



Interpreting building-level Covid-19 wastewater monitoring data

Scott Olesen <olesen@biobot.io>

Key points

- **Building-level wastewater monitoring can identify outbreaks**, allowing mitigation measures to be put in place to prevent those outbreaks from growing.
- Wastewater concentrations of SARS-CoV-2 should be interpreted as a **risk index** that simultaneously estimates both the likelihood and the severity of a Covid-19 outbreak. Biobot-measured wastewater concentrations greater than a certain quantitative threshold (10^7 normalized genome copies per liter divided by the number of people) merit action. Lower concentrations may also merit action.
- 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.
- Biobot has reliably detected outbreaks in facilities with as many as 1,000 occupants. In community settings, **wastewater monitoring can detect a single case among 3,600 people**, which indicates its suitability even for very large buildings.

Using building-level wastewater data to trigger action

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 influent sampled from municipal wastewater treatment plants down to measuring sewage in individual buildings, such as **university dormitories, hospital wards, and correctional facilities** (Betancourt et al. 2021; Karthikeyan et al. 2021; Harris-Lovett et al. 2021; Reeves et al. 2021; Targeted Wastewater Surveillance at Facilities, Institutions, and Workplaces 2021).

When monitoring at the level of individual buildings or small groups of buildings, the resulting data can be used to trigger a swift Covid-19 response. For example, detection of high levels of SARS-CoV-2 in wastewater can be followed by individual-level mass testing to identify and isolate infected people. Because many infected people do not show symptoms but still shed virus in stool, **wastewater testing may be able to identify outbreaks among a building's inhabitants before that outbreak would be detected by symptoms checks or diagnostic tests alone** (Bibby et al. 2021; Gibas et al. 2021; Olesen et al. 2021).



Despite this promise, it is more challenging to interpret building-level wastewater data, compared to community-level data collected at a municipal wastewater treatment plant (Harris-Lovett et al. 2021). In this white paper, we provide a quantitative risk index approach that can help contextualize wastewater data for use in building-level decision-making. We also review the challenges to interpreting wastewater data.

Wastewater can detect outbreaks but not necessarily individual infections

The purpose of Covid-19 surveillance is to provide information that can inform public health action, such as mitigating an outbreak. Ideally, surveillance can detect a single infected person in the monitored population. In practice, this kind of precision is often not possible because of technological and resource constraints, but it is also not necessary to mitigate outbreaks. So long as at least one person in the outbreak cluster is detected, the outbreak can be mitigated.

For example, one surveillance approach is to perform diagnostic tests on some members of the monitored population at some interval, say testing 10% of people twice a week. This approach is not guaranteed to detect a single infection, but it will likely detect a large outbreak.

Wastewater monitoring is not guaranteed to detect an individual infection because of:

- **Technological and biological factors:** Methods to detect virus in sewage have a finite limit of detection, and rates of fecal virus shedding can vary dramatically from person to person (Li et al. 2021), so much so that some people appear to not shed virus in their stool at all (Jones et al. 2020; Kitajima et al. 2020; Li et al. 2020). In community-level sampling at wastewater treatment plants, Biobot's methodology is able to detect as few as one case per 3,600 people, and at the building level, Biobot has robustly detected outbreaks in facilities with populations of approximately 1,000 people.
- **Behavioral factors:** Not everyone will have a bowel movement in the building they nominally occupy during a time when a sampling is being collected. For example, if a 24-hour composite sample is collected twice a week and an infected person does not deposit material on either of those two days, they may not be detected at all.

In a congregate living environment, where transmission rates are high and outbreaks will grow quickly, **building-level wastewater monitoring may be more valuable as a tool for**



detecting outbreaks, rather than individual cases. While there is no guarantee that wastewater monitoring can detect a single infection, 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, 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).

In buildings with lower transmission rates, like office settings, wastewater monitoring's potential ability to detect single cases may be useful, for example, to confirm that the safety policies and protocols that are in place are ensuring that no infected person is in the workplace.

Not all detections of virus in wastewater necessarily merit a response

As discussed above, Covid-19 surveillance methods like wastewater monitoring are more reliable for detecting outbreaks rather than individual infections. Conversely, detectable but low levels of virus in wastewater may not necessarily merit a response.

Low levels of virus 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, so that 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. Convalescent individuals shed at lower –but still potentially detectable– levels relative to infectious people.
- **Infrastructure factors:** Residual stool may remain in toilets or pipes, so that a detection with low concentration is potentially possible even after all infected individuals are removed from the building. 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 was 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.



Wastewater concentrations relate to both the likelihood and the size of an outbreak

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 or an issue of minor cross-contamination, that is, that there is a real risk of transmission and an outbreak.

