urine for several weeks after rash onset. This extended urinary shedding window is particularly relevant for wastewater surveillance, as it means wastewater signals may persist longer than clinical infectiousness.

Wastewater detection: Measles RNA is detected in wastewater through reverse transcription-polymerase chain reaction (RT-PCR) testing targeting wild-type (i.e., non-vaccine strain) measles virus (MeV). Measles signal in wastewater is primarily attributed to viral shedding in urine and respiratory secretions. Importantly, RNA from the live attenuated measles vaccine can also be detected in wastewater following vaccination. Vaccine strain measles has no current public health risk. However, vaccination-associated shedding occurs at lower rates, and PCR testing can distinguish vaccine-derived RNA from wild-type RNA.

Federal case notifiability: Measles cases are immediately notifiable through NNDSS. Given its high transmissibility and the public health consequences of delayed response, confirmed and suspected measles cases should be reported to public health authorities within 24 hours.

Wastewater surveillance considerations: Measles wastewater surveillance can complement clinical and case-based surveillance by providing an additional community-level signal, particularly where infections may not yet be reflected in reported cases. Any confirmed wild-type detection in wastewater warrants immediate investigation regardless of signal magnitude. The use of wild-type-specific PCR assays or follow-up genomic sequencing of wastewater-derived measles RNA is strongly recommended to distinguish wild-type from vaccine-derived virus. A non-detect should not be interpreted as confirmation of absence, particularly in large sewersheds where a small number of cases may not produce detectable concentrations.

Additional resources:

- CDC – Wastewater Data for Measles
- Water Environment Federation – Measles Information for Water Professionals
- WHO – Wastewater and Environmental Surveillance Summary for Measles, Mumps and Rubella

Respiratory Syncytial Virus (RSV)

RSV is an RNA virus transmitted primarily through respiratory droplets, direct contact with infected individuals, and surfaces or objects (fomites). RSV is endemic in the United States, and typically has seasonal activity that begins in fall, peaks in winter, and ends in spring, though timing can vary. While most infections cause mild cold-like illness, RSV is a leading cause of severe lower respiratory tract disease in the United States, with infants, young children, and older adults incurring higher risks of severe outcomes.

Clinical presentation: Typical symptoms include runny nose, cough, sneezing, fever, wheezing, and decreased appetite; young infants may present with nonspecific symptoms such as decreased activity, breathing difficulties, and irritability. RSV can progress to bronchiolitis or pneumonia. Most otherwise healthy adults experience mild symptoms that may be indistinguishable from a common cold and are often not diagnosed or tested.

Incubation period: Symptoms typically appear four to six days after exposure but can range from two to eight days.

Shedding: Shedding typically begins three days after infection and may continue for up to eight days in adults; certain infants and immunocompromised people may shed virus for several weeks or longer. RSV RNA is shed primarily through respiratory secretions (e.g., nasal discharge and sputum).

Wastewater detection: RSV RNA is detected in wastewater through PCR testing that targets RSV genetic sequences. Because fecal shedding occurs in only a minority of infections, the wastewater signal is thought to reflect contributions from both fecal shedding and respiratory secretions entering the waste stream through handwashing and other routes. Despite the limited fecal shedding, RSV concentrations in wastewater have shown strong correlation with clinical case trends, and wastewater signals have in some cases preceded rises in clinical cases.

Federal case notifiability: RSV is not routinely notifiable at the federal level. Participating laboratories voluntarily report positive tests through NREVSS. Additional federal surveillance systems track RSV hospitalizations and emergency department visits through RSV-NET and NSSP. Wastewater monitoring of RSV is conducted through NWSS.

Wastewater surveillance considerations: Because RSV often causes mild illness in adults that goes undiagnosed and untested, clinical surveillance substantially undercounts RSV burden in the community. Wastewater surveillance can provide a more complete picture of population-level RSV circulation, particularly for tracking the onset and peak of seasonal activity. However, some populations with the highest disease burden, including infants and older adults who use diapers or other absorbent products, may contribute less consistently to the wastewater signal. As with influenza, RSV wastewater signals should be interpreted primarily as trend indicators rather than direct proxies for case counts, given the variability in fecal shedding across individuals. RSV vaccines are available for older adults and during pregnancy, and a monoclonal antibodies are available to protect infants and some young children from severe disease. A non-detect should not be interpreted as confirmation of no RSV transmission; non-detects may reflect multiple factors such as low prevalence or wastewater dilution.

Additional resources:

- CDC – National Wastewater Data for Respiratory Viruses
- Water Environmental Federation – Respiratory Syncytial Virus Information for Water Professionals
- WHO – Wastewater and Environmental Surveillance Summary for a Combined Suite of Respiratory Viruses

SARS-CoV-2 (COVID-19)

SARS-CoV-2 is an RNA virus transmitted primarily through respiratory droplets and aerosols. SARS-CoV-2 is endemic in the United States following the COVID-19 pandemic and continues to circulate year-round with seasonal variation. The virus evolves quickly, with new variants emerging regularly that may differ in transmissibility, clinical severity, and immune evasion.

