

Brief: Interpreting Covid-19 wastewater data from buildings

Key points

- **Building-level wastewater monitoring can identify outbreaks**, allowing mitigation measures to be put in place to prevent those outbreaks from growing.
- Biobot has **reliably detected outbreaks in facilities of varying sizes**, from only a few hundred, to more than 1,000 occupants.
- Even in congregate living settings where outbreaks can occur in the span of a few days, **a single detection with a sufficiently high concentration can merit action**, and in our experience has reliably indicated that an outbreak is occurring.
- When paired with clinical testing, **consistent wastewater monitoring prevents asymptomatic cases from turning into outbreaks**.
- Biobot's Covid-19 wastewater concentration tiers are designed to provide an initial guide to interpreting wastewater data. Interpretation of wastewater data depends on the sampling location, the monitored population, and the local public health situation. Wastewater data complements other important local indicators, including clinical case, hospitalization, and vaccination rates.

How does wastewater monitoring detect Covid-19 outbreaks?

Covid-19 wastewater monitoring is a powerful complement to diagnostic testing. Wastewater monitoring has been used at multiple geographic and population scales, from measuring community-level disease activity using wastewater treatment plant samples, down to measuring sewage in individual buildings, such as university dormitories, correctional facilities, and places of work.

Wastewater testing can detect outbreaks because at least one infected person in the outbreak will likely shed enough virus in stool to be detected. For example, in a study at the University of North Carolina at Charlotte in late 2020, when wastewater monitoring was in place, clusters of 1 to 3 infected people were routinely detected. When wastewater monitoring was not available, clusters grew to 5 to 10 people before they were detected (Gibas et al. 2021).



Why does Biobot use wastewater concentration tiers?

Wastewater data for SARS-CoV-2 detection and quantification can span a wide range, from less than 10,000 virus copies per liter of wastewater to over 1 million copies per liter. To help guide interpretation of wastewater data, Biobot divides wastewater virus concentrations into four tiers. These tiers were developed using empirical data from Biobot's samples, from academic studies, and from tiers derived by academic groups (e.g., Clemson University). For more information on how these tiers were developed, see the Appendix.

Tier	Wastewater virus concentration (copies/L)	Examples of when this tier has been observed in Biobot's data	
1	Less than 10,000	Few or no infected individuals. Active transmission is unlikely.	
2	10,000–100,000	Increasing numbers of infections, or decreasing numbers of infections after an outbreak.	
3	100,000–1,000,000	Some building outbreaks peak at this tier. Larger outbreaks continue to rise to tier 4.	
4	> 1,000,000	Numerous infected individuals. A large outbreak may be in progress.	

Biobot Covid-19 wastewater tiers

For buildings, low concentrations of virus are consistent with very few, if any, people in the monitored population being infected. Higher concentrations are an indication that the presence of the virus in the building is not a one-off and that there is a high risk of within-building transmission and an outbreak.

Note that any wastewater virus tiering system involves selecting breakpoints that do not have intrinsic biological significance. There is effectively no difference between a sample with 10,001 copies per liter and one with 9,999, even though they fall into different tiers.

How are wastewater concentration tiers used in...

University dormitories?

 Wastewater monitoring at the University of Arizona prevented an outbreak in fall 2020. When a wastewater sample from one dorm tested positive for SARS-CoV-2, all



311 people who lived and worked in the dorm were clinical tested. Two students, infected but asymptomatic, were identified and isolated, preventing an outbreak (Peiser, 2020).

- The University of Michigan used wastewater monitoring to trigger mass testing of studies in dormitories, and to close public locations such as gyms, when wastewater virus levels in those facilities were too high (EPA, 2022).
- Administrators at Rollins College (Winter Park, Fla.) used wastewater data to monitor Covid-19 in specific residence halls. When wastewater indicated increased infections, administrators directed "pop-up" clinical testing to those dorms, allowing infected students to be quickly identified and isolated.

Congregate living facilities?

- The sheriff for Middlesex County, Massachusetts, which includes the city of Boston, explained: "The results of the samples analyzed by Biobot show the processes and procedures we have put in place – including testing, mask wearing and quarantining of newly admitted individuals – continue to be effective in mitigating the impact of COVID at the Middlesex Jail & House of Correction, [...] providing us with an additional crucial layer of confirmation" (Middlesex Sheriff's Office, 2021).
- At the J. Paul Taylor Center, a juvenile detention facility in New Mexico, an increase in SARS-CoV-2 levels was detected in the wastewater sample and the facility was notified of the increase (NMED, 2021a). In response, the Center tested all of its residents and staff, finding three staff members who tested positive. The positive staff members quarantined off site, and the subsequent wastewater results returned to non-detectable levels (NMED, 2021b).

Places of work?

• An enterprise customer samples wastewater five times a week at some of its offices to monitor Covid-19. When the virus is detected in wastewater, administrators open an incident report to determine whether the detection can be connected to a specific incident or event. If three consecutive wastewater samples yield detections, an internal review committee is notified and creates an action plan. Actions include offering clinical Covid-19 tests to employees and sending infected employees home.



