CRISPR-based diagnostic technology rapidly detects different COVID variants and other pathogens

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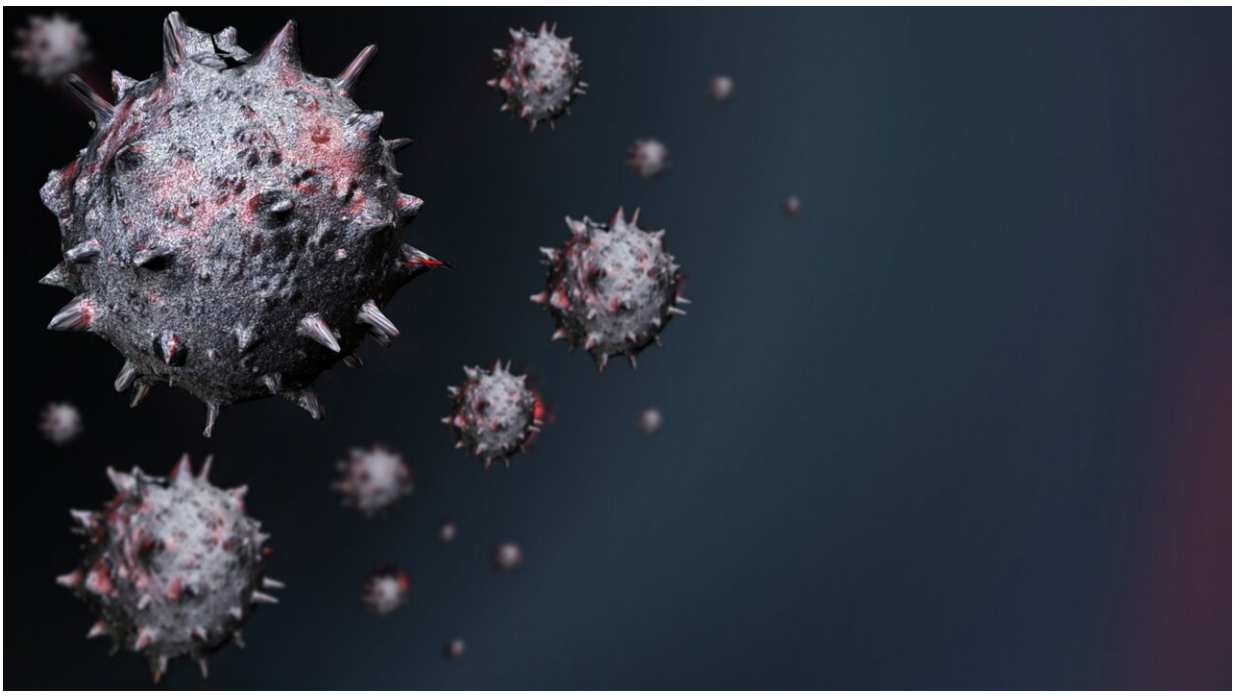

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Researchers at the Broad Institute of MIT and Harvard and Princeton University have developed a CRISPR-based technology that can rapidly differentiate between omicron, delta, and other COVID-19 variants, as well as other respiratory viruses including flu. The team used the

method, known as mCARMEN, during the beginning of the omicron surge in December 2021 to get a provisional look at the rising prevalence of the variant in Massachusetts. Based on those results, the Massachusetts Department of Public Health (MDPH) shared this information with hospitals in the state to help guide treatment options for COVID-19 patients.

To design mCARMEN, the researchers adapted CARMEN, the CRISPR-based diagnostic technology developed at the Broad in 2020, to be faster, more sensitive, and more easily implemented in clinical and surveillance labs, with the goal of using the optimized platform more widely during future outbreaks of SARS-CoV-2 or other pathogens.

mCARMEN, an acronym for "microfluidic Combinatorial Arrayed Reactions for Multiplexed Evaluation of Nucleic acids," is described in *Nature Medicine.*

"The COVID-19 pandemic shows us that we need more testing, more often, particularly early on in a pandemic," said co-senior author Cameron Myhrvold, who helped develop CARMEN as a postdoctoral researcher at the Broad and is now an assistant professor of molecular biology at Princeton University. "COVID-19 shows us that challenging viruses will keep emerging, so we have to keep looking for them and come up with better ways of doing that."

"It's been wonderful to work on this big collaborative project," said study first author Nicole Welch, a graduate student in the Virology Program at Harvard University and in the lab of Pardis Sabeti at the Broad. "We're excited that it's already making a difference in this pandemic, and we hope to see its continued use to improve public health."

