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Evaluation of Neutralizing Antibodies against SARS-CoV-2 Variants after Infection and Vaccination Using a Multiplexed Surrogate Virus Neutralization Test

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Guests: Dr. Shuxia Zhou from Bio-Rad Laboratories and Dr. Alan Wu from the University of California, San Francisco.

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

Over the past two years, since the start of the COVID-19 pandemic, distinct variants of the SARS-CoV-2 virus have been identified and are driving new infections globally. Serological tests for monitoring antibody responses, post-virus infection and vaccination have been developed and are used widely in clinical settings. Neutralizing antibody assays are important for providing information on protective immunity against SARS-CoV-2 after infection and vaccination. However, the traditional or gold standard virus neutralizing assay plaque reducing neutralizing test requires Biosafety Level 3 facilities and well-trained investigators because of handling live pathogenic viruses. Furthermore, the turnaround time of the assay is rather long and it can take three to five days to complete the test.

A paper appearing in the May 2022 issue of *Clinical Chemistry* examined issues with these antibody tests that article, titled "Evaluation of Neutralizing Antibodies against SARS-CoV-2 Variants after Infection and Vaccination Using a Multiplex Surrogate Virus Neutralization Test," was authored by researchers at the University of California, San Francisco and Bio-Rad Laboratories. We are pleased to have two of the authors of that paper with this as guests in this podcast.

Dr. Alan Wu is professor of laboratory medicine at the UCSF and Co-Core Laboratory Director at the Zuckerberg San Francisco General Hospital. Shuxia Zhou is a senior staff scientist at Bio-Rad Laboratories Clinical Diagnostic Group working on assay development and we'll start with you Dr. Zhou. Would you tell us how this multiplex surrogate virus neutralization assay works?

Shuxia Zhou: Sure. Before I talk about how the multiplex surrogate virus neutralization assay works, I would like to expand on neutralizing antibodies and the virus neutralization assay in general. A neutralizing antibody is an antibody that helps host cells defend [against] a pathogen or infectious particle

by neutralizing the biological effects. Neutralization makes the particle no longer infectious or pathogenic. Neutralization antibodies are part of the humoral response of the adaptive immunity against viruses or bacteria or microbial toxins. By binding specifically to the surface structure, or antigen, of the infectious particle, neutralizing antibodies prevent the pathogens from interacting with the target cells. However, not all antibodies that bind pathogenic particles are neutralizing. Non-neutralizing antibodies, or binding antibodies, bind specifically to the pathogen, but do not interfere with the infectivity because they don't bind or block the critical region.

Regarding the virus neutralization assay, the virus neutralization assays major neutralizing antibodies in serum and plasma. For example, the plaque-reduction neutralization test (PRNT) is considered the gold standard method for measuring levels of neutralizing antibodies for many viral diseases. SARS-CoV-2 virus enters target cells through the interaction of its surface spike protein with the host cell angiotensin-converting enzyme 2, called ACE2 receptor. Antibodies that can bind to the virus spike protein have the potential to neutralize viral entry into cells and play an important role in the protective immune response to stop this COV-2 infection.

As demonstrated in Figure 1 of the article, our new multiplexed surrogate neutralization assay is a bead-based competition assay and performed on the fully automated BioPlex 2200 System. In this assay, the full-length trimeric spike proteins of SARS-CoV-2 wild-type or variants are coupled separately to the spectrally distinct magnetic beads. The neutralizing antibodies present in serum or plasma compete with biotinylated human recombinant ACE2 receptor [to] bind to the trimeric spike proteins that are coupled to the bead. After washing, the beads are incubated with streptavidin-PE conjugate to generate the fluorescent signal.

And then the reactive beads are streamed through a flow cell and the rate for the bead reading and fluorescent response. So, the presence of neutralizing antibody in the serum samples blocks biotinylated ACE2 binding to the spike protein of bead and reduces the signal. The percentage inhibition of neutralizing antibody is calculated based on the relative fluorescence intensity we call the RFI of the test samples that was normalized by normal serum samples without any anti SARS-CoV-2 antibodies. So, I want to emphasize here that this assay is multiplexed. So, it can simultaneously measure neutralizing antibody inhibition levels to wild-type, as well as multiple variants, in the same reaction.

Bob Barrett: What are the advantages compared to gold standard PRNT assay or other commercial surrogate neutralization assays?

Shuxia Zhou: So, we all know the PRNT assay has several limitations such as requiring a higher biohazard level laboratory, it is technically demanding, low throughput, difficult to automate, and has a long turnaround time because it takes time for preparing cells and for [the] virus to form visible plaques. So all these limitations make it unsuitable for a routine clinical test or large scale studies, for example, vaccine clinical studies. So, to support the limitations of PRNT, many alternative neutralization assays were developed, such as for size reduction, or called micro neutralization assay, pseudotype virus neutralization assay, and the ELISA plate-based surrogate neutralization assays. However, these alternative assays either still need to utilize a pathogenic virus, such as in micro neutralization assay, or involve a manual process with low throughput, such as in pseudovirus neutralization, or use fragments of spike proteins. For instance, the GeneScript ELISA plate-based, surrogate VNT assay.

