Developing Wastewater Sampling Plans to Monitor Public Health

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The choice of where, when, and how to collect wastewater samples should be based on the goals and purpose of the monitoring system you use. The choice of where to sample is critical because it determines the population represented and captured by the wastewater measurement as well as the sensitivity of the assay (the laboratory test that measures the amount of a specific substance), in terms of number of people who need to be infected to observe a positive signal. Although you can collect samples from anywhere in the sewer system, in practice, samples must be collected at locations where you can access wastewater safely and reliably, such as wastewater treatment plants, lift stations, or manholes. In addition, the frequency and type of sample collected will affect the resolution and quality of information generated from the wastewater system. This brief highlights the following key considerations when developing a wastewater sampling program: (1) Designing a sampling plan - where and when to collect wastewater; (2) Sample collection types; (3) Adaptive sampling; and (4) Data quality and variability.

Designing a sampling plan

Wastewater samples should be collected in a manner that aligns the resulting information with the public health goals of the monitoring program. This will depend on aspects of the public health goals, including the size of the community you want to monitor, the financial resources available for performing the sampling and acting on the results, and the layout of the community's sewage system. For example, if you want early detection of viral outbreaks at a specific school or nursing home facility, then you would have to sample that facility daily because doing so aligns with the goal of catching outbreaks as soon as possible at a specific facility. But if your goal is to understand the general trends of the virus in a region in which you are sampling multiple wastewater treatment plants within that municipality, then a weekly sampling plan at the geographically dispersed treatment plant level is suitable because it aligns with the goal of monitoring larger

Sample collection types

Sample type	Examples	Advantages	Disadvantages
Grab	Placing a 500mL collection bottle in wastewater and filling it.	· Low cost · Minimal equipment required · No external power or batteries required	· Diurnal variability affects sensitivity and representativeness · Less quantitative than composite samples
Passive	Suspending a Moore Swab, medical gauze on a string, for 24 hours in a pipe carrying wastewater. Once the Moore Swab is retrieved, collecting liquid that is squeezed out.	· Low cost · Minimal equipment required · No external power or batteries required	· Performance depends on material, sewage characteristics, and time deployed · Less sensitive and quantitative than composite samples
Composite	A battery powered, automated sampler's hose sits in a manhole and is automatically collecting 15 mL of wastewater every 15 minutes for 8 hours.	· More representative because each sample comprises aliquots collected at defined flow or time intervals. · Representative samples enable sensitive and quantitative measurements of disease targets in wastewater samples.	· Autosamplers are expensive, require external power, and require maintenance

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community samples for an entire region. Another way to think of the sampling frequency is that large systems will change more slowly because they support more people, so you don't have to sample the treatment plants daily for you to understand the trends for a region.