Clinical presentation: Symptoms range from mild to severe and include fever, cough, shortness of breath, sore throat, congestion, loss of taste or smell, fatigue, body aches, headache, nausea, and diarrhea. Many infections are asymptomatic or mildly symptomatic. Severe disease and death occur most commonly in older adults and immunocompromised people, though population immunity through vaccination and prior infection has substantially reduced severe outcomes.

Incubation period: Typically, symptoms appear two to five days after exposure. Incubation periods vary by variant and have generally shortened with more recent variants.

Shedding: Shedding begins up to one week before symptom onset, peaks around the time of symptom onset, and typically continues for at least two weeks after symptoms resolve. Infectious viral shedding declines more rapidly than RNA detectability. SARS-CoV-2 RNA is shed abundantly in feces, making it one of the most reliably detected pathogens in wastewater surveillance. Fecal shedding is consistent across infected individuals regardless of symptom status, which makes wastewater a particularly effective tool for capturing both symptomatic and asymptomatic cases.

Wastewater detection: SARS-CoV-2 RNA is detected in wastewater through RT-PCR targeting conserved regions of the viral genome. The primary contributor to the wastewater signal is fecal shedding. Wastewater surveillance programs often monitor specific variants by targeting variant-defining mutations or through whole-genome sequencing of wastewater-derived RNA. Because many SARS-CoV-2 infections are asymptomatic or mild, wastewater surveillance may capture a substantially larger proportion of community SARS-CoV-2 infections than clinical reporting alone.

Federal case notifiability: COVID-19 is not routinely notifiable at the federal level. COVID-19-associated pediatric mortality is routinely notifiable through NNDSS. Participating laboratories report confirmed cases to RESP-Net and NREVSS.

Wastewater surveillance considerations: SARS-CoV-2 is the pathogen for which wastewater surveillance is most extensively validated. Because fecal shedding is high and consistent, wastewater signals correlate well with community transmission trends and a non-detect is a relatively reliable indicator that significant community circulation is not occurring. Given reduced clinical testing, case counts are no longer a reliable benchmark for calibrating wastewater signals. Wastewater genomic sequencing can detect emerging variants at the population level before they are well represented in clinical sequencing databases, providing advance warning of variant shifts.

Additional resources:

- CDC – National Wastewater Data for Respiratory Viruses
- WHO – Wastewater and Environmental Surveillance Summary for a Combined Suite of Respiratory Viruses

Hepatitis A (HAV)

HAV is a highly contagious RNA virus transmitted primarily through the fecal-oral route, which can be transmitted through close personal or sexual contact with an infected person, or through ingestion of contaminated food or water. HAV is considered a low-endemicity disease in the United States, with most infections occurring among adults with identified risk factors. People experiencing homelessness, men who have sex with men, and people who inject drugs are at highest risk.

Clinical presentation: Symptoms include dark urine, clay-colored stools, diarrhea, fatigue, fever, joint pain, loss of appetite, nausea, and jaundice. Many infections are asymptomatic or mild, particularly in young children. Illness typically resolves within two months, and most people recover fully without permanent liver damage. Severe disease, liver failure, and death are rare but occur most commonly in older adults and individuals with chronic liver disease.

Incubation period: Typically, symptoms appear 28 days from exposure, ranging from 15 to 50 days.

Shedding: HAV is shed primarily in feces. Shedding begins about two weeks before symptom onset and continues for about one week after symptoms appear, with the highest levels of shedding occurring before symptoms develop. This pre-symptomatic shedding window means wastewater signals are likely to precede clinical detection by one to two weeks.

Wastewater detection: HAV RNA is detected in wastewater through RT-PCR targeting HAV genetic sequences. Infected people consistently shed high levels of HAV in stool, resulting in readily detectable viral RNA in wastewater. Because many HAV infections are asymptomatic or mild, wastewater surveillance may capture a substantially larger proportion of community HAV burden than clinical reporting alone.

Federal case notifiability: Hepatitis A is routinely notifiable through NNDSS. Given the long incubation period and high rate of asymptomatic infection, clinical case counts are likely to substantially undercount true HAV burden in the community.

Wastewater surveillance considerations: HAV is one of the pathogens for which wastewater surveillance has the clearest advantage over clinical surveillance alone. The long incubation period, high rate of asymptomatic infection, and concentration of cases in populations with limited healthcare access all mean that clinical case counts are a lagging and incomplete indicator of community transmission. Wastewater signals can precede clinical detection by one to two weeks, providing meaningful lead time for public health response including vaccination campaigns and provider alerts. HAV vaccination is highly effective, and a confirmed or sustained wastewater signal in a jurisdiction with known high-risk populations should prompt consideration of vaccination outreach as a primary response tool. Wastewater data have been used successfully to trigger and geographically focus vaccination campaigns before clinical cases were widely identified.

Additional resources:

- WHO – Wastewater and Environmental Surveillance Summary for Hepatitis A and E Viruses

Norovirus

Norovirus is a highly contagious RNA virus and the leading cause of vomiting and diarrhea from acute gastroenteritis in the United States, accounting for over half of all foodborne illness outbreaks. Norovirus is endemic in the United States with a seasonal pattern, with cases peaking in winter months. Transmission occurs through the fecal-oral route, through consumption of contaminated food or water, direct contact with infected people, or contact with contaminated surfaces.