This assay uses the RBD fragment of the S1 spike protein, which is very different from the native protein, which has S1 and S2 fragments and RBD is only part of [the] S1 fragment. So, the native SARS-CoV-2 spike protein is the trimeric format that contains three identical subunits. Furthermore, these alternative neutralization assays only measure wild-type virus or [one] variant at a time. So, the multiplex surrogate virus neutralizing assay reserves all these limitations. First of all, it doesn't require the use of pathogenic, live viruses and a Biosafety Level 3 lab, but instead uses full-length trimeric spike proteins, which are the same as the native structure of spike proteins on the surface of the virus. So, the multiplex format enables simultaneously measuring neutralizing antibodies, wild-type virus, and multiple variants of concern or variants of interest in the same reaction, and it is a fully automated assay with a throughput of eighty samples per hour and the first results could be generated in 52 minutes and most importantly, it also demonstrates in our article [that] it has very good correlation or concordance to the standard PRNT assay.

Bob Barrett: Doctor, in the article, it was demonstrated that neutralizing antibodies to all variants were statistically lower than wild-type and COVID-19 patients. Is it because the COVID-19 patients included in the study were all wild-type virus infected patients? Have you ever compared the neutralizing antibodies or genotype confirmed variant infected patients?

Shuxia Zhou: This is a very good question. So, we have RT-PCR amplification test results but no genotype sequence test results for the COVID-19 patients in this study. However, the majority of the patients, if not all, were probably infected by

wild-type virus at the time of the sample collection, which was before July 2020.

So, at that time, variants of concern had not been circulating and vaccination had not been started in the US. So the COVID-19 patients in our study cohort are all non-vaccinated, wild-type virus infected patients. Actually, we did the genotype sequencing for the patients who had nasopharyngeal swab samples available and confirmed that they were infected with wild-type virus.

We did tests for the neutralizing antibodies in a group of genotype- confirmed delta-variant-infected patient samples subsequent to the study. As expected, the neutralizing antibodies were higher for the delta variant than wild-type for the genotype-sequence-confirmed delta-variant-infected COVID-19 patients.

Bob Barrett: Well, since the omicron variant had not yet emerged at the time of your study, is it possible to include the omicron variant in this multiplex surrogate neutralizing assay?

Shuxia Zhou: Oh yes. It is actually one of the advantages of the multiplex assay. We have included [the] omicron variant trimeric spike protein coupled beads in our assay and tested samples from vaccinated subjects. The results demonstrated that the neutralizing antibody level, in our case percentage inhibition, after vaccination is significantly lower for omicron compared to wild-type and all other variants including alpha, beta, gamma, and delta. This may explain the relatively high breakthrough infection rates for omicron variant in fully vaccinated individuals.

Bob Barrett: Thank you so much, doctor. Dr. Wu, I would like to turn to you for the final question. From a clinical laboratory medicine perspective, how do you position the surrogate VNT in the future SARS-CoV-2 pandemic or COVID-19 management?

Alan Wu: Well, COVID is moving from a pandemic to an endemic. So, the important question moving forward is determining whether an individual is protected against a future infection. This is applicable for patients who have been previously infected by the virus and those who have received a vaccine and/or the various boosters that have been given. We know that antibody titers to SARS-CoV-2 decline over time and therefore, there will be a need for tests to determine immune status on a regular basis. You can't just assume that because you've been infected or that you have received a vaccine that you are immune. Neutralizing antibodies provide more information than the standard antibody tests that are used just to determine that somebody has been exposed to viral proteins. These neutralizing antibody tests help us determine

if they are better protected against a future immune than just the presence of the antibodies themselves.

And in this BioPlex 2200 assay, we have developed a family of assays against the various variants of concern and therefore, it gives us a better idea of where we might be in terms of immune status moving forward. These neutralizing antibody tests, specifically those that are directed towards these variants of concern, can also be very important for epidemiology studies. We can get some idea as to the likelihood of which variant is positive and therefore be useful for scientists in tracking the history of the epidemic. I need to emphasize, however, that assessing B-cell function is only one arm for determining an individual's immune status, that the neutralizing antibody test does not replace the need for assessing T-cell function.

Bob Barrett: That was Dr. Alan Wu, professor of Laboratory Medicine at the University of California, San Francisco. He was joined by Dr. Shuxia Zhou from Bio-Rad Laboratories and they have been our guests in this podcast on neutralizing antibodies against SARS-CoV-2 variants. Their paper on that topic appears in the May 2022 issue of Clinical Chemistry. I'm Bob Barrett. Thanks for listening.