Moccasin Bend Wastewater Treatment Plant Influent

Sample collection date: **July 1, 2021**

SARS-CoV-2 virus in wastewater

NOT DETECTED	Virus concentration (genome copies per liter of sewage)	Normalized* virus concentration (genome copies per liter of sewage)
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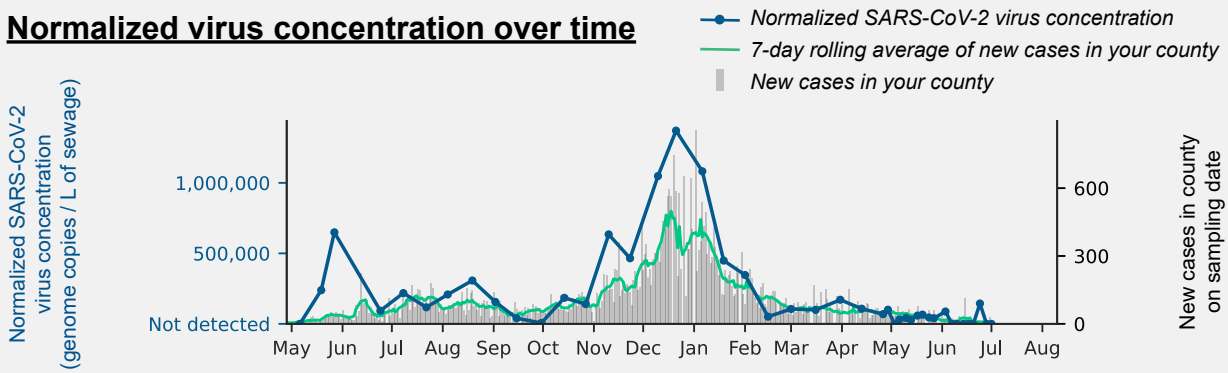
**Normalized virus concentration value is derived by adjusting the raw virus concentration to the PMMV fecal marker in order to account for dilution*

Normalized virus concentration over time



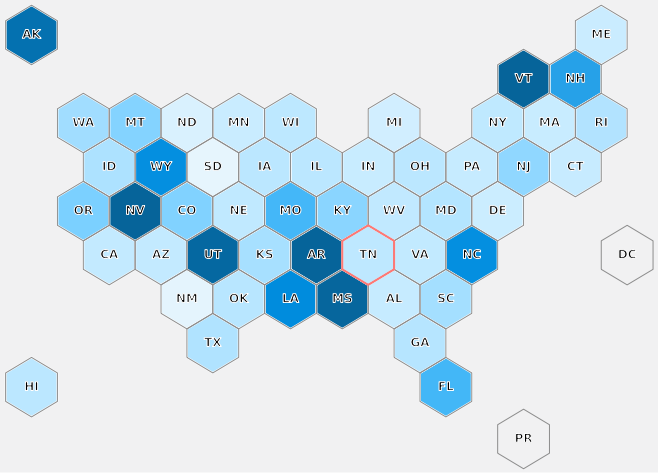
Overview of normalized virus concentration levels

State-level mean of samples (collected in the past 6 weeks)