Higher concentrations are a potential indication, but not definitive proof, that more people are infected. Shedding rates vary across infected people, and although there is insufficient data to make a definitive statement about this variation, **it is likely that one person can shed ten times as much virus as another person**. In other words, the same concentration of virus in wastewater could be due to 10 infected people who shed an average amount of virus, or it could be due to just 1 single infected person who sheds large amounts of virus.

When monitoring wastewater at a community level, there are usually enough infected people that the variation between individuals averages out, so that the concentration of virus in wastewater tracks the number of infected people. However, at the building level, where only a small number of people might be infected, variability between individuals makes the relationship between virus concentrations and number of infected people more uncertain (Wade et al. 2021).

Thus, for small populations, high levels of virus in wastewater indicate a high probability that *someone* is infected, but the level does not necessarily indicate how many people are infected (Harris-Lovett et al. 2021). As outbreaks grow in size, wastewater virus concentrations better track the number of infected individuals. **We recommend interpreting wastewater concentrations as a risk index that provides information both about the likelihood that an outbreak is occurring and also the likely size of the outbreak.**

An outbreak risk index for triggering action

We recommend interpreting wastewater concentrations using a four-tier scheme that roughly classifies the risk of a sizeable outbreak:

1. Very low risk: Undetectable levels of virus
2. Low risk: Detectable levels of virus below a **threshold concentration of 10^7 normalized genome copies per liter (copies/L), divided by the number of people contributing to wastewater**
3. Medium risk: 1-10x the low-risk threshold concentration

4. High risk: >10x the low-risk threshold concentration

These concentrations were calibrated using Biobot’s laboratory methodology. Because different methodologies can produce substantially different SARS-CoV-2 concentration measurements, **these tiered concentrations are not applicable to measurements made using methodologies other than Biobot’s.**

These values are summarized in the table for two representative population sizes.

Population size	Very low risk	Low risk	Medium risk	High risk
100	Not detected	<10 ⁵ copies/L	10 ⁵ –10 ⁶ copies/L	>10 ⁶ copies/L
1,000	Not detected	<10 ⁴ copies/L	10 ⁴ –10 ⁵ copies/L	>10 ⁵ copies/L

In our experience, **high-risk concentrations as measured by this index are a reliable indicator that there is at least one infected person in the monitored population.** To avoid more people being infected, a response like quarantine or mass testing should be taken.

In all cases, it should be remembered that wastewater-based monitoring is rapidly evolving, and no algorithm for triggering action can have perfectly rigorous support at this time. **We recommend that you use these tiers as an initial guide,** adjusted according to your specific risk-reward trade-offs. Depending on an organization’s risk-reward trade-offs, **taking action at lower risk tiers could also be justified.** As you develop a better understanding of the relationship between wastewater data and the risk of an outbreak, you may also be able to adjust the tiered concentrations based on data specific to your site.

The data used to develop these tiered concentrations is summarized in the Appendix.

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 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. We caution, however, the sampling frequency may need to be adjusted based on the sewer system's hydraulics, which can be quantified using a dye test, described below. A dye test can help quantify the needed pumping frequency.

Increase the frequency of sampling

In our experience, the value of building-level monitoring is closely related to 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 rather than linearly, weekly or twice-weekly sampling may suddenly transition from non-detect to high risk concentrations. Thus, depending on the setting, it may not be feasible to confirm an outbreak using a trend analysis.

Perform dye tests

In a dye test, dyed water is placed in a monitored toilet and flushed, and the sampling location is monitored to measure the delay between the flush and the measurement as well as the amount of time the dye is present at the sampling location after the flush.

Dye tests are helpful because:

- There is a chance that a sampling location does not include material from the target population. For example, in a study of effluent from a Covid-19 hospital ward, after a series of false negative results led researchers to question the original sampling locations, dye tests showed that none of the manholes initially thought to be downstream of the ward actually were receiving wastewater from the 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.



