



What is wastewater-based epidemiology?

Wastewater-Based Epidemiology (WBE) or wastewater monitoring of COVID-19 is a rapidly evolving area of research that holds great promise as an unbiased leading indicator of the presence of COVID-19. Clinical diagnosis is a lagging indicator of the level of SARS-CoV-2 within a community and does not fully account for asymptomatic or pre-symptomatic cases, which constitute a significant proportion of any given infected population.

Evidence suggests that 40-70% of people start shedding the virus in their stool five to seven days before exhibiting symptoms, most frequently immediately after contracting COVID-19, and that infected individuals shed SARS-CoV-2 in their stool regardless of exhibiting symptoms.

WBE has been used reliably for decades to detect polio in countries where the disease remains endemic and more recently to monitor opioid use levels. Current methods can reliably detect increases in virus, however decreases are more difficult to track because of the persistence of virus shedding after a person recovers.

What is Burlington's SARS-CoV2 Wastewater Monitoring Program?

Since August of 2020, the City of Burlington has been implementing SARS-CoV-2 Wastewater Monitoring Program as an effort to help mitigate the spread of COVID-19. The technology is used to analyze wastewater samples to look for the presence of SARS-CoV-2 RNA gene copies/fragments. The initiative is a collaboration between the City of Burlington, the Vermont Department of Health (VDH), New York based GoAigua Inc., Colorado based GT Molecular LLC, and participating research partners at Dartmouth Hitchcock Medical Center and the University of Vermont.

Wastewater entering treatment plants is sampled for fragments of the non-infectious RNA (ribonucleic acid). The wastewater flows from homes and businesses into the City's three wastewater treatment plants. A wastewater sample (24-hour composite) is then collected in an area where all the sewage from a service area enters the plant. This sample is analyzed by a laboratory to determine the number of virus gene copies present, related to the wastewater flow that occurred on the sample day and the population that contributed to the flow.

What are the objectives of Burlington's Wastewater Monitoring Program?

1. Provide an additional dataset on City-level total infection trends over time; and
2. Serve as a leading indicator of potential infection increases used to inform decision making by the Vermont Department of Health, City of Burlington Emergency Operations Center, and other community stakeholders.

Where is the City Monitoring?

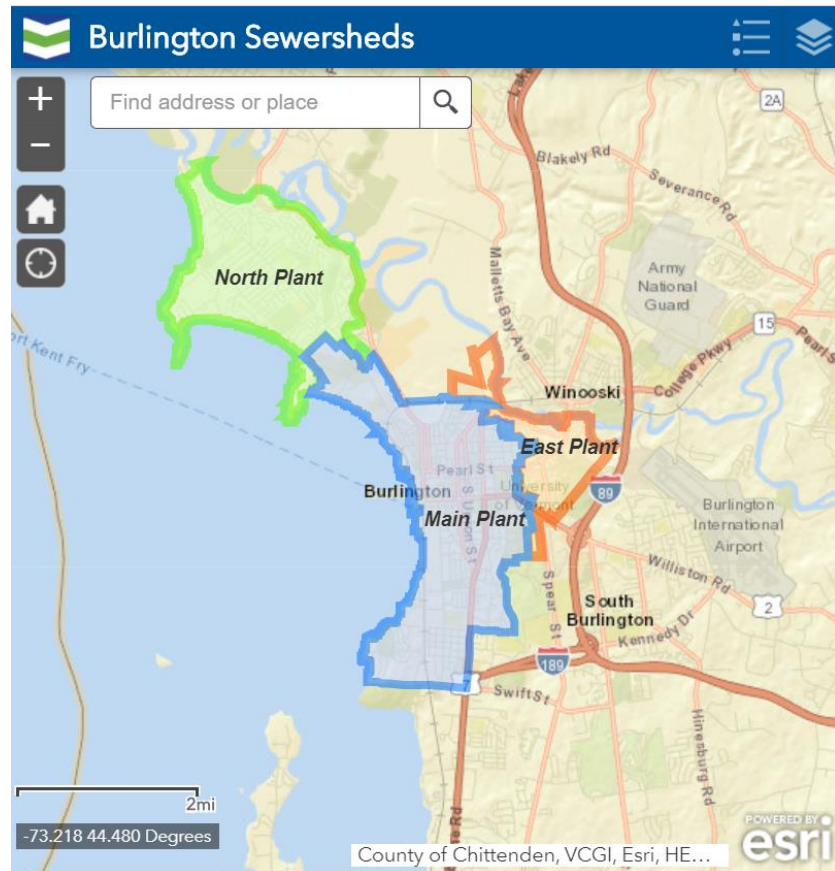
Currently, the City is sampling wastewater three times per week from each of the City's three wastewater treatment plants in order to report RNA levels at the sewershed level. A sewershed is an



Burlington's SARS-CoV2 Wastewater Monitoring Program

FREQUENTLY ASKED QUESTIONS

area of land where the raw sewage from homes, businesses, and industries flows through a series of sewer pipes to a single downstream point, where it enters a wastewater treatment plant. Samples of the raw wastewater are being collected and monitored for the virus's RNA before going through treatment.