Clinical presentation: Symptoms include sudden onset of nausea, vomiting, diarrhea, and stomach cramps, sometimes accompanied by fever, headache, and body aches. Symptoms typically resolve within one to three days in otherwise healthy people. Norovirus can cause more severe and prolonged illness in older adults, young children, and immunocompromised people. Norovirus can occur year-round.

Incubation period: Typically, symptoms appear 12 to 48 hours from exposure.

Shedding: Norovirus is shed in high concentrations in feces and vomitus. Shedding can begin before symptom onset and may continue for two weeks or longer after recovery from illness. This post-symptomatic shedding window indicates that wastewater signals may remain elevated after clinical cases have resolved, and persistence of a wastewater signal does not necessarily indicate ongoing acute transmission.

Wastewater detection: Norovirus RNA is detected in wastewater through RT-PCR targeting genogroup-specific sequences (GI and GII). Shedding can be high, but shedding magnitude and duration vary across individuals. Both symptomatic and asymptomatic infections contribute to the wastewater signal, and because many norovirus infections do not result in healthcare visits, wastewater surveillance captures a substantially larger proportion of community burden than clinical reporting.

Federal case notifiability: Norovirus is not routinely notifiable at the individual case level. Health departments voluntarily report norovirus outbreaks to CDC through the National Outbreak Reporting System (NORS), while participating public health laboratories submit outbreak-associated genotype and basic epidemiologic data through CaliciNet. NoroSTAT combines timely NORS and CaliciNet reporting from participating states to support near-real-time outbreak surveillance. Separately, participating laboratories report testing data to NREVSS to track broader laboratory activity. Because individual cases are not reportable, outbreak-level reporting substantially undercounts true community burden.

Wastewater surveillance considerations: Norovirus circulates year-round and is often detectable in wastewater. Trend analysis is the primary interpretive tool. Programs should focus on identifying departures from expected seasonal patterns. Because norovirus has a short incubation period, longer moving average windows can obscure early increases in transmission. Wastewater surveillance may also show a longer tail at the end of a seasonal outbreak than clinical data. This extended signal could reflect a combination of prolonged post-symptomatic shedding and continued low-level transmission. These patterns can provide useful context for healthcare providers and local health departments. Norovirus circulates as multiple genogroups and genotypes, and the dominant circulating strain can shift over time.

Poliovirus

Poliovirus is a highly contagious RNA virus transmitted through the fecal-oral route. Due to high vaccination rates, poliovirus has been considered eliminated in the United States since 1979, and cases are rare and usually travel-related or associated with vaccine-derived virus. Despite elimination of wild-type disease, wastewater surveillance has emerged as a critical tool for detecting silent poliovirus circulation in under-vaccinated communities before paralytic cases occur.

Clinical presentation: About 75% of poliovirus infections produce no symptoms. About 25% cause mild flu-like symptoms lasting two to five days. In fewer than 1% of infections, the virus invades the nervous system and causes weakness or paralysis in the arms or legs, which can be permanent or fatal. The rarity of paralytic disease means that clinical surveillance misses the vast majority of poliovirus circulation.

Incubation period: For non-paralytic symptoms, the incubation period is typically three to six days from exposure. For paralysis, it is typically seven to 21 days.

Shedding: Poliovirus is shed in high concentrations in feces. Shedding begins within days of exposure, peaks approximately one week after exposure, and continues for four to six weeks. This prolonged shedding window — combined with the high proportion of asymptomatic infections — means wastewater surveillance can detect community circulation before clinical cases are identified, and signals may persist for weeks after an outbreak has been brought under control.

Wastewater detection: Poliovirus RNA is detected in wastewater using a pan-polio RT-PCR assay, typically performed on cell culture concentrates rather than directly from the wastewater matrix. For all positive results, a critical second step is sequencing to determine whether the detected virus is wild-type poliovirus (WPV), circulating vaccine-derived poliovirus (cVDPV), or RNA shed from the live attenuated oral polio vaccine (OPV). These distinctions have fundamentally different public health implications and must guide the response. Note that while OPV is not used in the United States, it remains in use internationally and may be detected in wastewater from recent travelers.

Federal case Notifiability: Poliovirus infection is immediately notifiable through NNDSS. Report suspected cases to public health authorities without waiting for laboratory confirmation.

Wastewater surveillance considerations: CDC does not recommend routine wastewater testing for poliovirus in jurisdictions with high vaccination coverage and no known outbreak. However, wastewater surveillance is strongly recommended as part of the response to any confirmed or suspected poliovirus case, and may be warranted in communities with documented low vaccination coverage even in the absence of a clinical case. Because poliovirus is non-endemic in the United States, any confirmed wild-type or cVDPV detection in wastewater warrants immediate investigation regardless of signal magnitude. Poliovirus wastewater testing also requires advance planning for containment. Laboratories, wastewater treatment plants, and other facilities that collect or handle samples that may contain poliovirus should follow applicable requirements for potentially infectious materials. Vaccination coverage data for the sewershed population provide essential context for interpreting wastewater signals. A non-detect for poliovirus should not be interpreted as confirmation of absence, particularly in large sewersheds.