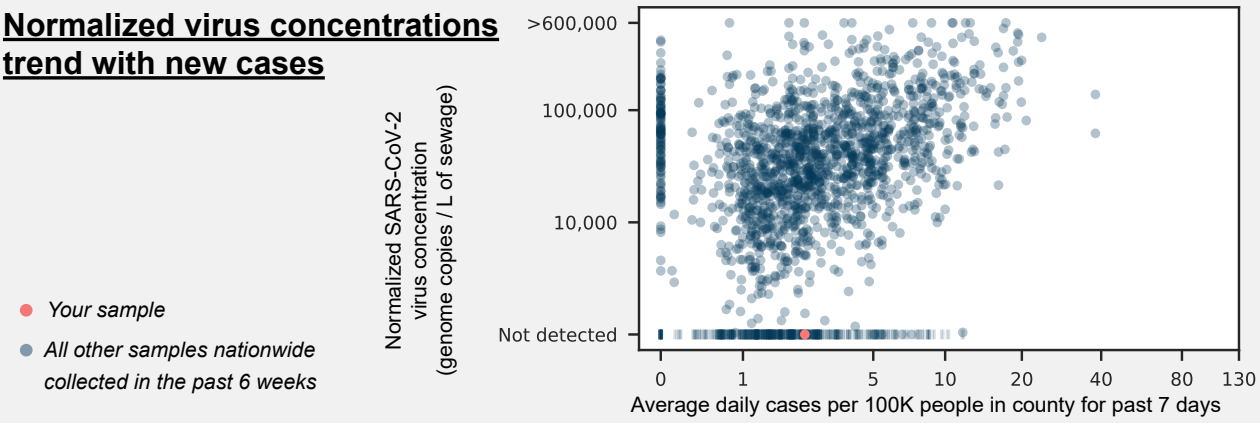
lower — higher

Color indicates normalized virus concentration level

Your state outlined in red

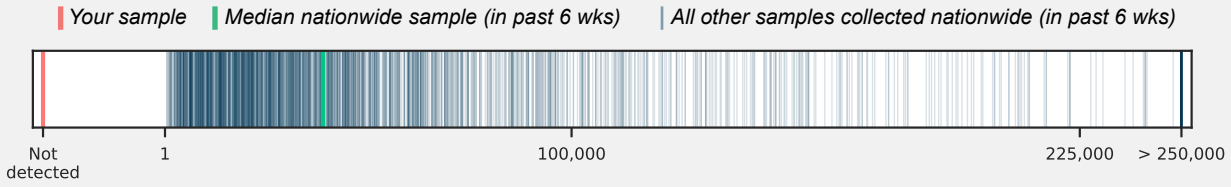


Normalized virus concentrations trend with new cases



Normalized virus concentrations in comparison

Your sample had no detectable levels of SARS-CoV-2. **21%** of all samples collected in the past six weeks also had no detectable levels.



Biobot COVID19 incidence estimate*

** using Biobot's current analysis model v2.0, which reflects active R&D and will change over time with developing research*

-- new cases / day

(--% incidence rate)

Using a reported flow rate of 43.24 MGD

For more information, read the whitepaper:
https://doi.org/10.1101/2020.06.15.20117747

This incidence estimate represents the projected average of **confirmed new clinical cases (per day)** that will be reported in your community 7 days from the sampling date. This estimate reflects active R&D.

This number is derived from Biobot's latest proprietary case model, leveraging thousands of samples analyzed for Covid-19. For context, USA Facts reports **7 new cases** on this sampling date and an average of **8.7 cases** per day for the past 7 days in **Hamilton County, TN**.

Visit our website for more details behind the process:
https://www.biobot.io/case-estimates
https://www.biobot.io/updated-model

Biobot's Covid-19 wastewater testing product

Lab protocol
Our methods for detecting SARS-CoV-2 in sewage are adapted from CDC protocols. Our approach relies on detecting genetic fragments of the virus that are excreted in stool by qPCR analysis, which does not determine if the virus is dead or active.

Limit of detection (LOD)
The LOD for our lab protocol is 3,600 copies/L of sewage (see more details in Release Notes below). In terms of case estimates, we reliably detect the virus (>99%) when there is at least 1 infected person in a population of 6,500 people.

Data use
Biobot's wastewater data provides an alternative metric to guide response to the Covid-19 outbreak. We recommend sharing this information with local public health officials. We believe this work has the greatest impact on a statewide level, and hope that you will reach out to your local officials and encourage the expansion of our partnership across your state.

Questions and support
For questions specific to your report, email **support@biobot.io.**

Biobot's QA/QC protocol

Biobot has an in-house lab facility with a team of scientists dedicated 100% to Covid-19 wastewater testing. All reported data passes our QA/QC protocol:

1) Sample collection
- 3 x 50 ml samples are shipped with a frozen pack to keep 4C temperature control.
- Documentation collected via online form: location, date, time, flow rate on sampling day, sampling type, precipitation events.