Is it safe?

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. Before being discharged from the treatment plants, wastewater is treated and disinfected with hypochlorite (bleach) to remove viruses and bacteria and is monitored to meet all state and federal discharge limits. Additionally, our drinking water is filtered and also disinfected prior to being pumped out to our taps.

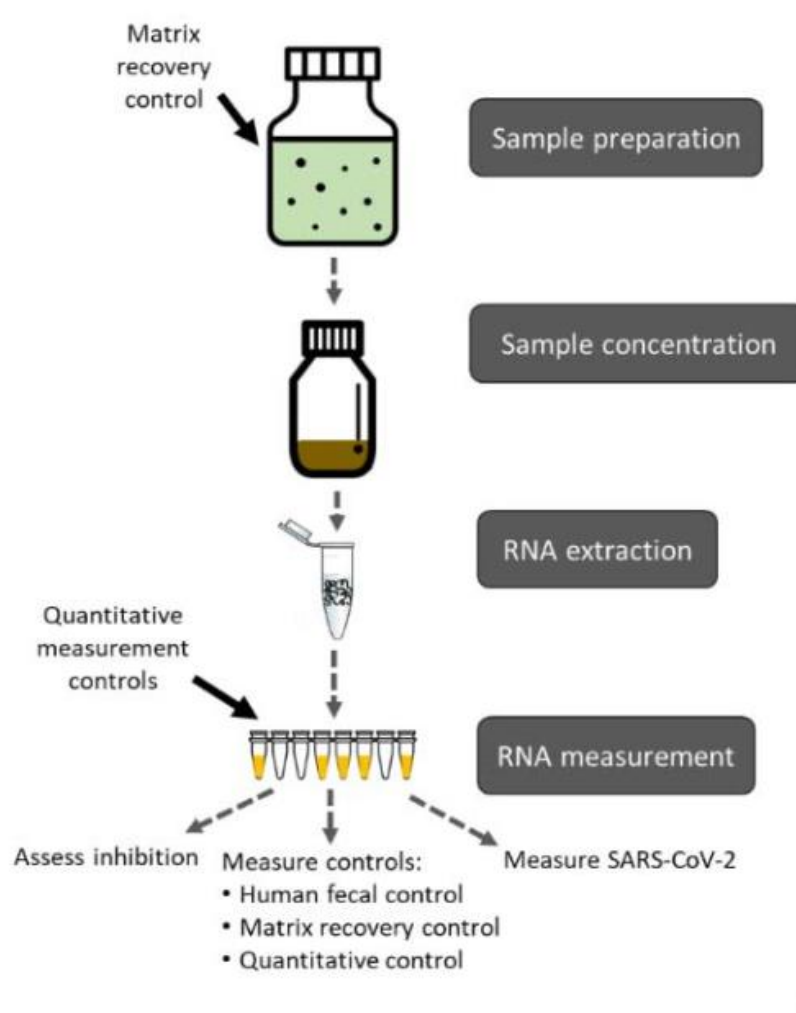
How are samples analyzed?

Wastewater samples are analyzed 3 times per week by GT Molecular, a Fort Collins based laboratory that has extensive experience performing wastewater testing in the United States. GT Molecular in collaboration with Colorado State University, developed and deployed the state of Colorado's state-wide surveillance program, comprising twice weekly testing at 22 wastewater treatment plants. GT Molecular also provides this service to a growing list of other municipalities and facilities.



Burlington's SARS-CoV2 Wastewater Monitoring Program

FREQUENTLY ASKED QUESTIONS



Overview of wastewater sample processing and testing for SARS-CoV-2:

After sample collection, the first step in SARS-CoV-2 wastewater testing is sample preparation. A matrix recovery control should be spiked into the sample during this step. The second step is sample concentration. The third step is RNA extraction from the concentrated wastewater sample. The final step is RNA measurement. Along with measurement of SARS-CoV-2 RNA in this step, several laboratory controls should also be measured, including matrix recovery controls, human fecal normalization, quantitative measurement controls, and controls to assess molecular method inhibition.

How can results be interpreted?

There are several factors to consider when interpreting viral data in wastewater. Because researchers are still learning about the timing and rate of shedding of the virus RNA in feces of infected people (and not all people appear to shed viral RNA in their stool), it is most appropriate to monitor and observe the trends of viral gene copies detected in a community over time. In Burlington, wastewater samples are collected three times per week from each wastewater plant (using a 24-hour composite sample) and



only reflect a snapshot of that single day's flow. Based on a three-times-weekly wastewater sampling frequency, short-term trends could then be calculated from samples collected over a week-plus timespan, and sustained trends from the five or more samples collected over a 15-day timespan.

It's also important to note that readings of zero (0) genomes per liter of viral concentration indicate that viral levels are lower than the limits of detection. This does not indicate that there is no SARS-CoV-2 virus in the community.

