

## **COVID** patients exhale up to 1,000 copies of the virus per minute during first eight days of symptoms

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(A) Levels of exhaled SARS-CoV-2 RNA copies per minute over the course of infection. Each dot represents the daily average number of exhaled SARS-CoV-2



RNA copies exhaled per minute, for each participant. The overlaid black line represents the rolling average of exhaled viral RNA copies over days since symptom onset. The overlaid red line is the fitted S curve ( $R^2 = 0.7657$ , adjusted  $R^2 = 0.7567$ , RMSE: 0.3755). The green vertical dashed line indicates the inflection point of the S curve. (B) Viral RNA copy numbers binned over days since symptom onset. Each dot represents results from one sample. Mean and standard error are represented by red lines within each bin. \* indicates P T equivalent. Credit: (2023). DOI: 10.1101/2023.09.06.23295138

COVID patients exhale high numbers of virus during the first eight days after symptoms start, as high as 1,000 copies per minute, reports a new Northwestern Medicine study.

It is the first longitudinal, direct measure of the number of SARS-CoV-2 viral copies exhaled per minute over the course of the <u>infection</u>, from the first sign of symptoms until 20 days after.

On day eight, exhaled levels of <u>virus</u> drop steeply, down to near the limit of detection—an average of two copies exhaled per minute.

Northwestern investigators tested <u>breath samples</u>—collected multiple times daily from 44 individuals—over the entire course of infection to determine when a person is most infectious.

The study has been accepted for publication in *eLife* and has been posted as a preprint on <u>medRxiv</u>.

Mild and moderately symptomatic patients with COVID still exhale large amounts of virus, though severely symptomatic cases exhale higher levels on average, the study reports.

Vaccinated and unvaccinated patients exhale similar levels of virus over



the course of infection, the research shows.

The amount of virus being exhaled while infected was the same no matter which variant a person was infected with—people infected with alpha exhaled just as much as those infected with omicron, the study reports.

"An important question in understanding transmission of SARS-CoV-2 is how much virus a patient is exhaling into the environment over the course of their infection and for how long," said senior author Christina Zelano, assistant professor of neurology at Northwestern University Feinberg School of Medicine. "During COVID-19 infection, at what point are you exhaling a lot of virus, and when do you stop breathing it out?"

"The vast majority of research on <u>viral loads</u> over the course of a COVID-19 infection has been based on nasal or oral swabs, which measure virus in the nose or throat," said lead study author Gregory Lane, senior research project manager in Zelano's lab. "However, SARS-CoV-2 is spread through breath, and virus on the breath may not match virus in the nose. The dynamics of viral shedding on breath over the course of infection are poorly understood, despite the fact that this is how the virus spreads."

Also unknown is the relationship between levels of viral RNA on breath and symptom severity, vaccination status, virus variant and patient age and sex. To answer these questions, Zelano said, we needed a method to directly measure viral RNA on breath, repeatedly in the same patient over the course of their infection.

"We developed this easy, cheap method and used it to collect over 300 breath samples from 44 patients over the course of their infections—multiple samples a day over multiple days," Lane said.



With this new device, investigators detected viral RNA in 100% of specimens collected from COVID-positive patients who were within 10 days of symptom onset and in none of the specimens collected from COVID-19 negative patients—a very high rate of accuracy.

The study findings could be used to calculate the amount of time it takes for an individual to exhale an infectious dose of SARS-CoV-2, Lane.

"For example, if we assume the infectious dose for COVID is 300 copies, then a person who is exhaling 1,000 viral copies per minute would exhale an infectious dose in 20 seconds (highly risky in an elevator), whereas a person who is exhaling two viral copies per minute would exhale an infectious dose in about two hours (probably safe in an elevator)," Lane said.

It is not yet known what an infectious dose of viral airborne particles is.

For the study, Northwestern scientists created and patented a portable, non-invasive, inexpensive device to collect breath samples from infected people, and used qPCR to measure SARS-CoV-2 in those samples. They used this device to test for the presence of the virus over 10 minutes of natural, relaxed breathing, establishing a conservative baseline of exhaled virus amount. Talking, singing or shouting would likely increase amounts of exhaled virus.

## How the study was conducted

Scientists shipped the device to patients' homes, allowing them to selfcollect samples from their own home, which were mailed back to the lab for analysis. They recruited patients testing for COVID-19 from Northwestern Medicine. Participants watched a video online to learn how to collect samples. Patients provided multiple samples per day over the entire course of infection.



Samples were mailed back to the lab, where qPCR was used to quantify numbers of viral RNA copies in breath specimens.

"Once you can answer the question of how many viruses you are exhaling for each day after your symptoms start, critical pieces of the puzzle of how to avoid the spread of COVID-19 fall into place," Lane said. "This information speaks directly to when someone with COVID-19 should isolate; when they are more likely to infect other people by breathing out virus into the air around them; and when they become much less likely to spread the infection. These two pieces of information are very important for infection control and for informing public health recommendations."

The study measured virus on breath from the first day symptoms start, which is day zero. From that day until day eight, <u>infected people</u> exhaled lots of virus, the scientists found. The amount varied, with peaks of 1,000 viral copies a minute, but the average was high. Then, on day eight, it dropped steeply to levels that hovered around the limit of detection (about two copies a minute), with few exceptions.

Because the device is scalable (the paper provides instructions to make it), it can be used for other respiratory diseases to obtain measurements to find out how much of a pathogen is on the breath.

**More information:** Gregory Lane et al, Quantity of SARS-CoV-2 RNA copies exhaled per minute during natural breathing over the course of COVID-19 infection, *medRxiv* (2023). <u>DOI:</u> <u>10.1101/2023.09.06.23295138</u>

Provided by Northwestern University



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