Coronavirus Disease 2019 (COVID-19)

Developing a Wastewater Surveillance Sampling Strategy

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Use this guidance to implement wastewater-based disease surveillance. Wastewater-based disease surveillance is a rapidly developing science, and CDC will continue to update guidance and information as it becomes available.

Sampling strategy overview

A COVID-19 wastewater surveillance sampling strategy should be driven by state, tribal, local, and territorial public health needs with strong engagement from wastewater treatment plants. Wastewater surveillance data are intended to complement other COVID-19 surveillance indicators that inform public health actions. No interventions or public health actions should be based solely on wastewater data. A sampling strategy should balance available resources and testing capacity with public health data needs, and it may need to be updated over time with changing scientific knowledge and public health needs.

What is a sewershed?

A **sewershed** is the community served by a wastewater collection system.

Developing a COVID-19 wastewater surveillance sampling strategy includes three steps:

1. **Identify public health data needs.** The public health data needs depend on the status of the local epidemic and other available health indicators. Based on the current state of the science, wastewater surveillance can be used to support the following response objectives:
    - **Detect the presence of COVID-19 within a sewershed**, potentially earlier than with established case surveillance. The virus has been detected in wastewater several days prior to reported cases within the community.

Knowing the virus is present in wastewater can be an important indicator when monitoring higher-risk communities with no known case patients. However, not detecting viral RNA in wastewater can never be used to rule out the presence of infections in a community.

- **Monitor trends in COVID-19 within a sewershed**, including both reported cases and unreported infections. Analyzed wastewater data can provide trends in SARS-CoV-2 concentration over time. Trends in viral concentrations in wastewater have been demonstrated to lead trends in new reported cases within a sewershed by days. Using wastewater surveillance as a leading indicator of COVID-19 trends may be useful when trends in the number of new case patients are fluctuating or for assessing possible impacts of community mitigation efforts.

SARS-CoV-2 RNA concentrations in wastewater cannot currently be used to determine the total number of infected persons in a community or the percent of the population that is infected. More data on SARS-CoV-2 concentrations in the feces of infected individuals are needed to be able to understand the relationship between SARS-CoV-2 RNA concentrations in wastewater and how many people in a sewershed are infected.

2. **Assess wastewater sampling and testing capacity.** When evaluating wastewater sampling and testing capacity, it is critical to include people with expertise in environmental microbiology and wastewater systems. Methods for accurately and precisely sampling and quantifying viral RNA in wastewater that are representative of viral shedding within a community are still under development. For this reason, there is limited testing capacity across the United States and much of that is housed in academic institutions that lack capacity for sustained surveillance. Effective use of limited wastewater testing capacity will require balancing population coverage and timely trend information that requires more frequent sampling. Supply chain issues can also limit testing capacity and should be evaluated prior to selecting a testing method.
3. **Develop a sampling plan**. Address the following questions in your sampling plan:
   - Where to sample?
   - How often to sample?
   - What to sample?
   - How to sample?
   - How to safely collect, store, and ship samples?

Where to sample

Community wastewater surveillance

Sampling wastewater for SARS-CoV-2 as it enters a treatment plant (referred to as untreated influent) is used to evaluate trends in infection within the community contributing water to the sewer system. Select the number of treatment plants for community-level wastewater disease surveillance based on the public health data needs in the region and availability of resources. COVID-19 rates and trends in the community, distribution of the population, and characteristics of the sewer system may also influence your selection.

Wastewater treatment plants may be selected for community wastewater surveillance to:

- Cover a certain percentage of the population.
- Provide data on communities at higher risk for COVID-19 or at increased risk for severe illness from COVID-19.
- Provide data on communities where timely COVID-19 clinical testing is underutilized or unavailable.
- Represent several sewersheds that serve a larger interconnected population, such as in dense urban areas.