How to use these tiers for your facility

Wastewater monitoring provides information that can inform public health action, such as mitigating an outbreak. Wastewater levels of SARS-CoV-2 correlate with the number of infected individuals in the building, and wastewater virus tiers are the starting point for planning mitigations. However, **any mitigating actions taken to prevent the spread of virus will need to be decided according to an organization's specific risk tolerance, resource availability, population served, and nature of services.** Additionally, mitigation measures will depend on whether samples remain at elevated concentrations or whether there is a single sample increase followed by a return to baseline levels.

Each facility and community operates in a unique context, so different actions are suitable for different communities. For this reason, suggested mitigation strategies are presented as a spectrum of possible options.

Wastew	ater Concentration Lev	el Paired with Trending	Direction		
Spectrum of Possible Mitigation Actions					
 Test wastewater at baseline frequency 	 Encourage mask wearing and social distancing Reduce group activities Improve air ventilation and filtration Increase frequency of wastewater testing 	 Encourage working from home Require mask wearing Reduce facility occupancy Test wastewater daily 	 Offer clinical testing to everyone in the facility Offer more protective masks (N95, KN95, KF94) Prioritize working from home 		



Considerations when interpreting wastewater data

Wastewater tiers are designed to provide an initial guide, the interpretation of which may be dependent on specific sampling location and population served. Several factors beyond those discussed above may impact what the tiers mean in a specific context.

Low levels of virus may not always indicate an outbreak

Low but nonzero wastewater virus concentrations could be due to a number of factors not related to a growing outbreak:

- **Behavioral factors:** An infected person might briefly visit the monitored building but still deposit virus in the wastewater system. Some people use the bathroom outside their residences and workplaces. Small amounts of virus may also make their way into wastewater via saliva. Simply spitting in a sink may be sufficient to cause a detection (Döhla et al. 2020).
- **Biological factors:** A person recovering from infection may return to the building. Recovering individuals shed at lower level but are still potentially detectable in wastewater (Corchis-Scott et al. 2021)
- Infrastructure factors: Detections are potentially possible even after all infected individuals are removed from the building because residual stool may remain in toilets or pipes. Also, small amounts of wastewater from other buildings may end up at the sampling point. For example, in a study of Spanish nursing homes (Davó et al. 2021), virus concentrations were consistently detected in wastewater from one nursing home, despite all residents and staff in that facility testing negative. Later investigation showed that the sampling location actually included wastewater from an adjacent building that housed some infected individuals.

Building concentration levels correlate with but do not exactly measure number of infected individuals

• Virus shedding in feces can vary from person to person by a factor of 100, and virus shedding changes over the course of an individual's infection. This means that the timing of sampling and the presence or absence of a "high shedder" contributing to the sample can cause rapid rises and falls in building-level data.



Tracking consecutive wastewater concentration levels over time, multiple times a week, is the best way to determine the presence of an outbreak.

- In a congregate living environment, where transmission rates are high and outbreaks will grow quickly, wastewater virus levels as low as Tier 2 can serve as an early warning sign of an outbreak. Sampling multiple times a week is the most effective way to confirm trends in virus activity.
- In buildings with lower transmission rates and smaller populations, such as office settings, Tier 2 virus levels may not warrant any action other than to monitor subsequent samples to determine if infections are increasing. More frequent sampling is still preferable, but sampling even just once a week can still be informative.

Potential actions to improve performance of building-level wastewater monitoring

Wastewater monitoring practitioners can take multiple steps to improve the quality of their monitoring program's data:

Increase the sampling frequency of sampling

The value of building-level monitoring strongly depends how often samples are taken. If sampling is too infrequent, then by the time a wastewater measurement is taken, the cluster of infected people will have grown large enough to be detected by other means. By collecting samples more often, an outbreak can be detected more quickly.

Frequent sampling may be especially important in congregate living settings, where outbreaks can occur over the course of only a few days. Because outbreaks can grow exponentially, weekly or twice-weekly sampling may suddenly transition from non-detect to very high concentrations. Thus, depending on the setting, it may not be feasible to confirm an outbreak using a trend analysis.

Tailor the sampling schedule

Design your sampling schedule based on the population being served and your facility's occupancy patterns to capture the most useful data. For example, if you have a Friday telework policy, sampling on other days of the week will provide more useful data.

Increase pumping frequency

After flushing, material may take only a few minutes to reach and pass a sampling location (Colosi et al. 2021). To maximize the chance that material from every flush will be captured, autosamplers should be set to their maximum practicable pumping frequency, as close to a



5-minute pumping frequency as possible. Pumping frequency may need to be adjusted based on the sewer system's hydraulics, which can be quantified using a dye test.

Perform dye tests

In a dye test, dyed water is placed in a toilet and flushed. The delay between the flush and the appearance of the dye at the sampling location is measured, and the amount of dye that makes it to the sampling location is measured. Dye tests are helpful because:

- There is a chance that a sampling location does not include material from the target toilet. For example, in a study of effluent from a Covid-19 hospital ward, a series of negative wastewater results led researchers to question the original sampling locations. Dye tests showed that none of the sampled manholes were actually receiving wastewater from the hospital ward in question (Colosi et al. 2021).
- Dye tests can also help determine what frequency of pumping is required to ensure good confidence that virus-positive material will be sampled and detected. If the transit time between a toilet flush and the sampling point is very fast, then an autosampler might not collect material from every flush.



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