2) Storage
- Raw sewage samples are received at Biobot and immediately pasteurized. Pasteurized samples are stored at 4C for up to 3 days before viral concentration.
- Extracted RNA is stored at 4C for no longer than 24h before analysis by RT-qPCR.
- Extracted RNA is stored at -80C for the next 12 months.

3) Sample processing
- 15 mL of sewage sample is used for viral concentration and RNA extraction.
- Second and third replicates are kept at 4C for 30 days as back-up.
- Pepper Mild Mottle Virus, PMMoV, is a fecal indicator used as internal control.
- CDC Primers N1 and N2 are used to target SARS-CoV-2.
- Each test primer (N1 and N2) is run in triplicate in the qPCR assay.
- Four positive controls (synthetic SARS-CoV-2 N gene) are run in each 96-well plate.
- Two negative controls (no template) are run in each 96-well plate.
- Standard curves (synthetic SARS-CoV-2 N gene) are run once a week.

Biobot's data interpretation

Raw viral concentration (genome copies per L of sewage)
The raw SARS-CoV-2 viral concentration is directly measured by the laboratory qPCR assay.

Normalized viral concentration (genome copies per L of sewage)
We normalize the SARS-CoV-2 viral concentration to a fecal indicator, to account for differences in dilution. We use PMMV as this fecal indicator, which is an RNA virus that is commonly excreted in stool.

Biobot's Covid-19 incidence estimate
Our latest Covid-19 incidence estimation model is built from Biobot's dataset, the largest Covid-19 dataset in the world to date. We mined this dataset to derive an empirical relationship between the amount of virus in sewage samples and the number cases reported in the associated communities over the next 7 days. This means that our model provides an estimate of the number of cases that will be reported in your community in the next week.

We convert the raw viral concentration into a Covid-19 case estimate using the following equation:

$$Number\ of\ Covid19\ cases = \frac{Viral\ concentration^{*} \times Flow\ rate\ on\ sampling\ date}{Virus\ shed\ per\ reported\ case\ per\ day^{**}}$$

* We use the raw viral concentration in this calculation because dilution is accounted for by multiplying the raw concentration with the daily flow.
** Parameter currently derived from Biobot's dataset. Learn more at: **https://www.biobot.io/updated-model**

The incidence estimation equation uses your reported flow rate and the measured virus concentration, but does not use your reported catchment population. If we do not have a reported flow rate for your sample, we use your location's average influent flow rate that you provided during onboarding in the calculation.

You can interpret this incidence estimate as the number of cases that will be reported in your community in the next 4-7 days. For more information on the predictive nature of wastewater data, read our blog post: **https://www.biobot.io/case-estimates.** For more information on our new incidence estimation model, read our blog post: **https://www.biobot.io/updated-model.** For more technical details on both aspects, read the preprint: **https://doi.org/10.1101/2020.06.15.20117747.**

Our model is built in part on data from clinically confirmed cases. Reported cases include only patients who sought out Covid-19 testing and received a positive test result. This population likely does not include most asymptomatic patients or individuals without access to testing. Because reported cases are an undercount of the true number of infected individuals, our estimate is also likely a conservative projection of the number of new cases in your population. We are working actively to update our model based on the latest science and data sets available.

Incidence rate (%)
The incidence rate is calculated by dividing the Biobot case estimate by your reported catchment population.

Release notes

Lab protocol versions
We are continuously working to improve our protocols to increase the sensitivity of our measurements and reduce variability. You can find the protocol that was used to generate your data at the bottom of each page of this report:

Lab Protocol Version	Limit of detection (LOD)	Description
v3.1 (current)	4,800 copies/L	Kit-based virus concentration and extraction with multiplex one-step RT-qPCR for all targets, including PMMoV and controls.
v3.0-3.0.1	4,800 copies/L	Kit-based virus concentration and extraction with multiplex one-step RT-qPCR and algorithmic Ct call. Additional controls introduced.
v2.3.1	3,600 copies/L	Kit-based virus concentration and RNA extraction in duplicate, followed by RNA pooling and one-step RT-qPCR at Biobot and algorithmic Ct calling.
v2.3	3,600 copies/L	Kit-based virus concentration and RNA extraction with one-step RT-qPCR at Biobot and an improved algorithmic Ct calling method.
v2.2	2,100 copies/L	Kit-based virus concentration and RNA extraction with one-step RT-qPCR at Biobot laboratory.
v2.1	1,700 copies/L	Kit-based virus concentration and RNA extraction with one-step RT-qPCR at our MIT partner laboratory.
v2.0	34,000 copies/L	Kit-based virus concentration and RNA extraction with two-step RT-qPCR at our MIT partner laboratory.
v1.0	6,400 copies/L	PEG virus concentration and Trizol RNA extraction with two-step RT-qPCR at our MIT partner laboratory.