Prior to selecting a wastewater treatment plant for community wastewater surveillance, it is critical to consult with wastewater engineers and utility managers to understand:

- Geographic area and population served by the utility
- Relative contribution of the types of waste inputs (industrial, commercial, residential)
- Operating factors that could influence the detection of SARS-CoV-2 (e.g., pre-treatment of incoming wastewater or diversion of wastewater to adjust flow upstream of the sampling site)
- Available sampling locations at the treatment plant
- Utility capacity for sample collection, documentation, and shipping
- Availability of utility meta-data needed for public health interpretation (e.g., influent flow measurements, chemical/physical water quality measurements, service area shapefile)

Targeted wastewater surveillance

Targeted wastewater surveillance entails sampling wastewater from upstream in the wastewater network (e.g., lift stations, interceptors, manholes). Targeted wastewater surveillance may provide a better understanding of how SARS-CoV-2 infections are distributed within a sewershed. However, there are currently little data demonstrating the application of this approach.

When deciding whether targeted wastewater surveillance would be useful for public health action, it is important to consider the following:

- SARS-CoV-2 RNA concentrations are more variable upstream from the wastewater treatment plant than at the plant intake because upstream wastewater has had less time to mix and contains feces from fewer people.
- Access to sewer lines serving only the intended target population may require infrastructure alterations or may not be possible.
- Depending on the size of the target population, conducting effective targeted wastewater surveillance may be more costly and logistically challenging than case surveillance.

How often to sample

Wastewater sampling frequency depends on how the data will be used for public health and the prevalence of COVID-19 in the community. With sufficient testing frequency, wastewater testing may be used to track trends over time. Single samples or very infrequent (e.g., monthly) sampling will likely not be informative for establishing trends, but could be used for establishing presence of COVID-19 in a community.

If the goal of wastewater surveillance is to screen for the presence of SARS-CoV-2 in wastewater, sampling once per week may be adequate. If the goal is early indication of infection trends, at least three sampling points are needed within a trend period of interest for surveillance. For example, if samples are collected once every seven days, 15 days is the minimum timespan over which a trend can be confirmed. There are little data available describing how rapidly wastewater concentrations may change under various epidemic scenarios.

Consider the following when determining sample frequency at a specific location:

- **A minimum of three samples is required to detect wastewater trends over time.** The time between consecutive wastewater samples determines the minimum length of time over which a trend may be detected. For example, if samples are collected once every seven days, 15 days is the minimum timespan over which a trend can be confirmed.
- Laboratory testing capacity and supply chain shortages may limit the maximum sampling frequency.
- One-time sampling will not provide actionable data beyond presence of SARS-CoV-2 infection within the sewershed.

What to sample

Sample type is an important consideration for collecting representative samples and will depend on the sample collection location and factors specific to the wastewater treatment plant. Closely consult with treatment plant staff to determine appropriate sample types that will best represent the target population. Samples should be collected at locations that precede addition of chemicals or mixing of waste streams at the wastewater treatment plant.

There are two wastewater surveillance sample types

1. **Untreated wastewater:** Untreated wastewater includes waste from household or building use (e.g., toilets, showers, sinks), which contains human fecal waste, as well as waste from non-household sources (e.g., rainwater, industrial use). Untreated wastewater may be sampled from wastewater treatment plant influent (prior to primary treatment) or upstream in the wastewater collection network. Changes in SARS-CoV-2 RNA concentrations in wastewater samples collected from wastewater treatment plant influent have been shown to correlate with trends in reported cases. In most cases, untreated wastewater will likely require concentration prior to RNA extraction. The number of infections needed to detect the virus in wastewater without concentration is difficult to determine because it depends on both the

method detection limit and the amount of virus in feces, for which there are few data.