Additional resources:

- CDC — Wastewater Testing Considerations
- Water Environment Federation — Revised Guidance on Wastewater Surveillance for Poliovirus
- WHO — Wastewater and Environmental Surveillance Summary for Poliovirus

Mpox

Mpox is a DNA virus transmitted primarily through direct contact with bodily fluids, skin lesions, or mucous membranes of an infected person, as well as through contact with contaminated materials such as bedding or clothing. Respiratory transmission can occur but requires prolonged close contact. Mpox virus is divided into two major clades: Clade I, which has historically been associated with more severe disease, and Clade II, which caused the 2022 global outbreak. Cases in the United States are most often travel-associated or linked to close-contact networks. Programs in several jurisdictions implemented mpox wastewater monitoring during the 2022 global outbreak.

Clinical presentation: Symptoms typically begin with fever, muscle aches, headache, fatigue, and swollen lymph nodes, followed by a characteristic rash with lesions that progresses through defined stages over two to four weeks. Flu-like prodromal symptoms typically precede the rash by one to five days. Severe disease can occur, particularly in immunocompromised people. Clade I and Clade II have different clinical presentations and epidemiological characteristics; Clade I is generally associated with more severe disease and higher mortality.

Incubation period: Symptoms appear between one and 21 days after exposure, typically between days seven and 14.

Shedding: Mpox DNA is shed in feces, in urine, and from skin lesions. Shedding begins before symptom onset and continues until about four weeks after symptom onset, with the highest levels of shedding occurring in the two weeks immediately following symptom onset. In addition to fecal and urinary shedding, lesion materials may significantly contribute to wastewater through bathing, wound care, and direct contact with surfaces.

Wastewater detection: Mpox DNA is detected in wastewater through PCR. Wastewater testing can be designed to be clade-specific or to detect mpox broadly without clade distinction. Programs should be aware of which clade their assay targets and what circulating strains are relevant in their jurisdiction. Clade distinction matters for public health response, with Clade I warranting a more urgent response than Clade II. Programs whose assays do not distinguish between clades should have a plan for follow-up sequencing when a signal is detected to determine which clade is circulating.

Federal case Notifiability: Mpox is immediately notifiable through NNDSS. Report suspected cases to public health authorities immediately without waiting for laboratory confirmation.

Wastewater surveillance considerations: Mpox is non-endemic in the United States, and any confirmed detection warrants immediate investigation regardless of signal magnitude. Wastewater surveillance for mpox is particularly valuable in jurisdictions where cases may be underreported due to stigma or limited healthcare access among affected populations. A vaccine has been available in the United States since 2019. Programs should interpret wastewater signals alongside current information on vaccination coverage in the relevant population. A non-detect should not be interpreted as confirmation of absence, particularly given that mpox transmission may be concentrated in specific social networks rather than distributed uniformly across the sewershed population.

Additional resources:

- CDC – Wastewater Data for Monkeypox
- Water Environment Federation – Mpox Information for Water Professionals
- WHO – Considerations for Wastewater and Environmental Surveillance for Monkeypox Virus: Interim Guidance

Candidozyma auris (C. auris)

Candidozyma auris — also known as *Candida auris* — is an emerging multidrug-resistant fungus that has spread rapidly across healthcare settings worldwide. *C. auris* is of significant public health concern due to its resistance to one or more classes of antifungal medications, its ability to persist on surfaces and spread within healthcare environments, and its high mortality rate among invasive and/or medically complex cases. Unlike the other pathogens in this section, *C. auris* transmission is primarily associated with healthcare settings pathogen rather than community transmission, which has implications for how wastewater surveillance is designed and interpreted.

Clinical presentation: *C. auris* can cause a range of invasive infections including bloodstream infections (candidemia), wound infections, ear infections, and urinary tract infections. Asymptomatic colonization is common and plays a significant role in transmission within healthcare settings. Colonized people can shed *C. auris* and serve as a reservoir for transmission to other patients, making detection of both colonization and infection relevant for healthcare infection control.

Colonization and infection timing: The time from exposure to detectable colonization ranges from days to weeks, and the time from colonization to active clinical infection ranges from days to months, though both times vary depending on the patient's immune status and clinical condition.

Shedding: *C. auris* can enter wastewater via contributions from colonized or infected individuals in contaminated healthcare environments. The shedding window and relative contributions of shedding sources (e.g., urine, feces, tissue) are not yet well characterized. More research is needed to determine the duration and consistency of shedding from colonized versus infected patients, and how shedding levels vary across individuals and clinical contexts. Programs should interpret wastewater signals in light of this uncertainty and avoid drawing precise quantitative conclusions from concentration data alone.

Wastewater detection: *C. auris* DNA is detected in wastewater through PCR targeting *C. auris*–specific sequences. Because it is a fungus, *C. auris* requires adapted laboratory protocols for DNA extraction from wastewater matrices. Detection methods for *C. auris* in wastewater are still evolving, and programs should verify that their laboratories are using validated methods.

Federal case notifiability: *C. auris* clinical cases and screening cases are routinely notifiable through NNDSS. Both types of cases should be reported to state or local health departments in accordance with jurisdictional requirements and CSTE/CDC case definitions and guidance.