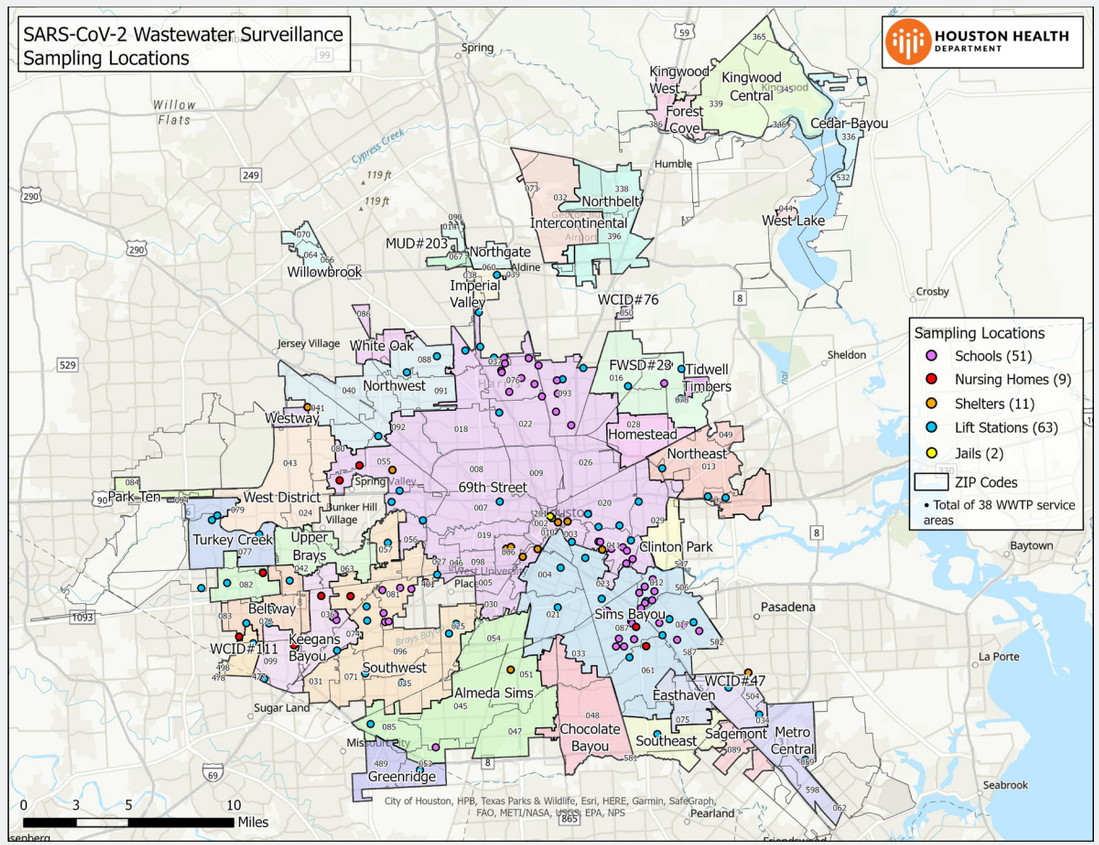
Sampling at the community level

Consider establishing a wastewater monitoring system for a city, municipality, county, or other communities served by multiple wastewater treatment plants with the two-pronged goal of developing regional trends and identifying hot spots of increased viral activity. A helpful first step is to collaborate with the wastewater utility entity (or entities) for the region and to map the sewage system. See Figure 1 for an example of mapping the wastewater treatment plants. Working with utility partners is crucial because they have the technical expertise to understand the abilities and limitations of the sewage system in your region and will typically be the team performing the physical sample collecting. Through your collaboration with the utility partner, you can decide the type of sample and the collection frequency. At the community level, a common sample design is a 24-hour composite measurement collected at wastewater treatment plants at least once a week. For sample site location, it is common to sample at all wastewater treatment plants in the region. At this point, decide what your wastewater monitoring goal is and how you will adjust a sampling plan to create a sustainable sampling plan for your program and its financial resources. There are many ways to iterate on the sampling plans that will still allow for high-quality data. The following are insights into selecting sites and sampling frequency:

Site selections

- Selecting sites based on largest population size will allow you to monitor the largest number of people in the region but will not provide insights into hot spot regions because the geographic area is too large. For example, if a region can only monitor one site, select the largest site to maximize the area monitored.
- Review the population covered by each wastewater treatment plant and decide whether any sites contain certain populations that align with your program's goals. Sampling sites that serve vulnerable communities and those without access to the health care can guide resources to help these communities. For example, if you are interested in monitoring areas with low testing rates, review the testing rates of the population served by each wastewater treatment plant and select accordingly.
- Understand the geographical makeup of the wastewater treatment plant so you can better decide on which sites to monitor. For example, if a site is in a remote location, consider incorporating extra resources to ensure the sample can be transported to the lab properly.
- Consider selecting lift stations, or other intermediary sites that wastewater travels through as it makes its way to a wastewater treatment plant, to obtain a more geographically refined approach. The sites allow for more granularity in geographic wastewater viral trends, but they often do not have the same resources available at wastewater treatment plants. For example, flow rate from wastewater treatment plants is easy to obtain and can be used by the data analysis team for normalization. Flow rate might not be as easy to obtain at sites such as lift stations, so they must be estimated based on population size.

Figure 1. Map of sampling locations in Houston, Texas



Note: The Houston Health Department coordinated with the utility partner, Houston Public Works, to create the map of the wastewater treatment plants, lift stations, and facility sampling locations.

