

# SARS-CoV-2 Delta Variant PCR Could Help Surveillance Efforts, Developers Say

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NEW YORK - Diagnostics developers have recently created simple, fast PCR tests for the Delta variant of SARS-CoV-2 with hopes of enabling quick screening of virus-positive samples to find signature variant SNPs that could be used to focus sequencing efforts and save time and money.

But with Delta now making up nearly all of the approximately 1 million new cases in the US, the utility for such PCR screening has become more nuanced.

Delta variant assays have been developed by labs at Hackensack Meridian Health (HMH) and Rutgers University, as well as by commercial teams at BGI, SpeedX, and DiaCarta.

A mere 12 weeks ago, the Delta variant accounted for 6 percent of sequenced SARS-CoV-2 samples in the US. It now makes up almost 99 percent of infections, according to [data](#) from the US Centers for Disease Control and Prevention. But, the US surveillance system data comes from "thousands" of whole genome sequences provided every week through the agency's national genomic surveillance efforts, according to CDC's website, and in the past seven days CDC [data](#) shows that there have been more than 1 million new infections diagnosed in the US.

This month, the World Health Organization published [guidance](#) for SARS-CoV-2 variant surveillance that suggested targeted sequencing of specimens with a higher pre-test probability of being a variant of concern might be beneficial, in addition to traditional approaches. The WHO guidance lists PCR-based single SNP detection assays among potential triggers for targeted sequencing.

The HMH team routinely screens all positive patient samples from its more than 130 clinics in New Jersey using high-throughput PCR-based testing.

"We do a deeper dive here," said David Perlin, chief scientific officer and senior vice president at HMH's Center for Innovation and Discovery. "We want to provide a more detailed look as to what is going on with the pandemic in our region," he said, adding that sequencing everything became impractical for this purpose during peaks in cases which necessitated testing thousands of samples per week.

The HMH team had been screening for Alpha, Beta, and Gamma variants since the beginning of 2020, as [previously reported](#). The lab added a Delta assay in May. It also sends out samples for whole-genome sequencing, in part to identify clades and other mutations in Delta that are now popping up.

In May, 15 percent of the viruses the HMH team tested were Delta, Perlin said. By June it had risen to 50 percent, and by July Delta had swamped out everything else.

Although Perlin had been expecting Delta's arrival in the US, and had been collaborating with colleagues in India and the UK, "The speed with which Delta overtook all the existing resident viruses

here surprised me — I didn't think that was going to be possible because I've never seen anything like that," he said. "This is evolution in real time."

Surprisingly, there are few publications of Delta variant allele-specific PCR tests, though there had been many pre-prints for other variants of concern. Perlin thinks that at this stage, it may be more difficult to get a methodology publication accepted, but his lab expects to publish its Delta assay with NJ case data in the future.

The lab of David Alland at Rutgers University has also developed an assay for the Delta variant. The *Journal of Clinical Microbiology* [publication](#) of this assay in July, co-authored with scientists at Cepheid, noted that a Delta test was in the works, and senior author Alland confirmed in an email last week that the lab has now finished the development process.

Commercial Delta variant tests are also available from developers such as BGI, SpeedX, and DiaCarta, and one is in development from ID Genomics.

### Enabling innovations

The Delta variant assays are essentially basic PCR genotyping tests. Both the BGI and HMH assays target the L452R and E484Q mutations, the DiaCarta Delta test targets L452R only, and the SpeedX Delta test detects a mutation called P681R.

The tests can run independently or in conjunction with other variant assays. For example, the SpeedX [variant assay](#) to detect Alpha, Beta, and Gamma can be adapted to add Delta, while the DiaCarta Variants Detection Kit detects mutations associated with Alpha, Beta, Gamma, and Delta in one test.

But, although they are basic allele-specific PCR tests, these assays for Delta each offer enabling technologies or elements that are somewhat unique in the PCR space.

For example, the SpeedX assays use the firm's proprietary [PlexPCR](#) multiplexing chemistries, while the DiaCarta kit uses xenonucleic acids, or XNAs, in its probes, as recently described in a [medRxiv](#) pre-print.

"XNAs hybridize tightly to complementary DNA target sequences only if the sequence is a complete match," said Michael Sha, chief technology officer and senior VP of research and development at DiaCarta. This "clamping" effectively blocks strand elongation of non-mutant viruses, improving specificity.

The in-development test from ID Genomics purports to 'fingerprint' all currently known COVID-19 variants of concern or interest, including Alpha, Beta, Gamma, Delta, Delta-plus, Epsilon, Lambda, simultaneously, and offers the potential to detect the emergence of novel variants using a technology called multi-band dipstick DNA chromatography.

The BGI tests, meanwhile, are adaptable to large-scale testing, such as in the firm's more than 80 Huo-Yan inflatable labs that it has set up in nearly 30 countries and regions.

The Rutgers team's assay for Alpha and Beta variants uses sloppy molecular beacon probes, which purportedly enhance specificity as well.

And the unexpected element of the HMH assay is that the high-throughput workflow is powered by the [Mic](#), a tiny thermal cycler instrument from BioMolecular Systems. Indeed, the HMH team got its Emergency Use Authorization for a SARS-CoV-2 test from the US Food and Drug Administration in March of 2020 using its Mic-based workflow. The overall workflow also includes of a brief heat treatment, beacon molecular probes, and melt curve analysis.

