Bench to Bedside

Virus Surveillance and Diagnosis With a CRISPR-Based Platform

Tracy Hampton, PhD

A newly developed technology draws on the latest genome editing advances to detect viruses at scale—including the one that causes coronavirus disease 2019 (COVID-19)—for surveillance and diagnostic purposes. The innovation, recently described in *Nature*, uses clustered regularly interspaced short palindromic repeats (CRISPR) and CRISPR-associated protein 13 (Cas13), gene editing tools based on a bacterial defense system against invading viruses.

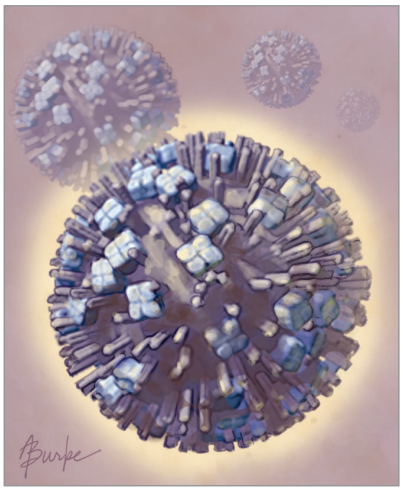
Unlike previous CRISPR-Cas13–based diagnostics, the new approach can complete thousands of tests simultaneously on a single microfluidic chip through a process that gives results in a matter of hours. Dubbed CARMEN (short for the combinatorial arrayed reactions for multiplexed evaluation of nucleic acids), the technology enables more than 4500 tests on a massive-capacity chip.

The nimble platform can be used to detect a single type of virus from more than 1000 patient samples or to look for more than 160 different human-associated viruses in a small number of samples. In one analysis, CARMEN's developers tested dozens of patient samples for 169 different human-associated viruses with published genome sequences.

The process uses randomness. Typically, if patient samples are being tested for many different viruses, each sample must be tested against each virus. Instead of forcing these pairs, the researchers let them associate randomly. Doing this enough times ensured that, statistically, every possible pair was achieved.

"We were pleasantly surprised to see that the method performed very well, both in terms of sensitivity and concordance with other methods for nucleic acid detection, such as next-generation sequencing," the study's co-lead author Cameron Myhrvold, PhD, a postdoctoral fellow at the Broad Institute of MIT and Harvard, told *JAMA* in an email.

The new approach incorporates microfluidics with a CRISPR-based detection technology developed in 2017 called the Specific High-Sensitivity Enzymatic Reporter Unlocking (SHERLOCK) platform. CARMEN relies on tens of thousands of microwells, small compartments that each hold 2 nanoliter-sized droplets: 1 containing viral genetic material from a sample and the other containing virus detection reagents.



The viral genetic material is first amplified into multiple copies and then labeled with a unique fluorescent dye. When a detection droplet containing CRISPR-Cas13 identifies its specific viral sequence target in a sample droplet in the same microwell, a fluorescence microscope detects the resulting color-coded signal.

"We realized that there was a need to perform multiplexed nucleic acid testing at scale, which pressures both the technical and cost performance of a test platform," Myhrvold said. "We reasoned that a CRISPR-based technology, with its exquisite sensitivity and specificity, might be able to perform well enough in a microfluidic context to enable that vision to be realized—so we embarked on developing the CARMEN technology."

Myhrvold said CARMEN even discovered a few viruses in patient samples that his team didn't know were there, including an anellovirus and a pegivirus. They confirmed these discoveries with next-generation sequencing. The scientists also quickly incorporated detection mixtures for the novel coronavirus that causes COVID-19 when it emerged and used the technology to successfully test for drug-resistance mutations present in blood samples from patients with HIV.

The technology's ability to detect subtle differences in viruses represents an especially promising application. The method successfully differentiated between subtypes of influenza A strains in patient samples. "This would come in handy, for example, in monitoring which flu strains are gaining traction in a given year," said Richard Sherwood, PhD, an assistant professor of medicine at Brigham and Women's Hospital who conducts CRISPR-related research and was not involved with the study.

Sherwood, as well as the study's investigators themselves, noted that CARMEN may be useful as an additional method for detecting pathogens but that other current and investigational techniques will continue to be important. "CARMEN will complement them, not replace them," Sherwood said in an email.

In its current incarnation, the platform requires microfluidic chips, fluorescence microscopes, and other special laboratory equipment and supplies, but the team is working to adapt the technology to expand its use in settings without these resources. Regardless, Sherwood said that screening locations such as airports or office buildings may be more suited to testing methods such as SHERLOCK that could be run on a strip of paper like a pregnancy test.

The new platform's biggest promise may be its high-throughput capacity. More than 400 patient samples can be tested for coronaviruses at the same time using the team's custom chip, for example. "CARMEN could be useful to surveil populations for signs of whether an outbreak is emerging or waning since it is so much cheaper per test than other strategies and can be rapidly adapted on the fly based on new sequence data," Sherwood said. ■

Note: Source references are available through embedded hyperlinks in the article text online.