Wastewater Surveillance Data Reporting & Analytics

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Implementing Wastewater Surveillance

Developing a Wastewater Surveillance Sampling Strategy

Testing Methods for Wastewater Surveillance

Data Reporting and Analytics for Wastewater Surveillance

Public Health Interpretation and Use of Wastewater Surveillance Data

Targeted Wastewater Surveillance at Facilities, Institutions, and Workplaces

Wastewater Surveillance in Low-Resource Waste Systems

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.

Data reporting for public health

A minimum set of data is required to interpret SARS-CoV-2 wastewater measurements for use in the COVID-19 response. These data are collected during multiple steps of the sample collection and testing processes.

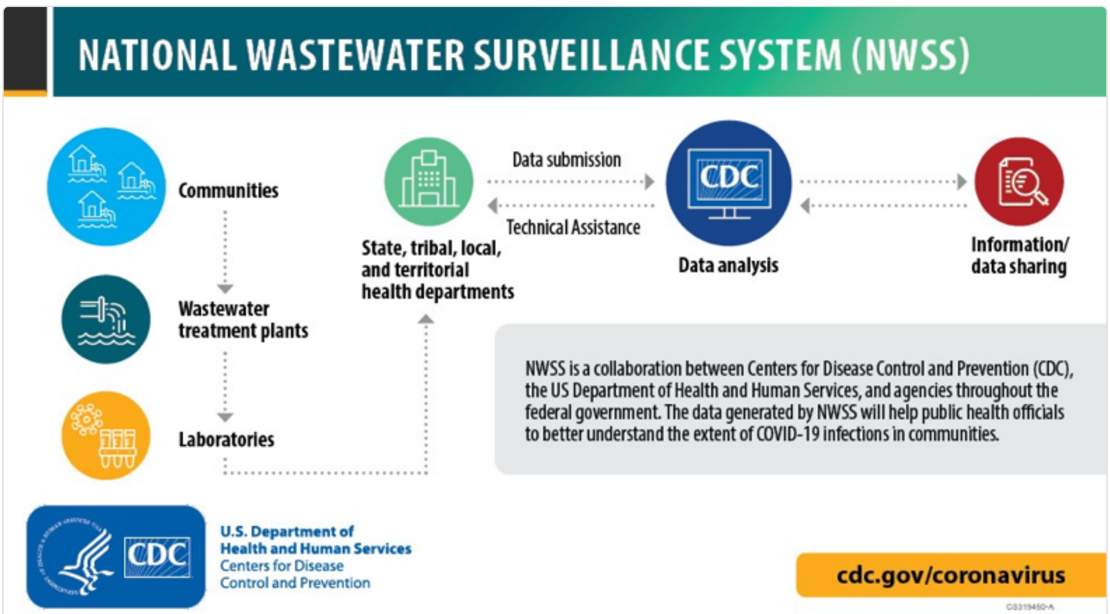
1. **Wastewater treatment plant:** Information on the wastewater treatment plant service area, number of people served by the utility, and treatment processes is needed to understand the wastewater source.
2. **Sampling:** The sample collection time, date, and location, as well as the sample type (grab or composite) and wastewater flow rate during sample collection are needed to understand sample collection conditions.
3. **Testing:** Information about sample concentration, extraction, and quantification

methods, as well as viral recovery efficiency and molecular inhibition measurements are needed to compare wastewater collected from multiple locations and analyzed by different testing laboratories.

Data submission to NWSS

To participate in the National Wastewater Surveillance System (NWSS), wastewater treatment plants and testing laboratories should coordinate with their state health department to assess their site's suitability for wastewater surveillance. NWSS partners must be able to collect the data needed for public health interpretation. Data are submitted to NWSS by state, tribal, local, or territorial (STLT) health departments using a standard collection instrument within the DCIPHER platform. CDC will analyze data reported to NWSS and return results to stakeholders through products such as state reports and dashboards for public health action.

CDC sewage surveillance data lifecycle



This flow chart shows how the NWSS system works. Wastewater from communities is collected by wastewater systems and transported to wastewater treatment plants. Participating utilities collect samples of untreated wastewater or primary sludge. These samples are sent to environmental laboratories for SARS-CoV-2 testing. The testing data, along with the associated utility metadata, is submitted to participating STLT health departments. Health departments submit these data to CDC through the NWSS DCIPHER portal. CDC analyzes the data in real time and reports results to the health department for use in their COVID-19 response.

Data analytics

To interpret SARS-CoV-2 wastewater measurements, polymerase chain reaction (PCR)-based measurements must be converted to sample concentrations and adjusted for testing and wastewater factors, which may change from sample to sample within a wastewater system, and between wastewater systems. Converting PCR measurements to wastewater concentrations must be done prior to submitting data to NWSS. Viral recovery and fecal normalization will be evaluated by the NWSS analytic engine as described below.

Sample concentration calculation

SARS-CoV-2 RNA is quantified using PCR technology, either reverse transcription quantitative PCR (RT-qPCR) or reverse transcription droplet digital PCR (RT-ddPCR). Laboratory staff should convert concentration estimates produced by PCR software (in units of copies per reaction or copies per reaction volume) to virus concentrations per volume of unconcentrated wastewater or sludge sample. This conversion accounts for the volume of template used in the PCR (and reverse transcriptase reaction if separate), the concentration factor of nucleic acid extraction, and sample concentration processes.