Sampling frequency

- Pick an acceptable data timeliness for your program and select sample frequency based on that. For example, the utility partner is able to sample three times a week, but the laboratories can only handle two samples per week. If the sample frequency abilities of different parts of the program do not match, then data timeliness will be an issue.
- Decide how important flexibility and consistency are to the program. For example, if the data analysis team compares the samples between weeks, collect the wastewater samples on the same day each week to maintain consistency.
- Consider time between samples and the corresponding days of the week. If sampling more than twice a week, it is common to allow at least one day between sample collection dates to gain a larger temporal scale. For example, if it is important to the goal of the program that you monitor the weekend trends and weekday trends in a region, sample on Saturdays and Wednesdays.

Sampling at the institution level

It is also important to consider monitoring at specific facilities, such as congregate living facilities, college dorms, schools, jails, or large community working environments. Generally, you will collect composite samples at manholes with flow coming from only the facility intended for monitoring. Unlike wastewater treatment plants, the facility composite samples can cover shorter time frames, overlapping with when the facility population commonly uses the restroom. For example, an elementary school only needs an eight-hour composite sample for the hours that schools are in session. The facilities selected for monitoring are commonly in areas of high viral load for the community or when the population is at high risk of poor health outcomes. See Figure 2 for an example of selecting school sites with school populations that draw from a ZIP code with high COVID-19 burden. There are many aspects you can tailor facility wastewater monitoring to, so review what aligns with your program's goal and financial resources.

Site elections

- Physically inspect the manholes associated with the site for sampling viability. The manhole will need enough flow for sample collection, to flow from the human waste (for example, from restrooms and not the kitchen sinks), and to be physically accessible for the team to safely sample.
- Consider what sites are associated with outbreaks in your region and the importance to your program of capturing those outbreaks. For example, if tourists and travelers are the first to bring the new variants to your region, select to sample at the airport.
- Collaborate with epidemiologists and public health outreach teams to identify facilities of vulnerable populations—for example, a large shelter has communal living instead of individual rooms and caters to people older than age 60.
- Consider deploying passive samplers to identify outbreaks in facilities where symptoms exist, but clinical sampling does not occur. On these types of deployments, it is important that the sampler is deployed in such a manner that it does not cause a clog in the pipe.

An environmental investigator from the Houston Health Department collects a sample from a manhole site in Houston, Texas.

Sampling frequency

- If the facility is in a highly affected area and interventions are underway, consider sampling multiple times a week because it is beneficial to catch changing environments early. Further outbreaks at specific locations might be an early indicator of outbreaks in the larger community.
- If the facility is part of a larger system (for example, it contributes to a wastewater treatment plan that is monitored), then weekly measurements provide a strong indicator of viral levels within each relevant subpopulation.
- If the decisions on daily testing of people in the facility are made based on the wastewater monitoring, then consider the importance of sampling daily. For example, the students in a college dorm will go to university testing sites based on the wastewater results.