Wastewater surveillance considerations: Because *C. auris* is primarily transmitted in healthcare settings, wastewater surveillance may be especially actionable when used at specific settings rather than applied at the community treatment plant level. Facility-level surveillance at hospitals, long-term care facilities, and other healthcare institutions can provide earlier warning of *C. auris* introduction or spread within a facility than clinical detection alone. Community-level treatment plant surveillance can still provide useful signals for detecting *C. auris* in jurisdictions where healthcare facility wastewater surveillance coverage is limited or where the organism may be circulating across multiple facilities within a sewershed. Programs detecting *C. auris* in wastewater should coordinate closely with healthcare infection control teams, as the response is primarily clinical and institutional rather than community-level. A non-detect at the community level should not be interpreted as confirmation of *C. auris* absence in local healthcare facilities, as the dilution effect of large sewersheds may mask signals from individual facilities with small numbers of colonized patients.

Additional resources:

- Water Environment Federation — *Candida auris* Information for Water Professionals
- CDC National Notifiable Diseases Surveillance System — Candida auris

6. Wastewater Surveillance Case Studies

Case Studies

This resource presents four case studies drawn from real public health programs across the United States, each illustrating how wastewater data have been used to support surveillance for a different pathogen in different epidemiological contexts. Together they demonstrate the range of ways wastewater signals can inform public health practice.

The table below summarizes each case study and its primary takeaway. Each study follows a consistent structure covering the wastewater data used, complementary data sources, spatial and temporal alignment considerations, data quality and processing decisions, and the public health actions taken or recommended. Readers can work through the studies sequentially for a comprehensive overview or navigate directly to the pathogen or scenario most relevant to their program using the table as a guide. Key concepts illustrated in each case study are cross-referenced to the relevant sections in this toolkit where more guidance can be found.

Case Study	Jurisdiction	Year	Primary Takeaway
Hepatitis A in North Carolina	North Carolina Department of Health and Human Services (NCDHHS)	2024	Predefined decision trees were a useful way to trigger staged community alerts and provider outreach
Poliovirus in New York	New York State Department of Health (DOH), in collaboration with local health departments	2022	A pre-existing surveillance network enabled rapid testing of an emerging pathogen; upstream sampling and retrospective testing of banked samples then revealed the true extent and timing of community circulation
Influenza and RSV in Wisconsin	Wisconsin Department of Health Services (DHS)	2022–2023	Meaningful correlations were observed between wastewater and clinical trends
Measles in Texas	Texas Department of State Health Services (DSHS) and City of Houston Health Department (HHD)	2025	Real-time sequencing was used to differentiate between distinct outbreaks and provide early warning in under-immunized areas

Hepatitis A in North Carolina

In 2024, NCDHHS detected hepatitis A virus (HAV) in wastewater from a local community over two weeks before the first clinical case was identified. The detection triggered a pre-defined public health decision tree that guided staged notifications to healthcare providers, local partners, and the community. This case study illustrates how a well-designed decision framework can translate wastewater signals into timely public health action — and how early wastewater detection can provide a meaningful lead time even when the eventual case count is small.

Wastewater data

NCDHHS monitored HAV in wastewater through a partnership with WastewaterSCAN, which processed samples and published results on a public-facing dashboard. Samples in this case were collected from a local wastewater treatment plant. Pathogen concentrations were normalized using pepper mild mottle virus (PMMoV). Results were made available on the public dashboard three to six days after sample collection.

Other data sources used

Following the third consecutive HAV detection, NCDHHS began monitoring syndromic surveillance through the Electronic Surveillance System for the Early Notification of Community-based Epidemics (ESSENCE) for corroborating signals in healthcare-seeking behavior. When a clinical case was subsequently identified, NCDHHS conducted standard case investigation including vaccination of family members.

Spatial alignment

Surveillance was conducted at the treatment plant level, generally covering a county. The cases ultimately identified were within the sewershed area, confirming spatial concordance between the wastewater signal and the clinical cases.

Temporal alignment

The predictive value of the wastewater data was demonstrated clearly in retrospective analysis. For Patient 1, HAV was detected in wastewater 12 days before symptom onset and 18 days before clinical identification, with concentrations peaking five days before symptoms appeared. For Patient 2, following a period of non-detection, HAV was detected again 12 days before symptom onset and 16 days before clinical identification, with concentrations peaking one day before symptoms.

Public health action

NCDHHS had developed a pathogen-specific public health decision tree in advance of any detection, including one for HAV. The framework uses consecutive wastewater detections as the trigger for escalating action, reflecting the understanding that a single detection may represent a false positive or a transient infected traveler.

Following the third consecutive detection, the local public health agency issued an alert to healthcare providers, emergency services, and surveillance partners. After the fourth consecutive detection, state and local partners held a coordinating call to evaluate the need for public notification and community vaccination efforts. When Patient 1 was subsequently hospitalized within the sewwershed area, the local health department initiated a routine case investigation and vaccinated the patient's family members.

After four non-detects, a second series of three consecutive HAV detections prompted notification of local health officials and the wastewater treatment plant. In response, the health department

distributed educational materials to congregate living facilities, long-term care facilities, and homeless shelters and launched a public awareness campaign on social media.

Key takeaway

The early warning provided by wastewater monitoring, detecting the virus up to 18 days before clinical identification, could be instrumental in containing a broader outbreak had the cases occurred in congregate care settings or among high-risk populations. This case study demonstrates the value of pre-defined decision frameworks that establish clear, pathogen-specific thresholds for action before a signal appears, enabling consistent and defensible responses without requiring real-time deliberation.

