Carmel Area WD (CA) - Facility Influent

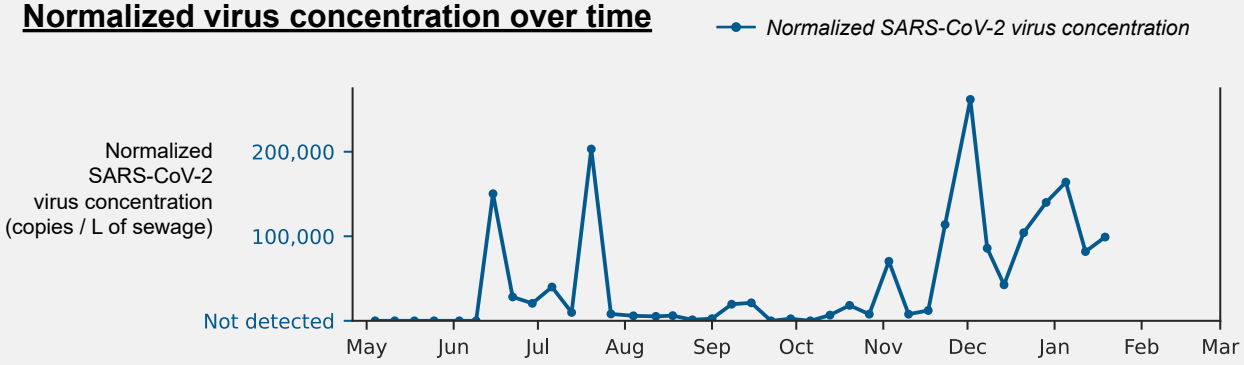
Sample collection date: **January 19, 2021**

SARS-CoV-2 virus in sewage

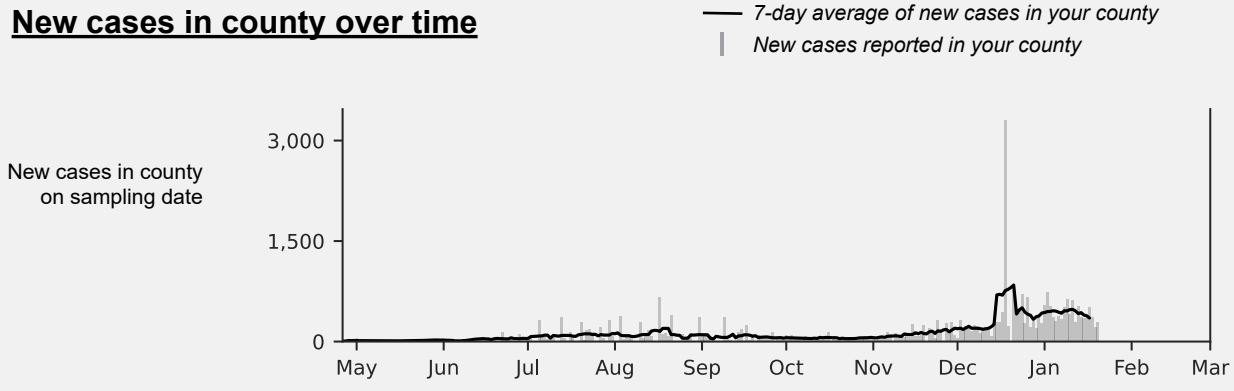
DETECTED	Virus concentration (genome copies per liter of sewage)	Normalized* virus concentration (genome copies per liter of sewage)
	234,808	**99,095**

**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



New cases in county over time



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 cases, 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, PMMV, 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.

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
v2.3 (current)	3.6 copies/mL	Kit-based virus concentration and RNA extraction with one-step RT-qPCR at Biobot and an improved algorithmic Ct calling method.
v2.2	2.1 copies/mL	Kit-based virus concentration and RNA extraction with one-step RT-qPCR at Biobot laboratory.
v2.1	1.7 copies/mL	Kit-based virus concentration and RNA extraction with one-step RT-qPCR at our MIT partner laboratory.
v2.0	34 copies/mL	Kit-based virus concentration and RNA extraction with two-step RT-qPCR at our MIT partner laboratory.
v1.0	6.4 copies/mL	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
v2.0 (current)	Full reports contain an updated case estimate, with a model derived from mining our proprietary wastewater dataset. This updated analysis does not affect any of the data shown in this one-page report.
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.

Data Analysis & Model version	Description
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 is reported. Full reports also report a case estimate, with a model derived from values reported in scientific literature.

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
v1.0 (current)	Covid-19 Community Report with virus concentration, normalized virus concentration, time series of normalized virus concentration, and time series of cases in county with 7-day rolling average.