The HMM lab has already purchased additional Mic instruments in order to scale up testing should the Delta surge get worse, which Perlin said it also used during a most recent peak in cases, and easily managed testing thousands of samples each week.

### Utility given Delta dominance

Viral sequencing-based surveillance is costly and time consuming, and perhaps consequently it can typically only sample a small portion of infections to extrapolate the proportions of different viral strains in the population.

But had high-throughput PCR-based variant surveillance been in use from the beginning, it may have been possible to more rapidly see the dangers of Delta and allow for the implementation of mitigation efforts, according to SpeeDx Senior VP of Clinical Affairs Charles Cartwright.

Futhermore, "We suspect that laboratories currently engaged in large-scale sequencing efforts could greatly decrease their cost basis and improve overall throughput by using a PCR method to screen viruses prior to sequencing," Cartwright argued.

Perlin's lab hopes to use PCR testing, in part, to better track variations of Delta that make it more dangerous.

And, although only about 1 percent of cases are not Delta at this point, the next dangerous variant could also potentially be detected by focusing sequencing efforts on samples that are negative for Delta.

"Variants have a way of sneaking up on researchers and the public health system, which mean that we all need to be vigilant," said Charles Bao, general manager of BGI Americas.

"PCR-based genotyping is rapid, sensitive, and cost-effective," Bao also said, noting that the firm's assay runs on PCR machines commonly found in most molecular diagnostic labs. On the other hand, next-generation sequencing "produces data that we may not need, and the NGS results may still need to be validated by a PCR method."

DiaCarta's Sha also emphasized that the more than 95 percent Delta dominance is an estimate that comes from sequencing a limited amount samples, which in turn is a cost- and time-saving strategy.

"However, a qPCR-based variant detection kit may help to overcome this bottleneck," he said, such that the variant identification of many more samples could be determined.

DiaCarta has submitted its test for combined SARS-CoV-2 detection and variant determination to the FDA for EUA, Sha said.

Still, Cartwright noted that as of yet there are no broad recommendations for the use of such assays in routine diagnostic procedures, "and thus it is unclear exactly how widely they will be used in the US."

Indeed, David O'Connor, a virologist at University of Wisconsin, Madison, noted in an interview that his lab has not used PCR-based testing for variants, in part because the genomic context that can be obtained by sequencing is important.

And, "If you target only specific mutations that you know, those that might be important but aren't part of your assay can be invisible," he said in an email.

O'Connor, who recently co-authored a [study](#) suggesting the Delta variant is shed at comparable levels by vaccinated people who get infected, said that it is difficult to know how much sequencing-based

surveillance is enough.

This is in part because "our understanding of how, when, and in what ways the virus will change remain so fluid," O'Connor said.

For example, his local community has high vaccination rates, and he would have thought this could act as "a firewall" against rapid Delta encroachment. Instead, "we saw it rise from a small minority of viruses to virtually all circulating viruses in less than a month," he said.

In terms of general surveillance, "we have to be alert to what is coming next," O'Connor said. "Continuing sequence surveillance will alert us to the presence and spread of the next variants of concern that are likely to emerge with a Delta 'backbone'."

That said, a use-case for PCR variant screening or testing might be more easily made when a variant impacts therapy.

For example, HMH screening during the era of dueling Beta and Gamma variants was useful to track proportions of these variants as well as to guide therapy.

"The E484Q or R mutations render the virus less sensitive to some of the antibody cocktails, so that was important information to relay to our clinicians," Perlin said. The health system even switched from one monoclonal antibody manufacturer to another when it found a quarter of the viruses it was detecting by surveillance were likely to resist treatment.

Now, Perlin worries about infections of immunocompromised people and those with waning immunity after vaccination. "We want to know what we are looking at — is it Delta, or some version of Delta that now is more successful, and if it is, is this regional, are there pockets of infection — there is a lot of epidemiology related to protecting those who are highly susceptible," he said.

It is possible to squelch infections, he added, when timely data can inform shutdowns and quarantines of healthcare units, for example.

Still, "Right now, there isn't a different treatment approach that one would use for Delta versus another variant," UW's O'Connor said, but further added, "It might not always be that way in the future."

And now, "The virus is now part of our natural world," O'Connor said. In addition to humans, it infects animals, and, "It is going to continue to spread through people and animals, and potentially change and shape-shift as time goes on," he said. "Better, faster, easier, cheaper diagnostics, including sequencing, and medical countermeasures can hopefully suppress the virus to low levels where community spread is sporadic and manageable, but there needs to be an understanding that flare-ups are all but inevitable," he also said.

SpeedX's Cartwright, meanwhile, said it would be naive to assume that Delta represents the summit of SARS-CoV-2's transmissibility and immune escape. Enhanced surveillance "via easily implementable PCR screening tests" could therefore be critical to prevent further waves of infection with new, more dangerous mutants, he said.

Perlin, for example, noted that Lambda is an immune escape variant that is both highly transmissible and has also demonstrated resistance to antibody therapies. "It is all throughout South America, yet it hasn't become predominant here — that's a major concern," he said.

The HMH team has already added the F490S target specific for Lambda to its high-throughput screening workflow.

Reducing the virus reservoir to a low enough level that there are fewer new variants may be the only way out of the pandemic, Perlin said, and this requires global vaccination efforts.

"I think we're talking at least another year," he said, suggesting that increased testing, even among asymptomatic and vaccinated people, will be needed.

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