For guidance on building decision trees for your program, see Section 4, Translating Wastewater Signals into Public Health Action. For HAV pathogen-specific shedding and surveillance considerations, see Section 5, Pathogen-Specific Considerations.

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Poliovirus in New York

In July 2022, a case of vaccine-derived poliovirus type 2 (VDPV2) was confirmed in an unvaccinated adult in New York. This was the first case of paralytic polio in the United States in nearly a decade. New York DOH already operated an established wastewater surveillance network, therefore they were able to activate polio-specific testing within about a week of the index case being confirmed. What followed was a rapid, multi-layered surveillance response: testing at wastewater treatment plants to assess the scope of circulation, upstream sampling within the county where it was detected to localize transmission within the community, and testing of banked wastewater samples from across the state to determine how long the virus had likely been circulating before detection. Genetic sequencing was used throughout this effort to confirm the strain and trace its origin back to the index case. This case study illustrates how a pre-existing wastewater surveillance infrastructure can be rapidly repurposed for an emerging threat, how upstream sampling can localize an outbreak's true geographic extent, and how vaccination coverage data can directly shape both response strategy and public communication.

Wastewater data

At the time the index case was confirmed, New York State had been operating an established wastewater surveillance network. As part of its routine operations, the surveillance network also retained nucleic acid extracts from prior wastewater sampling events, originally collected for COVID-19 surveillance. This combination of existing infrastructure and banked samples allowed the state to begin testing wastewater treatment plant samples for poliovirus within about one week of confirmation, and later to test the already-banked extracted samples retrospectively.

Between March and October 2022, more than 1,000 samples were analyzed across wastewater treatment plant and upstream sites in the county where the index case was detected, and in surrounding counties, New York City, and Long Island, collected as 24-hour composites typically twice weekly. About 8% tested positive for vaccine-derived poliovirus type 2 (an infectious adaptation from the oral polio vaccine) across multiple sewersheds, with sustained detections in the index case county and two neighboring counties throughout the surveillance period. This indicated ongoing community transmission rather than isolated introductions.

Upstream sampling within the index case county, at sites such as manholes and lift stations closer to individual neighborhoods, was initiated to help determine whether transmission was localized or more widespread within the community. Setting up this upstream sampling network took about four weeks, considerably longer than the treatment-plant-level response; this reflected the additional logistical work needed to identify and access appropriate upstream sampling sites.

Retrospective testing of the banked samples helped determine how far back poliovirus circulation extended. Of 260 banked samples analyzed from the affected counties, 20 (collected between May and July 2022) were genetically linked to the index patient's stool samples, providing evidence that the virus had been circulating in the region weeks before the paralytic case was identified.

Further details on the methods and findings from this upstream sampling effort are described in Godinez et al. (2025).

Other data sources used

Vaccination coverage data from the New York State Immunization Information System (NYSIIS) was essential for interpreting the wastewater findings and directly shaped both the public health response strategy and public communication. As of August 2022, three-dose polio vaccination coverage among children aged 24 months and older in the initial county of detection was 60.3% — well below the threshold needed to prevent sustained transmission. In some zip codes, it was as low

as 37.3%. These vaccination data were used to identify and prioritize the specific communities most vulnerable to ongoing transmission, directing where vaccination outreach and clinic resources were deployed, and to inform the messaging used to communicate risk to those communities. In a follow-up investigation, Larsen et al. (2024) also integrated CDC's Social Vulnerability Index (SVI) to identify communities with heightened vulnerability to adverse outcomes, helping to prioritize geographic areas for response efforts.

Spatial alignment

To align population estimates with the 48 sewershed boundaries, researchers used an area-proportional interpolation approach using 2010 Census and 2018 American Community Survey data. The proportion of each census block overlapping with each sewershed boundary determined the population estimate, assuming uniform population distribution within blocks. The total population covered by the surveillance network was estimated at about 11.4 million.

Upstream sampling within the index case county provided a much finer level of spatial resolution than this treatment-plant-level estimate. Sampling upstream from the primary treatment plant, which covered a service population of roughly 201,000 people, broke this population down into much smaller sub-areas ranging from roughly 3,100 to 78,300 residents, enabling far more precise identification of where transmission was actually occurring within the county.

For more detail on spatial alignment approaches, see Section 2, Analyzing Wastewater Data.

Temporal alignment

DOH notified CDC of the confirmed case on July 18, 2022. Treatment-plant-level testing began within about one week, using 24-hour composite samples collected twice weekly. Upstream sampling took roughly four weeks to become operational. Retrospective genomic testing of banked samples later showed poliovirus had likely been circulating for several weeks before the index case was diagnosed, with linked detections as early as May 2022.

Only one paralytic case was ever identified during the outbreak, even as wastewater documented sustained detections across 10 sewersheds and three counties. This is expected for VDPV2: paralysis occurs in about 1 in 2,000 infections among unvaccinated adults, meaning the overwhelming majority of infections produce no symptoms and are never captured by clinical surveillance. Two independent modeling efforts estimated that about 10,000 infections likely occurred across the affected area, underscoring how much broader the true scope of transmission was than the single clinical case would suggest.