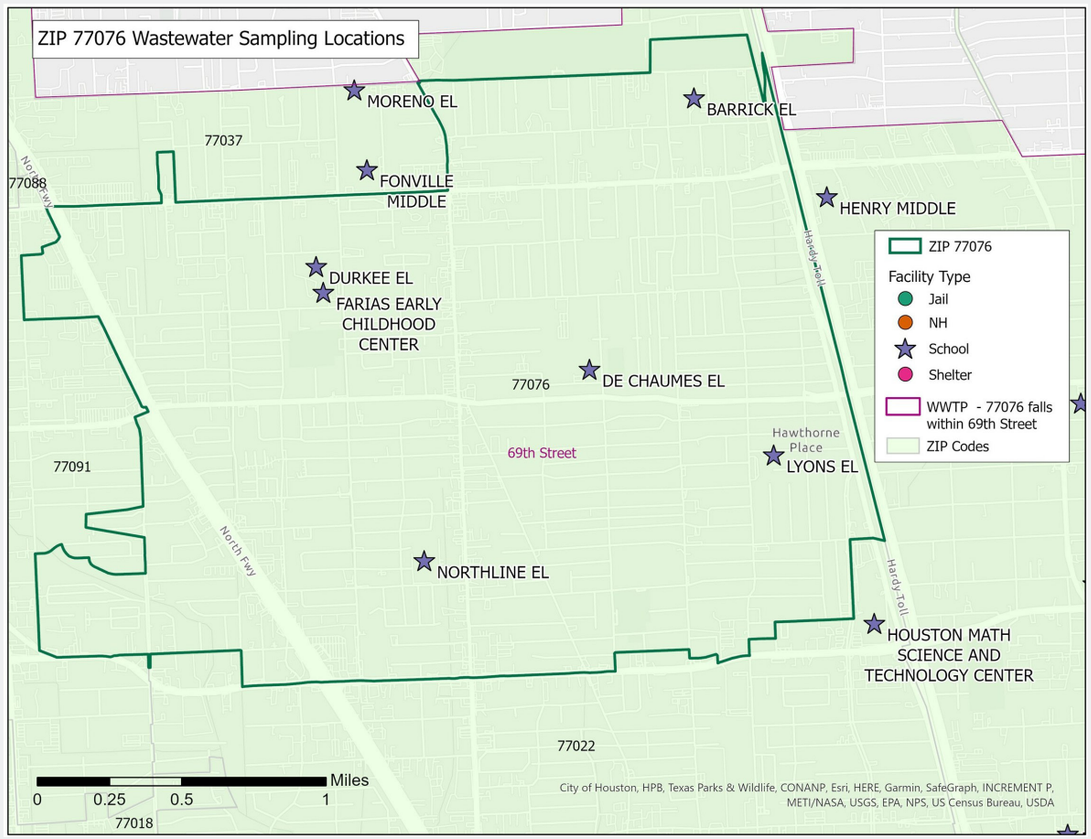
Adaptive sampling

Adaptive sampling provides the opportunity to optimize resources and maximize the information gleaned from the wastewater monitoring system. Take the last example of intense sampling for an institution, such as a university. Some universities rotated the locations of the limited number of autosamplers they had to cover all dorms within a week. This strategy kept them within budget and provided the necessary information to manage outbreaks at the university. For regional sampling, after you have established baselines, you can sample areas with low populations and low active virus levels less frequently and still maintain the integrity of the regional sampling program. Similarly, as virus levels trail off, less frequent sampling (for example, monthly) of individual facilities will still provide the opportunity to identify re-emergence of the disease at specific locations. Adaptive sample designs can be informed by other information regularly monitored by health departments, such as the community case counts, emergency room visits, or number of prescriptions issued. Adaptive sample designs can be simple, like deciding to decrease sampling frequency at all sites, or can entail more complicated models that optimize sampling frequency and location.

Data quality and variability

The are many ways to sample and quantify virus levels within wastewater, and the scientific and engineering community is quickly advancing this important technology. The current gold standard sample is a 24-hour flow-weighted composite sample from an autosampler, and the viable but inexpensive alternatives are grab, Moore swab, and tampon sample collection. For each sample collection method, there will be multiple sources of variability in the measurements that are independent of the viral load. For example, the flow at a wastewater treatment plant changes each day and hour depending on who contributes to the sewage system that day. This can lead to fluctuations in reported viral concentrations in the wastewater sample. For lower cost sampling approaches, such as grab samples, one would expect the variation from sampling to be higher because the variability of the snapshot of the sewage use will be higher

Figure 2. A map of ZIP code 77076 in Houston, Texas, and all nearby public schools with viable manholes for school-level wastewater monitoring



Notes: A goal of the wastewater monitoring program in Houston, Texas is to focus public health monitoring initiatives in ZIP codes with higher COVID-19 burden. As such, all schools with viable manholes for wastewater monitoring were selected to be part of the Houston wastewater monitoring program.

for a grab sample (one moment in time) than a composite sample (15-minute intervals over a 24-hour period). Variability might also be introduced from to the nature of sewage systems, which can be unique across municipalities. For example, gray water additions and industrial inputs into a sewage system are different in different areas, and both can contribute to the variability of wastewater data.