Case estimates of community infection based on wastewater measurements should not be used in wastewater monitoring as 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.

For more information about wastewater-based surveillance and its advantages see CDC guidelines: <https://www.cdc.gov/coronavirus/2019-ncov/cases-updates/wastewater-surveillance.html>

What are the limitations?

It's important to emphasize that this is an imperfect data set and should not be looked at in isolation. The City of Burlington is working closely with the Vermont Department of Health and the water engineers and epidemiology team at GoAigua and GT Molecular to interpret wastewater monitoring results. There are several known data limitations with this new application of wastewater-based epidemiology that are important to acknowledge. For example:

- These are limited point-in-time samples, so therefore not all people are “contributing to” the sample for that given day and we could also be picking up RNA from commuters during the week or people who may not necessarily live in Burlington.
- Not all people who have COVID-19 shed the virus (potentially 40 – 80 percent)
- Research demonstrates that more RNA is shed in the first several days after infection, but specifics about changes in levels of viral shedding during the course of the infection are still unknown. This is true for both symptomatic and asymptomatic individuals.
- Sampling variations (variation in flow, weather events, dilution)

Can we compare Burlington's results to other cities?

Comparison of the number of gene copies found between communities is discouraged due to the variability in wastewater flows and the various sources (industrial discharges or rainwater) that can contribute to each community's wastewater flow. It is better to use the information to observe trends in the data at a specific location.

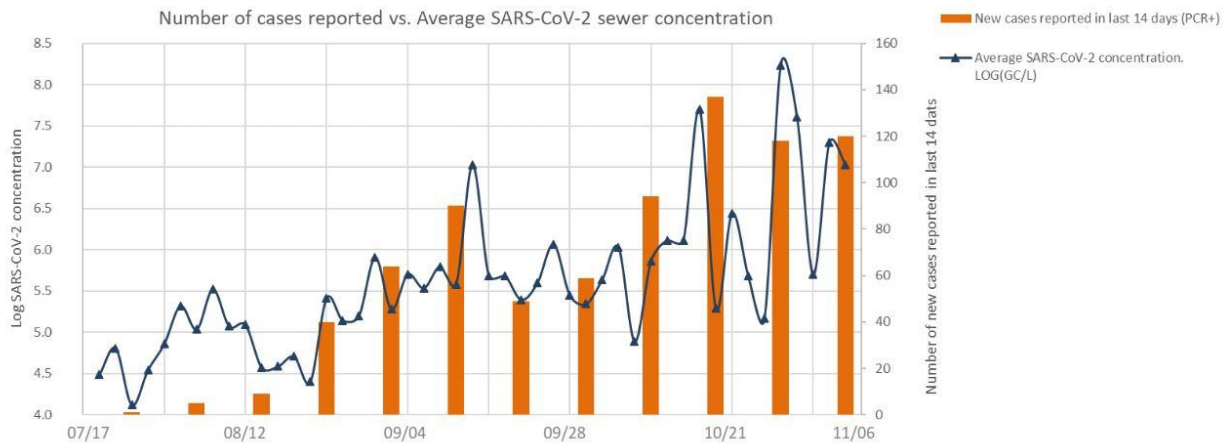
What should we expect to see?

When viral RNA is detected in wastewater, the results will vary sometimes significantly with each reading. The most important findings are the fact of detection at a significant level and the average concentration trend line over time. As you can see from this European City example below, where authorities have been consistently testing sewer and population since early July, when the viral RNA levels are detected, they fluctuate significantly but predict rising case growth.



Burlington's SARS-CoV-2 Wastewater Monitoring Program

FREQUENTLY ASKED QUESTIONS



Wastewater Based Epidemiology is an emerging technology that is being used with guidance from the Centers for Disease Control (CDC) and the Environmental Protection Agency (EPA). Additional examples of how other cities are using this technology are the Ohio Coronavirus Wastewater Monitoring Network and Massachusetts's Water Resources Authority.

For more information on the Burlington's Wastewater Monitoring program including results to date, please visit the [City's Wastewater Monitoring page](#).

Who is GoAigua?

GoAigua (www.goaigua.com) is a New York-based company, subsidiary of the Global Omnium Group, a water utility operator and environmental laboratory facility based in Spain serving 7 million people throughout 400 communities. They are a pioneer company in sewer surveillance of pathogens and viruses: 3 years ago, they started a partnership with the Spanish National Research Council (CSIC), a Spain State-owned Agency, to detect different infectious viruses (Hepatitis, Norovirus, and others) in sewage water. Through their Big Data Platform they also developed a system to integrate the results with data from the entire utility (GIS, water usage patterns, etc.), the healthcare system and other metadata to provide valuable insights to public officials in the way they make policy decisions to fight the spread of COVID-19.

In March 2020, GoAigua began working with the Government of Spain to collect and analyze sewer samples to detect the concentration of SARS-CoV-2 RNA. Since then GoAigua has scaled up their community level sewer testing to more than 20 cities and 10 million people across 1,000 testing locations.