2. **Primary sludge:** Primary sludge comprises suspended solids that settle out of wastewater during the first solids removal ("sedimentation") process at a wastewater treatment plant. Primary sludge is distinct from secondary sludge following primary treatment. Do not use secondary sludge for wastewater surveillance. Changes in SARS-CoV-2 RNA concentrations in primary sludge samples have been shown to correlate with trends in reported cases. An advantage of primary sludge samples compared to untreated wastewater is that SARS-CoV-2 concentrates in sludge, which reduces the sample volume required to detect the virus and may eliminate the need to concentrate the sample prior to quantification. However, the extent of SARS-CoV-2 RNA concentration in sludge is not well characterized. Sludge samples may also present challenges that must be evaluated for each wastewater treatment plant, such as chemicals added at the treatment plant, increased concentrations of compounds that can interfere with laboratory methods, or the addition of recycled waste streams from other parts of the treatment plant.

Selecting a sample type

Untreated wastewater and primary sludge are both acceptable community wastewater surveillance sample types. For upstream targeted wastewater surveillance, only untreated wastewater samples are available. If laboratory methods are available, sludge sampling is recommended to evaluate infection presence within a sewershed with few known case patients because the virus will be more concentrated in sludge. Untreated wastewater samples are recommended when wastewater treatment plants apply disinfectant before sludge can be sampled, sludge testing demonstrates high assay inhibition or poor virus recovery, or solids residence time within the primary clarifier is unknown.

How to sample

There are two sample collection methods for wastewater surveillance

1. **Grab:** Grab samples can be collected rapidly and do not require automated equipment. However, grab samples may be less representative of community fecal contributions than composite samples. For untreated wastewater and sludge, grab samples represent a single moment in time and are highly influenced by daily fluctuations in wastewater flow and composition. At the treatment plant level, grab samples may provide similar concentrations to composite samples if the proportion of the community that is infected is sufficiently high. However, at this time, the minimum proportion of the community that needs to be infected for grab and composite samples to be similar is unknown.
2. **Composite:** Composite samples are collected by pooling multiple grab samples at a specified frequency over a set time period – typically 24 hours for wastewater surveillance. You can collect composite samples of untreated wastewater manually or using automated samplers with refrigeration capacity that collect flow-weighted samples (e.g., one sub-sample per 200,000 gallons of flow). Continuous composite

samplers (versus flow-weighted) may improve how representative the sample is of the community contributing to the sewer. Composite samples are considered more representative of community fecal contributions than grab samples.

Selecting a sample volume

The volume of sample to collect will depend on the sample type (wastewater or sludge). A 1 liter (L) composite wastewater sample or 100 milliliter (ml) grab sludge sample volume should be adequate for testing. The maximum amount of sludge solids that may be directly extracted is typically around 2 grams. The remaining sample volume (if any) can be used for repeat measurement or to assess biological variability.

The volume of sample that is concentrated and quantified will determine the lowest amount of SARS-CoV-2 RNA that can be detected. Concentrating more than 1 L of wastewater may result in poor recovery or viral signal inhibition. If using grab samples, consult with wastewater treatment plant staff to collect representative samples that capture peak times of human fecal loading and to understand the solids residence time for sludge.

How to safely collect, store, and ship samples

Sampling safety: There is no evidence to date that anyone has become sick with COVID-19 because of exposure to wastewater. Standard practices associated with wastewater treatment plant operations should be sufficient to protect wastewater workers from SARS-CoV-2. These standard practices can include engineering and administrative controls, handwashing, specific safe work practices, and personal protective equipment normally required when handling untreated wastewater. Beyond CDC recommendations for how to protect against COVID-19, no additional COVID-19–specific protections are recommended for workers managing wastewater, including those at wastewater treatment facilities.

Storage: Never store samples at temperatures higher than refrigeration (4°C). Refrigerate samples during the collection process. If possible, process samples within 24 hours of collection, as effective actionable wastewater surveillance relies on rapid data collection. Remaining samples can be frozen at -70°C for archiving. Avoid more than one freeze-thaw cycle. Preliminary data have shown potential loss of signal following freezing.

Shipping: When sending samples to laboratories, CDC recommends packing samples with cold packs (4°C) and using same-day or overnight shipping. Package and ship samples as Category B infectious substance (UN 3373), in accordance with the U.S. Department of Transportation's Hazardous Materials Regulations and the International Air Transport Association Dangerous Goods Regulations.

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