Statistical methodologies estimating the virus level can overcome the inherent sampling and measurement error. See Figure 3 for an example of the variability seen in weekly 24-hour flow-weighted composite sample results at a sewershed in Houston, Texas and the statistical trend model fit to these results. If you are regularly sampling your wastewater system, the viral load trends will be apparent, but it is important to investigate the source of this change and not immediately assume that it indicates a real change in virus levels. To illustrate, a wastewater treatment plant in Houston went through a three-week process of purposefully re-processing the wastewater flowing through the plant. This in turn resulted in dramatically reduced virus levels in the weekly sample, falling well outside of what was expected for the wastewater treatment plant. Because of the sampling frequency and sampling locations of this regional wastewater monitoring system, the dramatic change was identified as a process change and not a change in the virus level for the community sampled, so the samples from that time could not be used.

Conclusion

A wastewater sampling plan for public health use can be designed in numerous ways that differ in the number of sites sampled, sample collection method, or sampling frequency. This flexibility allows wastewater monitoring to be a practical tool for public health monitoring at the local level (facility or community) up to the state level. As more places are looking to implement wastewater monitoring, it is important for them to consider the breadth of sampling schema available and to tailor them to what fits for their community and its goals.

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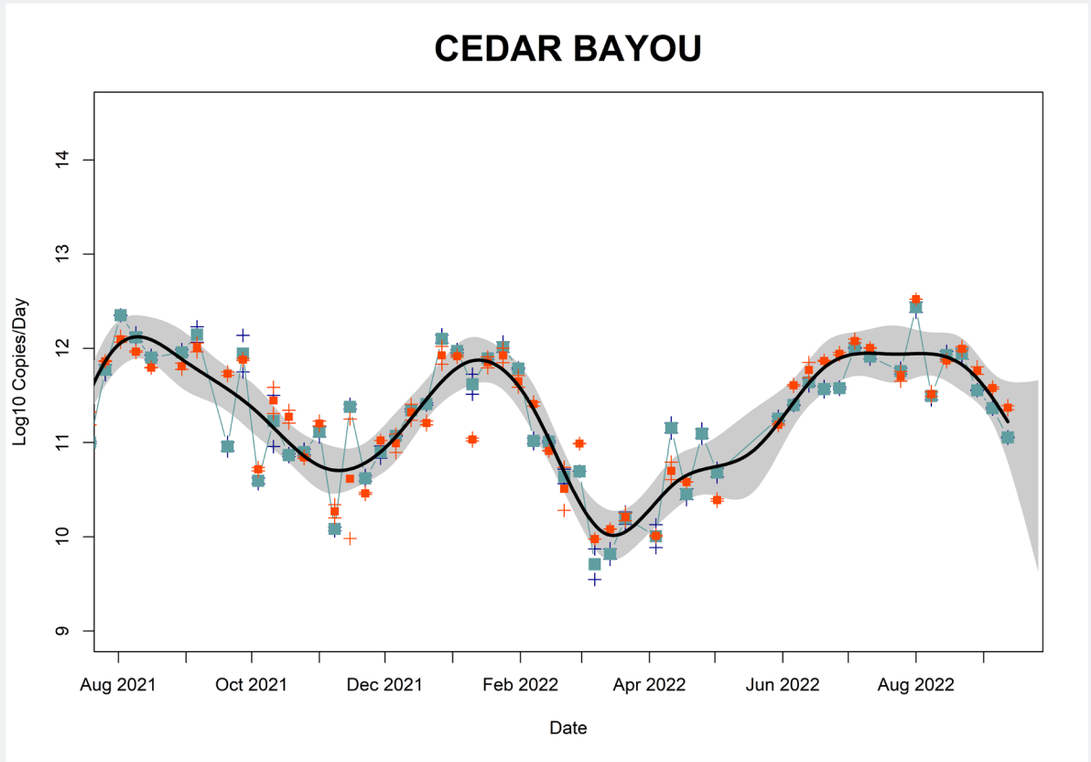
Figure 3. Measurements and trend from one sewershed in Houston, Texas



Notes: Teal and orange points represent aggregate measurements (in copies per day) from two different labs.