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Multiplex Fragment Analysis for Flexible Detection of All SARS-CoV-2 Variants of Concern

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Guest: Dr. Jeff SoRelle from the University of Texas Southwestern Medical Center in Dallas, TX.

Bob Barrett: This is a podcast from *Clinical Chemistry,* sponsored by the Department of Laboratory Medicine at Boston Children's Hospital. I am Bob Barrett.

Over the past two years, variants of concern of the severe acute respiratory syndrome coronavirus-2 have emerged as significant mutations that impact transmission of COVID-19, vaccine responses, severity of the disease, as well as other factors. Rapid identification of SARS-COV-2 variants is therefore needed in both clinical and public health arenas. Whole genome sequencing remains the current gold standard for variant identification but widespread use is challenging due to requirements for specialized equipment and bioinformatics expertise. Genotyping approaches are rapid methods for monitoring variants but require continuous adaptation. However, DNA fragment analysis may represent an approach for improved SARS-COV-2 variant detection. A paper appearing in the August 2022 issue of *Clinical Chemistry* examined a multiplex fragment analysis procedure for flexible detection of SARS-COV-2 variants of concern to help address this very issue. We are pleased to have the senior author of that paper with us as a guest in this podcast. Dr. Jeff SoRelle is an Assistant Professor in the Department of Pathology at the University of Texas Southwestern Medical Center in Dallas. He is a physician scientist with a research focus on the genetics of allergic disease. However, during the current pandemic, he has broadened his interest to create a technique that can detect all currently known variants of SARS-COV-2. So, to start with, Dr. SoRelle, what are the various ways laboratories currently use to detect variants of SARS-COV-2?

Jeff SoRelle: There's a variety of ways and they range from the fast to the more laborious. On the faster side are the sort of targeted PCR tests where normally a person that is being tested for COVID has just one test and it says positive or negative. These tests look at several different targets at the same time to look at several different mutations present in the variants. And then on the more laborious side is doing whole genome

sequencing. This is the equivalent of reading the genome of a COVID variant in an entire book. All of the different letters and nucleotides and it reads the entire book cover to cover. And for the comparison, it would be for the faster tests, they just look at a few words and say these words are most specific to the first edition or the third edition and try to interpolate with the variant based off of that.

Bob Barrett: How does the test described in the August 2022 issue of *Clinical Chemistry* compared to those methods? Is it faster, more sensitive, more comprehensive, or all of the above?

Jeff SoRelle: Yes, I'd sort of put it in between two ways because the usual fast method can only look at about four words or targets at one time. It can be pretty quick, about four hours. Our test is similarly quick and similarly affordable compared to whole genome sequencing, which does take about two weeks to do, requires quite a bit of expensive sequencing, and has to read the same sequence about a million times to really get a good idea of what's actually happening there. So, our test is definitely a lot faster. It looks at eight targets as I said, and it can look at them in a little bit of different ways. For instance, there are some areas that have deletions or insertions in the genome, and we can find differences in those insertions or deletions by changing the fragment size of the PCR product. We basically look at the size of a segment of DNA and if it gets shorter or longer, that's one of the signals that we have a variant. And most of the traditional ways of looking at that in the fast method of PCR aren't able to see those changes quite as easily. So, that's sort of what is unique about it is that it can adapt to a lot of the different variants because they all have these different sized insertions or deletions and we don't have to change how we're looking at it to see those differences.

Bob Barrett: Let's talk some more about how this test actually works. How complex is it and what sort of laboratory can competently carry it out?

Jeff SoRelle: For me, I've done an array of molecular testing and I'd say it's on the lower end of complexity for me, but probably in the higher end of complexity for most laboratories. The difficulty of it is that it requires instrumentation such as Sanger sequencers, that are not available in every single laboratory. Usually a molecular laboratory is going to have one of these, and usually more at larger centers, academic centers, or larger hospital networks. But once you have that and you get to the data, it's relatively easy to interpret because basically you have eight peaks and for five of those peaks you say, is it the right size or is it larger or smaller, is it shifted to the left or to the right?

And the other three peaks, they're sort of specific to similar mutations that occur in a receptor binding domain of the spike protein, which is important for infection. And the way we design those is it has a color that is specific to the mutant. So, it turns green if it's mutated and it's just blue if it's wild type. So, as far as that interpretation part goes, it's relatively simple and could be done in about five minutes for a single case.

Bob Barrett: So, you're saying that this is a very adaptable system. Does that mean it will be able to detect all future variants also?

Jeff SoRelle: I think so and there's no way to predict the future. But we've been able to detect all variants since Delta, even before Delta came about. And we haven't had to change a single thing on the assay to date. So, I think that we're able to see enough things that keep changing along the way that we continue to see any of the major variants of concern including those that you may not even remember, such as some from South America or India or some sub-variants of Omicron people may not be aware of but we've still been able to detect them all along the way.

Bob Barrett: Why would a patient or their physician need to know what particular SARS-COV-2 variant they have? How would that change their management?

Jeff SoRelle: It's a really good question and something that, aside from curiosity, is important for patients because when the Omicron variant came out, the previous monoclonal antibody therapies that were very effective for preventing worse illness were no longer effective against Omicron. They couldn't bind to it because the targets had changed and there was more mutations. But then they did come out with a new drug but was in limited supply and so to make sure the right patient could get the right drug, knowing the variant would allow us to give a Delta-specific or Omicron-specific antibody. And then when more sub-variants came out, such as BA.2, the original Omicron antibody was no longer working. So, we had to use a new one. And so, during these times when new variants come out, we have to sort of change our treatment recommendations based on the variant somebody has. And we have to know that in a pretty quick time frame for it to be effective. So, we can't wait for sequencing, which usually take at least one to two weeks. That's how this test is very useful as far as giving variant-specific information in a clinically actionable timeframe.

Bob Barrett: What about from an epidemiological point of view, tracking wise, how important is identification of a particular variant?

Jeff SoRelle: I think it's really important to try and stop the spread of many of them and people are getting new ones that come out all

the time. And it's been very helpful to partner with our local public health departments to provide them this information on a much faster basis whereas previously it was taking them almost four weeks to get variant results back, at which time, they could not really do much for the patient or anybody around them. So, it's important to track what things are happening, who you were contacted with and we can see this sort of thing being important going forward as new variants may have, maybe of more concern, arise.

Bob Barrett: Well finally, Dr. SoRelle, what are some potential challenges for other laboratories to implement this CoVarScan test?

Jeff SoRelle: One of the main challenges is that we don't have this test commercially available and I would love for it to be that way, just hasn't gotten a good partnership going yet but it's usually easy for labs to work with kits and have something out of the box they can open up and run. The other main thing would just be the instrumentation required. Currently, the Sanger sequencers that we're all used to are very large, taking up half of a bench, pretty expensive, but they're coming out with many smaller versions that are easier to use and much more affordable. And I think that would make this easier to use in other labs that would be able to get results within four hours from the time you find a positive COVID test result.

Bob Barrett: That was Dr. Jeff SoRelle from the Department of Pathology at the University of Texas Southwestern Medical Center. He has been our guest in this podcast on a new multiplex fragment analysis procedure for flexible detection of SARS-COV-2 variants of concern. His paper with other researchers from the University of Texas Southwestern Medical Center on that topic appears in the August 2022 issue of *Clinical Chemistry*. I am Bob Barrett. Thanks for listening.