Appendix

We draw evidence for the risk tiers from 6 building-level outbreaks and from community-level data

- In four incidents at four separate correctional facilities, wastewater concentrations reached the high-risk tier. These wastewater measurements prompted mass testing, which showed an outbreak was occurring. Those outbreaks affected between 0.5% and 20% of individuals at the facility.
- In two incidents at a fifth correctional facility, wastewater concentrations reached the medium-risk tier, without ever reaching the high-risk tier, when there were multiple infected individuals at the facility.
- In approximately 9,300 samples collected by Biobot from over 450 municipal wastewater treatment plants between March 2020 and August 2021, a 0.1% community-level prevalence was associated with concentrations of 10^6 copies/L, and 0.01% prevalence was associated with 10^5 copies/L. This relationship, if scaled down to a population of 1,000 or 100, would mean that a single case would produce a wastewater signal ten times greater than the minimum high-risk tier concentration.
- In a collection of university dormitories with a long history of sampling with Biobot, there was a fairly consistent prevalence of 1 case per 1,000 residents and a fairly consistent wastewater concentration of 10^5 copies/L, that is, at the bottom of the high-risk tier.
- Over one year of sampling in a collection of office buildings, Biobot developed a custom risk threshold, using a breakpoint of 10^5 copies/L for an office population of 100 individuals, which corresponds to the breakpoint between the low risk and medium risk tiers described above.

Evidence for risk tiers in the scientific literature is still sparse

A number of studies have reported on wastewater surveillance for buildings or other small catchment areas and how wastewater data correlates with detection of cases. However, we chose to develop breakpoints based on our data and experience because of multiple challenges in interpreting reports from the scientific literature:

- Many studies report only detection vs. non-detection, or they do not provide the raw wastewater data on virus concentrations and case counts.
- Even among those studies that do report wastewater data, the studies in general use different methodologies for sampling and virus quantification. Different methodologies for virus quantification can produce different results (Pecson et al. 2020).

- Confidence in a risk tiering system depends more on the number of outbreaks detected (or not detected) by wastewater monitoring, rather than on the number of samples collected (Lazic et al. 2018). To see why, imagine two buildings, one monitored for a whole year and the other monitored for only a month. Both buildings are Covid-free except at one point in time, when they each had one case. Even though there are many more samples associated with one of the buildings, both buildings each provide only one example of what wastewater threshold would be useful for triggering action.

Familiar calculations of sensitivity and specificity may be misleading when evaluating wastewater monitoring

We chose not to quantify the sensitivity and specificity of these tiers for multiple reasons:

- Strictly linking wastewater and case data on a building-level basis may be unrealistic given how buildings are used. For example, say an infected student officially residing in dormitory A uses a bathroom in dormitory B, leading to a “false positive” detection in dormitory B and a “false negative” nondetection in dormitory A. The straightforward calculation of sensitivity and specificity penalizes wastewater monitoring for being unable to distinguish the official residence of the individuals who deposited virus-positive material, when in fact the detection at dormitory A is precisely what we would expect wastewater monitoring to do.
- Computing sensitivity and specificity by comparing the presence or absence of known cases with detections or nondetections of virus in wastewater, as has been done multiple times in the scientific literature (Barich & Slonczewski 2021; Betancourt et al. 2021; Davó et al. 2021; Gibas et al. 2021; Karthikeyan et al. 2021; Wong et al. 2021), relies on case data as a “gold standard”. In our experience, case detection is not sufficiently robust to accurately evaluate wastewater monitoring’s performance. For example, if an infected person is asymptomatic and is never recognized as a case, then a wastewater detection would be erroneously labeled as a “false” positive.



Statements

SWO is an employee of Biobot Analytics.

References

Barich D, Slonczewski JL. Wastewater Virus Detection Complements Clinical COVID-19 Testing to Limit Spread of Infection at Kenyon College. medRxiv. 2021 Jan 12. doi: [10.1101/2021.01.09.21249505](https://doi.org/10.1101/2021.01.09.21249505).

Betancourt WQ, Schmitz BW, Innes GK, Prasek SM, Pogreba Brown KM, Stark ER, Foster AR, Sprissler RS, Harris DT, Sherchan SP, Gerba CP, Pepper IL. COVID-19 containment on a college campus via wastewater-based epidemiology, targeted clinical testing and an intervention. *Sci Total Environ*. 2021 Jul 20;779:146408. doi: [10.1016/j.scitotenv.2021.146408](https://doi.org/10.1016/j.scitotenv.2021.146408). Epub 2021 Mar 13. PMID: 33743467; PMCID: PMC7954642.

Bibby K, Bivins A, Wu Z, North D. Making waves: Plausible lead time for wastewater based epidemiology as an early warning system for COVID-19. *Water Res*. 2021 Jul 12;202:117438. doi: [10.1016/j.watres.2021.117438](https://doi.org/10.1016/j.watres.2021.117438). Epub ahead of print. PMID: 34333296; PMCID: PMC8274973.

Colosi LM, Barry KE, Kotay SM, Porter MD, Poulter MD, Ratliff C, Simmons W, Steinberg LI, Wilson DD, Morse R, Zmick P, Mathers AJ. Development of wastewater pooled surveillance of SARS-CoV-2 from congregate living settings. *Appl Environ Microbiol*. 2021 Apr 15:AEM.00433-21. doi: [10.1128/AEM.00433-21](https://doi.org/10.1128/AEM.00433-21). Epub ahead of print. PMID: 33858836.