Data quality and processing

A notable methodological contribution of this case study was the use of surveillance sensitivity modeling to assess what a non-detect actually means. Larsen et al. (2024) estimated the probability that communities with no wastewater detections were truly free of poliovirus by modeling infection prevalence and viral copy detection relative to treatment plant flow rates. This approach estimates the freedom from disease and allows public health officials to quantify the negative predictive value of wastewater data — providing the empirical basis needed to stand down active surveillance and shift resources back to routine monitoring once the probability of true absence reached a predetermined confidence level. This is a model for how programs can use sensitivity analysis to make defensible decisions about when an outbreak is over.

Public health action

On July 22, 2022 — the day after the index case was confirmed — DOH launched a provider advisory to increase clinical awareness of polio, enhance surveillance for potentially infected people, expand

wastewater testing, evaluate vaccination coverage in the affected community, and supply inactivated polio vaccine to county immunization providers. Vaccination clinics were launched throughout the index case county.

As wastewater surveillance findings accumulated, the state immediately made the operational decision to deploy upstream sampling to better localize where transmission was occurring. However, setting up and sustaining this upstream response proved challenging in practice: staffing and funding constraints limited how long the upstream network could be maintained, and it was labor-intensive to coordinate data sharing across the agencies involved in the response.

Widespread wastewater detections genetically linked to the index case, in the county where it was initially detected and in five surrounding jurisdictions, prompted New York State Governor Kathy Hochul to declare a state disaster emergency on September 9, 2022. Enhanced case surveillance identified individuals meeting clinical criteria for polio in nearby counties, and additional outreach to local providers and syndromic surveillance monitoring began. Despite the broad response, the number of doses administered at temporary and established clinics was insufficient to meaningfully increase population-level vaccination coverage in the most vulnerable zip codes.

Key takeaway

The New York poliovirus case study demonstrates that wastewater surveillance can detect what clinical surveillance cannot. Only one paralytic polio case was identified, while wastewater revealed the polio virus circulating silently across 10 sewersheds and six jurisdictions. Upstream sampling was essential to understanding the true extent of this community spread: without it, the response would have been limited to the much coarser picture provided by treatment-plant-level data alone, and would not have been able to confirm that transmission extended beyond a single household or neighborhood.

Genomic sequencing was essential not just for confirming detections but for mapping the transmission network and linking community-level findings to the index case. The sensitivity modeling approach used to determine when non-detects were meaningful enough to end the emergency response is a model worth adopting for other high-consequence pathogens.

For poliovirus-specific shedding and surveillance considerations, see Section 5, Pathogen-Specific Considerations.

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Influenza and RSV in Wisconsin

During the 2022 to 2023 respiratory illness season, the Wisconsin Department of Health Services compared wastewater concentrations of influenza A and RSV against emergency department visit data across the state's three most populous cities. The study found that wastewater signals for both pathogens often appeared before corresponding rises in emergency department visits, though a consistent lead time could not be defined. This case study illustrates both the potential and limitations of wastewater surveillance for monitoring endemic seasonal respiratory pathogens.

Wastewater data

Between August 2022 and March 2023, wastewater samples were collected approximately weekly from around 40 treatment plants across Wisconsin and analyzed for influenza A and RSV. For the comparative analysis with clinical data, results were restricted to four treatment plants serving Green Bay, Madison, and Milwaukee. In all three cities, sewershed boundaries and patient residential zip codes could be reliably aligned. Wastewater concentrations were log-transformed for analysis and were considered alongside emergency department visit data.

Other data sources used

Syndromic surveillance data were drawn from the Electronic Surveillance System for Early Notification of Community-Based Epidemics (ESSENCE), which receives data from 129 of Wisconsin's 139 emergency departments (93%). Emergency department visit data were included only for patients with residential zip codes falling within the three study cities, enabling a direct spatial comparison with the wastewater data from the corresponding treatment plants.

Spatial alignment

Spatial alignment between the wastewater and clinical datasets was assessed by comparing sewershed service area boundaries against patient residential zip codes from ESSENCE. The four treatment plants serving Green Bay, Madison, and Milwaukee were selected because their sewershed boundaries aligned reasonably well with the urban zip codes from which emergency department data were drawn. This determination was made by visual inspection rather than formal geographic analysis.

Temporal alignment

Clinical and wastewater data were paired by MMWR week across the August 2022–March 2023 analysis period. No data latency concerns were discussed in the published report. The study did not collect data before the start of the respiratory illness season, which limited the ability to establish baseline concentrations and define lead times before the first wastewater detections of the season. The authors note this as a limitation and recommend that future efforts begin data collection before the respiratory illness season to better characterize the relationship between early wastewater signals and subsequent clinical trends.

Data quality and processing

Both clinical case counts and wastewater concentrations were summarized by week. Wastewater concentrations were log transformed, with a value of one gene copy per liter added to all measurements before transformation to accommodate samples with zero detected concentration.

Public health action

No specific public health actions were taken directly in response to the wastewater data in this study, which was retrospective in design.

Key takeaway

The Wisconsin study demonstrates a meaningful correlation between wastewater signals and clinical trends for two endemic respiratory pathogens, while being transparent about what the data cannot yet reliably do. The alignment between clinical and wastewater data showed that the systems are complementary and wastewater can provide insight into levels of virus in the community regardless of healthcare seeking behavior.