CARMEN V1

Researchers in the labs of Sabeti and Broad core member Paul Blainey first developed the CARMEN platform using custom microfluidics chips and CRISPR-based guide RNA molecules that can detect specific sequences in a pathogen's genetic material. In a 2020 study, they showed that CARMEN could identify a single type of virus in more than 1,000 samples at a time, or search for more than 160 different viruses, including the COVID-19 virus, in a small number of samples.

However, the original CARMEN platform requires custom equipment, involves a manually intensive 8- to 10-hour workflow, and offers a lower throughput than what's needed during a pandemic. To better respond to public health threats such as the emergence of new SARS-CoV-2 variants, the researchers refined the technology in 2021 to make it more clinically useful and allow it to detect multiple viruses and variants quickly.

The team adapted CARMEN to work on the commercially available Fluidigm microfluidics and instrumentation platform, making it easier to run and cutting the run time in half. The researchers also streamlined the workflow for greater sensitivity, so that it can detect pathogens in samples with less genetic material. In addition, by using CRISPR-based enzymes Cas12 and Cas13 in combination, mCARMEN can not only spot the presence of a virus, but also measure the amount of virus in a sample—information that can indicate a patient's ability to infect others or whether a treatment is working.

Viewing variants

In their latest study, the scientists showed that mCARMEN could distinguish between 21 different human respiratory viruses in patient samples, including SARS-CoV-2, other coronaviruses, and influenza strains, performing as well as the original CARMEN technology.

Working with partners at Massachusetts General Hospital, they further demonstrated mCARMEN's ability to detect and distinguish between a number of human respiratory viruses in samples from patients.

To enable mCARMEN to both diagnose patients and monitor emerging COVID-19 variants, the researchers also developed a way for the platform to track changes in the SARS-CoV-2 virus. They devised a "variant identification panel" of CRISPR-based guide RNAs that recognize more than two dozen mutations in the virus's spike protein. Each variant has a unique pattern of these mutations that can serve as a "fingerprint" for the variant in a sample. With this panel, the team showed that mCARMEN can look for these fingerprints and distinguish between six SARS-CoV-2 variant lineages, including delta and omicron, as accurately as can be done with viral sequencing.

The researchers say that mCARMEN can also help flag new variants. If an unknown viral mutational fingerprint shows up in a sample, it can signal that a new variant may be emerging, which can be confirmed with more in-depth viral sequencing.

In partnership with the US Centers for Disease Control and Prevention and the MDPH, the Broad Institute has been conducting large-scale viral sequencing to support COVID-19 genomic surveillance since March 2021. With the emergence of the omicron variant at the end of 2021, the MDPH requested that Broad use mCARMEN to test COVID-19 specimens from across Massachusetts and provide a faster, preliminary analysis of omicron's prevalence for public health situational awareness. In late December 2021, the Genomics Platform handed off a subset of COVID-positive samples from its viral surveillance workflow to the team developing the mCARMEN platform.

The mCARMEN team used their platform to process nearly 1,000 samples in a day and quickly generate a picture of the variants

circulating within the state. They estimated that omicron had been the dominant COVID-19 variant in Massachusetts since mid-December. In addition to demonstrating the platform's ability to rapidly generate insights from a relatively small number of samples, the finding also had a real-time impact on the state's COVID response. MDPH was able to reach out to area hospitals and advise that when using monoclonal antibodies to treat COVID-19 patients, they should now choose ones better suited to omicron than to delta or other variants.

"With mCARMEN's quick turnaround time, we could help support that immediate public health response," said Welch. "The omicron outbreak shows the need for a diagnostic that gives more information than standard PCR-based testing, without the cost or time required with viral sequencing."

mCARMEN would require regulatory approval to be used to diagnose patients. For now, the team is continuing to use the technology in collaboration with colleagues in the Genomics Platform, the CDC, and MDPH to monitor for omicron and other COVID-19 variants. As new variants emerge, they can easily add new panels of variant mutations to the mCARMEN workflow, to learn what's circulating and support public health responses.

The team is also working with public health departments across the country and beyond to utilize mCARMEN for virus surveillance in those regions. In addition, they are supporting collaborating researchers who are developing new applications of the platform beyond the COVID-19 pandemic, such as detecting and discriminating bacterial infections and monitoring for antibiotic resistance.

"Our hope is to disseminate this technology much more broadly," said co-senior author Pardis Sabeti, institute member at the Broad, professor at Harvard University, and Howard Hughes Medical Institute Investigator.

"With this update to the CARMEN platform, we can design panels for a variety of uses, which will help us prepare for the next infectious disease threat."

More information: Nicole L. Welch et al, Multiplexed CRISPR-based microfluidic platform for clinical testing of respiratory viruses and identification of SARS-CoV-2 variants, *Nature Medicine* (2022). DOI: 10.1038/s41591-022-01734-1

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