Davó L, Seguí R, Botija P, Beltrán MJ, Albert E, Torres I, López-Fernández PÁ, Ortí R, Maestre JF, Sánchez G, Navarro D. Early detection of SARS-CoV-2 infection cases or outbreaks at nursing homes by targeted wastewater tracking. *Clin Microbiol Infect*. 2021 Feb 15:S1198-743X(21)00078-1. doi: [10.1016/j.cmi.2021.02.003](https://doi.org/10.1016/j.cmi.2021.02.003). Epub ahead of print. PMID: 33601008; PMCID: PMC7882920.

Döhla M, Wilbring G, Schulte B, Kümmerer BM, Diegmann C, Sib E, Richter E, Haag A, Engelhart S, Eis-Hübinger AM, Exner M, Streeck H, Schmithausen RM. SARS-CoV-2 in environmental samples of quarantined households. medRxiv. 2020 Jun 2. doi: [10.1101/2020.05.28.20114041](https://doi.org/10.1101/2020.05.28.20114041).

Gibas C, Lambirth K, Mittal N, Juel MAI, Barua VB, Roppolo Brazell L, Hinton K, Lontai J, Stark N, Young I, Quach C, Russ M, Kauer J, Nicolosi B, Chen D, Akella S, Tang W, Schlueter J, Munir M. Implementing building-level SARS-CoV-2 wastewater surveillance on a university campus. *Sci Total Environ*. 2021 Aug 15;782:146749. doi: [10.1016/j.scitotenv.2021.146749](https://doi.org/10.1016/j.scitotenv.2021.146749). Epub 2021 Mar 30. PMID: 33838367; PMCID: PMC8007530.

Harris-Lovett S, Nelson KL, Beamer P, Bischel HN, Bivins A, Bruder A, Butler C, Camenisch TD, De Long SK, Karthikeyan S, Larsen DA, Meierdiercks K, Mouser PJ, Pagsuyoin S, Prasek SM, Radniecki TS, Ram JL, Roper DK, Safford H, Sherchan SP, Shuster W, Stalder T, Wheeler RT, Korfmacher KS. Wastewater Surveillance for SARS-CoV-2 on College Campuses: Initial Efforts, Lessons Learned, and Research Needs. *Int J Environ Res Public Health*. 2021 Apr 22;18(9):4455. doi: [10.3390/ijerph18094455](https://doi.org/10.3390/ijerph18094455). PMID: 33922263; PMCID: PMC8122720.

Karthikeyan S, Nguyen A, McDonald D, Zong Y, Ronquillo N, Ren J, Zou J, Farmer S, Humphrey G, Henderson D, Javidi T, Messer K, Anderson C, Schooley R, Martin NK, Knight R. Rapid, Large-Scale Wastewater Surveillance and Automated Reporting System Enable Early Detection of Nearly 85% of COVID-19 Cases on a University Campus. *mSystems*. 2021 Aug 10:e0079321. doi: [10.1128/mSystems.00793-21](https://doi.org/10.1128/mSystems.00793-21). Epub ahead of print. PMID: 34374562.

Kitajima M, Ahmed W, Bibby K, Carducci A, Gerba CP, Hamilton KA, Haramoto E, Rose JB. SARS-CoV-2 in wastewater: State of the knowledge and research needs. *Sci Total Environ*. 2020 Oct 15;739:139076. doi: [10.1016/j.scitotenv.2020.139076](https://doi.org/10.1016/j.scitotenv.2020.139076). Epub 2020 Apr 30. PMID: 32758929; PMCID: PMC7191289.

Jones DL, Baluja MQ, Graham DW, Corbishley A, McDonald JE, Malham SK, Hillary LS, Connor TR, Gaze WH, Moura IB, Wilcox MH, Farkas K. Shedding of SARS-CoV-2 in feces and urine and its potential role in person-to-person transmission and the environment-based spread of COVID-19. *Sci Total Environ*. 2020 Dec 20;749:141364. doi: [10.1016/j.scitotenv.2020.141364](https://doi.org/10.1016/j.scitotenv.2020.141364). Epub 2020 Jul 31. PMID: 32836117; PMCID: PMC7836549.

Lazic SE, Clarke-Williams CJ, Munafò MR. What exactly is 'N' in cell culture and animal experiments? *PLoS Biol*. 2018 Apr 4;16(4):e2005282. doi: [10.1371/journal.pbio.2005282](https://doi.org/10.1371/journal.pbio.2005282). PMID: 29617358; PMCID: PMC5902037.