Data analysis & model versions

We are constantly iterating on and improving our data processing, analysis, and Covid-19 models to improve the interpretability of our data. You can see which version of our analysis and model was used in this report at the bottom of each page, and you can find more specific details in the release notes below.

Data Analysis & Model version	Description
v2.1 (current)	We report raw and normalized virus concentrations. To derive the normalized concentration, we multiply the raw lab concentration by a scaling factor (reference PMMoV divided by your sample PMMoV). The reference PMMoV is derived empirically from our entire database. All plots are generated using the normalized concentration.

Data Analysis & Model version	Description
v2.0	We launched an updated case estimation model which uses a viral shedding parameter derived from mining our proprietary wastewater dataset. The basic equation is the same as before, but the “viral shedding” parameter is now empirically derived as the amount of virus shed per reported case (rather than based on clinical studies of shedding in stool). As for previous versions, the case estimate is calculated using the raw SARS-CoV-2 concentration, and all other plots are generated using the normalized concentration, normalized with the same method as prior versions.
v1.2	We updated our normalization process for the virus concentration to retain units of copies/L of sewage. We multiply the raw lab concentration by a scaling factor ($scaling\ factor = \frac{reference\ PMMV}{kit\ PMMV}$). The reference PMMV is derived empirically from our entire database. As in previous versions, the case estimate is calculated using the raw SARS-CoV-2 concentration and accounts for dilution by using the flow rate provided.
v1.1	We updated detection thresholds to reduce the chance of false positives. Specifically, we’ve raised our limit of detection to ensure that all measurements can be confidently quantified, and are requiring two positive measurements per sample (out of six) to consider a sample detected.
v1.0	Raw viral concentration and Covid-19 case estimates are reported. The model parameter (virus shed per infected person per day) is determined from direct communications with Professor Kyle Bibby and Dr. Aaron Bivins and based on clinical viral shedding reported in Wolfel et al. *Nature* (2020).

Report design versions

We are continually making updates to our report based on internal R&D and feedback from our customers.

Report Design version	Description
v3.4.2 (current)	Minor changes: • Replaced regional geographical map with hexagonal map of the United States. • Removed circles indicating population of sampled catchments
v3.4.1	Minor changes: • Extended the scatter plot limits to display up to the 99th percentile of the normalized virus concentration trend with new cases. • Extended the rug plot limits to display up to the 95th percentile of the normalized virus concentration in comparison to other samples collected. • Added *Covid-19 Community+ Report* title to header

Report Design version	Description
v3.4	Major changes: • Implemented 7-day rolling average and daily cases in county for time series comparison plot. • Included comparison to historical 7-day average of reported cases in case estimate box and scatter plot.
v3.3.1	Minor changes: • Show Maryland and Delaware in Northeast map instead of South.
v3.3	Major changes: • All plots with aggregate data analyze samples based on a six week rolling window up to and including the date the sample was collected. Minor changes: • Added visual indicators to timeseries plot for new cases reported on the sampling date. • Regional geographic maps display the mean of the samples taken during the six week window, instead of the median.
v3.2.3	Minor changes: • Added the external Kit ID reference in addition to Biobot's internal Kit ID.
v3.2.2	Minor changes: • Updated the case estimate title to incidence estimate to better reflect the interpretation of our current model. • Changed the incidence estimate description to better reflect the updated model, and provided details around the methodology of how we arrived at the new model.
v3.2.1	Minor changes: • The case estimate is provided with an updated viral shedding parameter, and reflects the number of cases which will be reported in your community next week. • We've included information about your county's reported new cases back into the report.