For more detail on spatial and temporal alignment approaches, see Section 2, Analyzing Wastewater Data. For RSV and influenza-specific shedding characteristics and wastewater surveillance considerations, see Section 5, Pathogen-Specific Considerations.

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Measles in Texas

In January 2025, Texas experienced its largest measles outbreak in decades, one that ultimately resulted in 762 confirmed cases statewide, 56 hospitalizations, and two deaths. Wastewater surveillance played a key early warning role in two unrelated but simultaneous events: a large outbreak centered in West Texas (driven by one measles genotype) and a separate cluster in Houston (linked to a different genotype connected to international travel). This case study illustrates how genomic sequencing of wastewater samples can distinguish between concurrent outbreak strains, provide early warning before clinical cases are confirmed, and in some instances offer the only available sequence data when clinical specimens cannot be successfully analyzed.

Wastewater data

Between January 2 and March 17, 2025, DSHS's wastewater monitoring program collected weekly wastewater samples from two municipalities in West Texas — referred to as City A and City B in the published reports. Reverse transcription-polymerase chain reaction (RT-PCR) testing detected measles RNA on January 13 in City A and on January 6 in City B, ahead of the first confirmed clinical cases on January 23. Genomic sequencing confirmed that all wastewater-derived sequences were identical to the D8 wild-type strain driving the West Texas outbreak, confirming that the wastewater signal reflected active community circulation rather than vaccine-derived shedding.

Five hundred and fifty miles away, HHD's wastewater surveillance program detected measles virus in two treatment plant samples in Houston collected on January 7, 2025 — 10 days before two clinical cases were confirmed on January 17. Genotyping, performed by the Texas Epidemic Public Health Institute (TEPHI), revealed these detections belonged to the B3 genotype, entirely distinct from the D8 strain in West Texas, indicating two independent introduction events occurring simultaneously. Case investigations also identified that these cases were travel related, confirming the genotype results. Because direct sequencing of the Houston patients' clinical specimens was unsuccessful, the wastewater data provided the only definitive evidence of which genotype was circulating in Houston.

Other data sources used

Clinical case data from DSHS provided the comparison point for evaluating wastewater lead time. By April 2025, City A had confirmed 30 measles cases while City B had confirmed none — despite both cities having positive wastewater detections. This difference highlights the complexity of interpreting wastewater signals at the city level and the importance of not drawing direct equivalences between detection and case burden across different sewershed areas.

Spatial alignment

The wastewater treatment plants serving City A and City B in West Texas covered estimated populations of 266,750 and 102,664 respectively, and the two Houston treatment plants collectively covered about 218,000 people. Published reports do not specify the methods used to derive these population estimates. For guidance on population estimation approaches for sewersheds, see Section 2, Analyzing Wastewater Data.

Temporal alignment

Wastewater surveillance provided meaningful lead time ahead of clinical detection in both the West Texas and Houston events. In West Texas, positive wastewater signals preceded the first confirmed clinical cases. In Houston, a wastewater sample that was positive for measles virus ribonucleic acid (RNA) was collected on January 7, preceding clinical case reporting on January 17 by 10 days. In West Texas, the outbreak was formally declared on January 30 following additional cases; on the other hand, the two Houston cases were travel-associated and investigated by HHD without a formal

outbreak declaration. The Houston researchers noted that samples were not processed in real time, and estimated that real-time analysis could reduce the reporting lag by two to three days — further narrowing the gap between environmental detection and public health action.

Data quality and processing

For the West Texas cities, PMMoV was used as an internal fecal indicator to normalize results, and each sample was tested in triplicate to validate detection and account for variability in viral concentration. Genomic sequencing confirmed that all wastewater-derived sequences matched the D8 wild-type outbreak strain, distinguishing them from the vaccine-derived measles RNA that can also be detected in wastewater following immunization.

Public health action

On January 17, 2025, HHD confirmed two measles cases in unvaccinated adults with recent international travel histories. The department conducted case investigation and contact tracing, published a list of locations visited by the cases, issued a health alert to regional medical providers, and released a media advisory to the public.

Following this investigation, subsequent wastewater samples from Houston tested negative for measles virus RNA. HHD referenced this sustained non-detect in a public news conference as supporting evidence that the broader West Texas outbreak, driven by a genetically distinct strain, had not spread to Houston.

Key takeaway

The Texas measles case study demonstrates the early warning value of wastewater surveillance for a non-endemic, high-consequence pathogen. Measles RNA was present in the wastewater up to 10 days before clinical confirmation in both West Texas and Houston. Also, it illustrates how genomic sequencing of wastewater samples can resolve epidemiological questions that clinical surveillance cannot. In Houston, the wastewater data provided the only available evidence of which measles genotype was circulating when clinical specimens could not be sequenced. The simultaneous detection of two genotypically distinct strains in different regions of the same state underscores the value of sequencing as a routine component of measles wastewater surveillance.

For guidance on response protocols for non-endemic pathogens, see Section 4, Translating Wastewater Signals into Public Health Action. For measles-specific shedding and surveillance considerations, see Section 5, Pathogen-Specific Considerations.

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