Li X, Zhang S, Shi J, Luby SP, Jiang G. Uncertainties in estimating SARS-CoV-2 prevalence by wastewater-based epidemiology. *Chem Eng J*. 2021 Jul 1;415:129039. doi: [10.1016/j.cej.2021.129039](https://doi.org/10.1016/j.cej.2021.129039). Epub 2021 Feb 20. PMID: 33642938; PMCID: PMC7896122.

McClary-Gutierrez JS, Mattioli MC, Marcenac P, Silverman AI, Boehm AB, Bibby K, et al. SARS-CoV-2 wastewater surveillance for public health action. *Emerg Infect Dis*. 2021 Sep. doi: [10.3201/eid2709.210753](https://doi.org/10.3201/eid2709.210753).

Miura F, Kitajima M, Omori R. Duration of SARS-CoV-2 viral shedding in faeces as a parameter for wastewater-based epidemiology: Re-analysis of patient data using a shedding dynamics model. *Sci Total Environ.* 2021 May 15;769:144549. doi: [10.1016/j.scitotenv.2020.144549](https://doi.org/10.1016/j.scitotenv.2020.144549). Epub 2021 Jan 4. PMID: 33477053; PMCID: PMC7833251.

Olesen SW, Imakaev M, Duvallet C. Making waves: Defining the lead time of wastewater-based epidemiology for COVID-19. *Water Res.* 2021 Jul 15;202:117433. doi: [10.1016/j.watres.2021.117433](https://doi.org/10.1016/j.watres.2021.117433). Epub ahead of print. PMID: 34304074; PMCID: PMC8282235.

Pecson BM, Darby E, Haas CN, Amha YM, Bartolo M, Danielson R, Dearborn Y, Di Giovanni G, Ferguson C, Fevig S, Gaddis E, Gray D, Lukasik G, Mull B, Olivas L, Olivieri A, Qu Y; SARS-CoV-2 Interlaboratory Consortium. Reproducibility and sensitivity of 36 methods to quantify the SARS-CoV-2 genetic signal in raw wastewater: findings from an interlaboratory methods evaluation in the U.S. *Environ Sci (Camb).* 2021 Jan 20;7:504-520. doi: [10.1039/d0ew00946f](https://doi.org/10.1039/d0ew00946f). PMID: 34017594; PMCID: PMC8129921.

Reeves K, Leibig J, Feula A, Saldi T, Lasda E, Johnson W, Lilienfeld J, Maggi J, Pulley K, Wilkerson PJ, Real B, Zak G, Davis J, Fink M, Gonzalez P, Hager C, Ozeroff C, Tat K, Alkire M, Butler C, Coe E, Darby J, Freeman N, Heuer H, Jones JR, Karr M, Key S, Maxwell K, Nelson L, Saldana E, Shea R, Salveson L, Tomlinson K, Vargas-Barriga J, Vigil B, Brisson G, Parker R, Leinwand LA, Bjorkman K, Mansfeldt C. High-resolution within-sewer SARS-CoV-2 surveillance facilitates informed intervention [Internet]. 2021. Available from: <http://dx.doi.org/10.1101/2021.05.24.21257632>

Targeted Wastewater Surveillance at Facilities, Institutions, and Workplaces [Internet]. Atlanta (GA): US Centers for Disease Control and Prevention; 2021. Available from: <https://www.cdc.gov/coronavirus/2019-ncov/cases-updates/wastewater-surveillance/targeted-use-case.html>

Wade M, Jacomo AL, Armenise E, Brown M, Bunce J. Understanding and managing uncertainty and variability for wastewater monitoring beyond the pandemic: Lessons learned from the United Kingdom National COVID-19 Surveillance Programmes. *ESSOAr.* 2021 Jul. doi: [10.1002/essoar.10507606.2](https://doi.org/10.1002/essoar.10507606.2).

Wong JCC, Tan J, Lim YX, Arivalan S, Hapuarachchi HC, Mailepessov D, Griffiths J, Jayarajah P, Setoh YX, Tien WP, Low SL, Koo C, Yenamandra SP, Kong M, Lee VJM, Ng LC. Non-intrusive wastewater surveillance for monitoring of a residential building for COVID-19 cases. *Sci Total*



Environ. 2021 Sep 10;786:147419. doi: [10.1016/j.scitotenv.2021.147419](https://doi.org/10.1016/j.scitotenv.2021.147419). Epub 2021 Apr 29.
PMID: 33964781; PMCID: PMC8081581.