Presence

Presence of viral RNA in a wastewater sample is defined for RT-qPCR measurements as a signal that crosses the threshold at a cycle number <40 during the exponential phase of amplification. For RT-ddPCR measurements, presence is defined as three or more positive droplets. If multiple assays or multiple PCR replicates are run on a sample, the virus is considered present in the sample if there is detection in any one of the assays or replicates. Viral recovery and the amount of sample processed determine the lowest detectable quantity of virus in a sample.

Matrix recovery

A matrix recovery control (also called a process control) is a non-SARS-CoV-2 virus spiked into a wastewater sample at a known concentration prior to processing. This control is used to understand viral recovery, defined as the amount of virus lost during sample processing, and is important for comparing SARS-CoV-2 concentrations in wastewater over time. Viral recovery estimates can be incorporated into SARS-CoV-2 wastewater data by dividing the measured concentration of SARS-CoV-2 by the fraction of matrix recovery control recovered. The fraction of matrix recovery control recovered is the amount of non-SARS-CoV-2 virus measured after processing divided by the amount of non-SARS-CoV-2 virus spiked into the sample before processing.

Normalization

To compare viral wastewater concentrations over time, normalize estimated viral concentrations by daily wastewater flow to account for changes in wastewater contributions. This normalization provides data in units of viral gene copies per day. To compare viral levels across sampling locations, also normalize viral concentrations by the number of people served by the sewer system, resulting in units of viral gene copies per person contributing to the sewershed per day.

If the number of people contributing to the sewershed is expected to change over the surveillance period (due to tourism, weekday commuters, temporary workers, etc.), human fecal normalization may be important for interpreting SARS-CoV-2 concentrations and comparing concentrations between sewage samples over time. Human fecal normalization targets are organisms or compounds specific to human feces that can be measured in wastewater to estimate its human fecal content. While there is no consensus method, you can normalize by human fecal content by dividing non-normalized wastewater concentrations by the human marker concentrations, resulting in a unitless ratio. This ratio may also account for viral losses in the sewage system and viral recovery through laboratory processes.

Surveillance analytics

Trends

Wastewater trend classification is the statistical analysis of changes in the normalized concentration of SARS-CoV-2 in wastewater (i.e. not by qualitative visual assessment). Trends in these wastewater data can be used to assess COVID-19 trends (reported and unreported) within the community contributing to the sewer system. Trends of SARS-CoV-2 levels in wastewater cannot be determined from fewer than three sample points (e.g., consistent weekly sampling requires 15 days of data to estimate trends). You can classify trends into categories based on the duration and direction of change in virus levels for interpretation and public health use.

Trend calculation: The distribution of SARS-CoV-2 concentrations in wastewater is important to consider when calculating trends in virus levels. Normalize concentrations prior to calculating trends to account for changes in wastewater dilution and differences in relative human waste input over time.

- Trends can be calculated using linear regression with a minimum of three measurements, where the slope describes the trend.
- The independent variable in the trend regression should be date, not measurement number, to estimate changes per day rather than per measurement.
- As SARS-CoV-2 concentrations in wastewater are likely log-normally distributed, log-transform SARS-CoV-2 normalized concentrations prior to computing trends and other statistics.
- For trends that are calculated using log10-transformed concentrations, compute the percent daily change (PDC) in virus levels from the slope as: $PDC = (10^{slope}-1) \times 100$.
- Include wastewater samples with SARS-CoV-2 levels below the limit of detection in trend calculations. This can be done by assigning the sample a value of half the assay detection limit.

Measurement variability: For more precise evaluation of wastewater data, trend calculations can incorporate the variability in each SARS-CoV-2 measurement through statistical weighting using weighted least squares regressions, which can take into account variability in the sampling, processing, and quantification steps.

Trend classification: Trends may be broadly classified by duration—short-term or sustained—and direction—increase, decrease, or plateau.

- **Duration:** Trend classification schemes are dependent on sampling frequency. For example, short-term SARS-CoV-2 wastewater trends could be defined as trends spanning less than two weeks, and sustained trends could then be defined as trends spanning two weeks or longer. Based on a twice-weekly wastewater sampling frequency, short-term trends could then be calculated from three samples collected over an eight-day timespan, and sustained trends from the five samples collected over a 15-day timespan.

- **Direction:** You can classify normalized SARS-CoV-2 concentration trends into 'increasing', 'decreasing', or 'plateau' by testing trends for statistical significance. Statistical significance indicates that an increasing or decreasing trend exists, accounting for the variability in the SARS-CoV-2 data. You can also use a minimum percent daily change threshold in conjunction with statistical significance to assign trend direction.

Infection estimates

At this time, point estimates of community infection based on wastewater measurements should not be used. Such estimates depend strongly on clinical data describing the concentration of SARS-CoV-2 in feces over the course of infection and in individuals with varying levels of disease severity and few such clinical data are currently available. As more clinical data become available, using wastewater SARS-CoV-2 data to estimate the total levels of COVID-19 (i.e., symptomatic, asymptomatic, pre-symptomatic) in a community could be a useful application of wastewater surveillance.

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