Carmel Area WD (CA) - Facility Influent

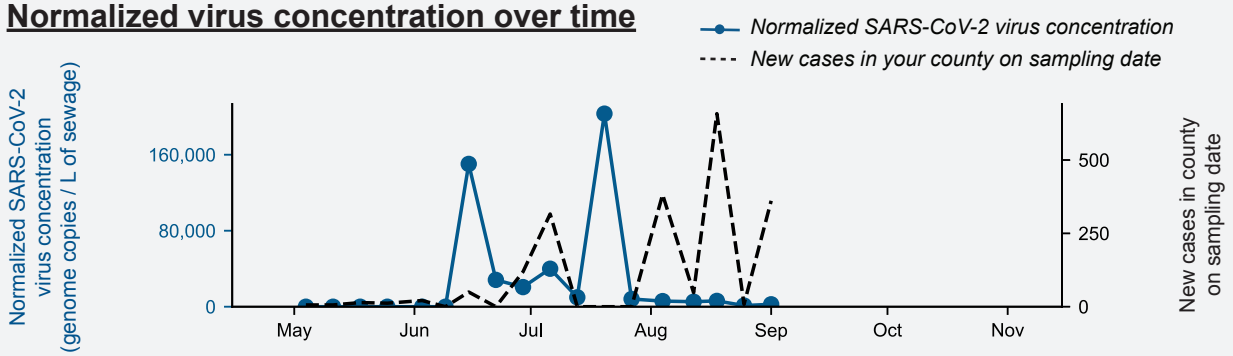
Sample collection date: **September 1, 2020**

SARS-CoV-2 virus in sewage

DETECTED	Virus concentration (genome copies per liter of sewage)	Normalized* virus concentration (genome copies per liter of sewage)
	14,813	**2,543**

**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



Regional overview of normalized virus concentration levels - West

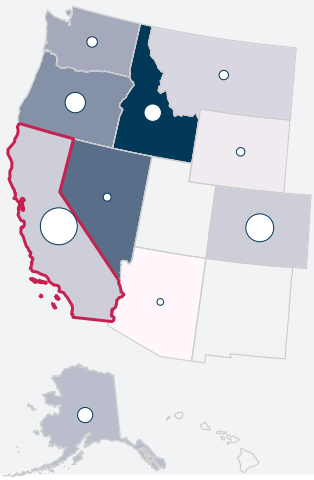
State-level median of collected samples (since June 1st)

lower — higher

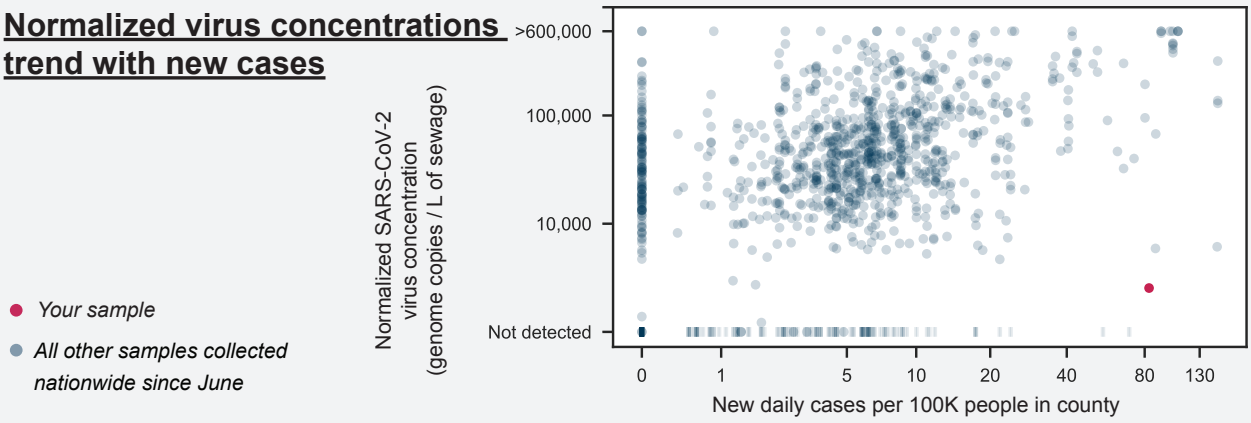
Color indicates normalized virus concentration level

Your state outlined in red

Size of circle indicates population served by participating facilities, ranging from 10k to 9mil

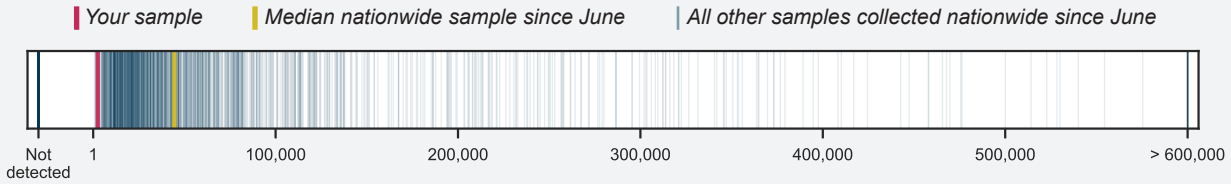


Normalized virus concentrations trend with new cases



Normalized virus concentrations in comparison

Your sample has higher concentration levels than **1%** of all quantifiable samples collected since June.



Case Estimates Reflect Active R&D

Evaluate as beta results when comparing to reported Covid19 cases. We will update our customers when we modify this model.

For more information, read the whitepapers:

https://doi.org/10.1101/2020.04.05.20051540
https://doi.org/10.1101/2020.06.15.20117747

Biobot COVID19 case estimate

80 new cases
(0.53% incidence rate)

Using a reported flow rate of 1.0 MGD

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 case estimate
We convert the raw viral concentration into a COVID19 case estimate using the following equation:

$$\textit{Number of Covid19 cases} = \frac{\textit{Viral concentration* x Flow rate on sampling date}}{\textit{Virus shed per infected person per day**}}$$

*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 estimated from research studying shedding in Covid-19 patients.

The case estimation equation uses your reported flow rate and the measured virus concentration, but does not use your reported catchment population.

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
v2.3 (current)	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 versions

We are constantly iterating on and improving our data processing and analysis to improve the interpretability of our data. You can see which version of our analysis 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 version	Description
v1.2 (current)	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
v3.2	Major changes: • The normalized viral concentration is shown in the report, so you can reproduce the time series plot on your own. • The sampling date shows the sampling end date (before it was the start date) to be in line with the industry standard. • We removed the box with county-level data from USA Facts on cumulative and new cases, so you can compare the Biobot data with your most relevant local datasets. Minor changes: • We changed the language around case estimates based on internal R&D to reflect new cases that will be predicted in your county by next week. • We updated language on charts to reflect the new normalization units, and removed redundant explanatory text. • We added legends to the timeseries and sample distribution plots. • We updated all visualizations to reflect data collected since June. • We removed the "percent change" callout in the time series and added axes with units.