Comparison of Two Automated Immunoassays for the Detection of SARS-CoV-2 Nucleocapsid Antibodies

Article:
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Guest: Drs. Jacqueline Hubbard and Robert Nerenz are both assistant professors and assistant directors of clinical chemistry in the Department of Pathology and Laboratory Medicine at Dartmouth-Hitchcock Medical Center.

Randi Kaye: Hello, and welcome to this edition of JALM Talk from the *Journal of Applied Laboratory Medicine*; a publication of the American Association for Clinical Chemistry. I'm your host, Randi Kaye.

The COVID-19 pandemic brought on an explosion of new diagnostics for SARS-CoV-2 to including serology assays to detect antibodies to the virus in blood samples. While there are numerous SARS-CoV-2 serology tests now available, only as subset of them have received emergency use authorization from the U.S. FDA, and there are differences in antibody targets and assay design across these tests. Robust analytical and clinical validation of these new tests is needed to aid laboratorians and healthcare providers with SARS-CoV-2 serology assay selection and result interpretation. An article in the March 2021 issue of JALM describes the performance characteristics of two automated immunoassays for the detection of antibodies to SARS-CoV-2.

On today's podcast, we are joined by two authors of this article. The first author, Dr. Jacqueline Hubbard and the senior author, Dr. Robert Nerenz. Doctors Hubbard and Nerenz are both assistant professors and assistant directors of clinical chemistry in the Department of Pathology and Laboratory Medicine at Dartmouth-Hitchcock Medical Center. Welcome to both of you. The first question, why did you undertake this study to evaluate and compare COVID serology tests methods?

Robert Nerenz: Well, I think it's first important to point out that there are two kind of general types of COVID test methods: you have those that are used for diagnosis in patients who are acutely symptomatic, and you want to detect the presence of either viral RNA or protein -- and that's not really what we worked with here. The other general type is for more of a retrospective analysis or surveillance type testing, and to answer this question, "Do I have antibodies to the SARS-CoV-2 virus?" So, really our target audience here where patients who had respiratory symptoms who for whatever reason didn't have access to diagnostic testing, and they're asking

this question, "Did I have COVID? You know, back when I was sick, was this COVID, or was this flu or was it just something else?" We recognized that these patients were going to get testing done somewhere; if we didn't bring it in-house, they would go elsewhere, and we wanted to make sure that patients being tested had access to high quality testing, but that is really the main driver. And we also recognized that it might be useful at some point down the road for surveillance epidemiology type of studies or evaluation of people who had been infected and wanted to provide plasma, and wanted to act as kind of convalescent plasma donors.

Randi Kaye: All right. Thank you. So, what qualities do you look for in a test method like this? What criteria did you use to determine whether the assays would be acceptable for clinical use?

Jacqueline Hubbard: So, I think the two main criteria that we were focusing on were sensitivity and specificity. So, first and foremost, we needed a test to be highly specific, meaning that if it generated a positive result, it would only do so if the patient had a previous COVID infection and developed antibodies to that that. But we also needed it to be sensitive, so that if a negative test result was generated that would only occur if a patient did not have antibodies to COVID. I think out of those two criteria, the one that we probably focused on or weighted a little more heavily was making sure that it was more specific than sensitive. Our rationale for that was: we would really rather report out a false negative result. Meaning a person who actually does have antibodies but the test says that they do not, versus reporting out a false positive result -- which would be a person who does have antibodies, but the test says they do not have them. So we thought that would be kind of a more conservative or safe approach.

Randi Kaye: That makes sense. How exactly did you go about assessing these assays? What types of samples did you use in your evaluation studies?

Jacqueline Hubbard: So in addition to the traditional validation experiments that we do like in precision and stability, we tested several hundred samples that I would say were divided into four major groups, so that we could assess specificity and sensitivity. So, for specificity, the two groups that we focused on were about 170 samples that were collected and frozen prior to, I think it was February 2019; and we call these kind of our pre-COVID cohorts. Then we also had over 200 samples that were collected from inpatients within one day of testing negative for SARS-CoV-2 via PCR. So all of those were expected to be negative, and were used to assess specificity. To assess sensitivity we had two additional groups: we had serum and plasma samples that were collected from 23 convalescent plasma donors, and then, we also had somewhere around 170 samples that were collected

sequentially from 21 inpatients with confirmed cases of SARS-CoV-2, and those were particularly useful because then we could track the antibody response over time after a recent infection.

Randi Kaye: So, what did you find? What were the general findings, and how did the assays perform?

Robert Nerenz: Yes, we found that that both assays really were very specific. We didn't see any false positives, which was great. Maybe a surprise was that not all patients who had PCR-confirmed infection and suggestive symptoms generated antibodies. Most patients who fit those criteria did, the vast majority, but we did see one or two patients who had PCR-confirmed disease and suggestive symptoms -- and it really looked like they had COVID -- but they didn't make antibodies. And so that that was a surprise. Also, we were also looking at the antibody trend over time in our hospitalized patients; we saw different patterns on the two assays and it indicated to us that maybe these assays are detecting qualitatively different types of antibody. It could be that the Abbot detects IgG only, which might explain the continuing-to-climb Abbot pattern, or it could be that the Roche assay detects all antibodies. So, it's a mix of IgG and IgA and IgM, which might explain the pattern that we often saw of a peak in antibody signal, which then kind of dropped off over time.

It could also be something about a change in the polyclonal antibodies mix that the Roche assay is detecting but not the Abbot. So, I don't have a great explanation for that, but it was an interesting finding. The other interesting thing was that we had two immunocompromised patients from whom we had sequential samples that were consistently negative on the Roche platform, and yet consistently positive on the Abbot. And again, we don't really know what this means; from the one perspective, it could be that Roche is telling us something about the quality of the antibodies that these patients are producing, or, on the other hand, it could just be something as simple as the Abbott assay is more sensitive. It's more able to pick up a low amount of antibody that's being made by these immunocompromised patients. And really until we study this further, and have some clinical context to apply to it, we won't really know which assay is "correct," so to speak, in immunocompromised patients.

Randi Kaye: Right. There are always more to learn. So, what message do you hope that the readers will take away from your article?

Robert Nerenz: Yes. I think the one big point is that the nucleocapsid antibody assays really are pretty useful to tell us if someone has had COVID. They're not useful for use in symptomatic patients; they're not diagnostic tests. They're really only useful after the fact, to give us a sense if somebody had

COVID infection or not. A positive result very strongly indicates that the patient did in fact have COVID. We talked about how they're very specific assays, and a negative result 14 days out from symptoms probably indicates that the patient didn't have COVID. It's just they probably had some other kind of infectious cause, but it doesn't absolutely exclude COVID infection -- because we did see some of these patients with the PCR-confirmed disease and symptoms who didn't make antibodies. The other main point is that we would not expect people who received the vaccine, but did not have actual viral infection, to be positive for the nucleocapsid antibodies. It's really only patients who are infected with the virus who will make antibodies to the nucleocapsid protein because the vaccines cause us to make antibodies to the spike protein, but not nucleocapsid.

Randi Kaye: Wow, so maybe relevant follow-up studies are called for. Are you performing any of those that you would like to share?

Jacqueline Hubbard: Well, I think we're always keeping busy. So, we're working on a couple different things. One thing we're doing is we're still following up on some of these patients that were in this initial study, so that we can kind of monitor or see how long antibodies last after a natural infection. So, I believe we tested some up to a couple hundred days post-infection, so that should be fun to watch and see how long they last over time. Another thing we've been working on is we're currently validating a spike antibody platform. So, that'll be useful not only in detecting antibodies from those people who have had a previous natural infection, but now we'll be able to tell if people who have been vaccinated have antibodies, and kind of monitor those antibody levels over time.

Randi Kaye: Wow, very interesting. So thank you so much for your work, and for joining us today.

Jacqueline Hubbard: Thank you for having us.

Robert Nerenz: Absolutely. Thank you.

Randi Kaye: That was Drs. Jacqueline Hubbard and Robert Nerenz from Dartmouth-Hitchcock Medical Center, describing the JALM article "Comparison of Two Automated Immunoassays for the Detection of SARS-CoV-2 Nucleocapsid Antibodies." Thanks for tuning in to this episode of JALM Talk. See you next time, and don't forget to submit something for us to talk about.