COVID-19 Antibody Testing – Buyer Beware

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The current global pandemic caused by the SARS-CoV-2 coronavirus remains uncontained and continues to spread throughout the United States and around the world. The demand for testing has driven a frenzy of activity, including eager venture capital investment, and has overloaded federal regulatory review, as the market has been inundated with dozens of ways to test for the presence of a SARS-CoV-2 infection. Just start typing "COVID-19" into your internet search engine and "COVID-19 testing" will inevitably come up as one of the top suggestions for auto-completion.

How do we make sound decisions about who, when, and by what method to test? As students return to schools and university campuses across the nation, it is clear that the prevalence, quality, and reliability of COVID-19 tests are highly variable, and pose a quagmire for educational institutions, businesses, healthcare systems, and individuals seeking to be part of the solution to monitor and overcome the pandemic.

Within months after COVID-19 was declared a pandemic, hundreds of commercial test kits for detecting SARS-CoV-2 antibodies were released into the market; however, the institutions desirous of antibody testing were not necessarily adequately educated on how to choose an appropriate test, when to use it or how to interpret it. Moreover, the accuracies of these kits were rapidly called into question, since they were developed through abbreviated procedures and sometimes without appropriate clinical test validation[1]. One study directly compared 12 different

antibody test kits, of which 10 of them were commercial rapid point-of-care tests, and found that some of these kits could have a false positive rate of greater than 10%[2].

Clinical test validation is highly regulated and intended to demonstrate the performance of the test using both reference materials (when available) and human-derived specimens. The results are often compared to a standard reference method, or one previously reviewed and approved (by the FDA, for example). In addition to specimens from healthy persons and those known to be infected with SARS-CoV-2, the validation must also include specimens from patients known to have other infections. This measures the test specificity, that is, the possibility of false positives that may be caused by other viruses that could mimic SARS-CoV-2 (such as other coronaviruses and influenza). Such a false-positive result from persons infected with other viruses is a phenomenon known as "cross-reactivity". Rigorous validations that include these and other quality control procedures were not performed by many antibody test kit manufacturers early in the pandemic. Instead, the responsibility to demonstrate the performance of the test kits (the formal validation) was left to individual laboratories planning to implement antibody testing, or simply not done at all.

Antibody tests, also known as serology tests, are performed on a blood draw and typically can detect SARS-CoV-2 antibodies after the second week of symptoms onset. This lag between disease onset and antibody detection is known as the "window period" in infectious disease testing. It reflects the amount of time required by the body to become sensitized to the infection and to mount an immune response by B cells to produce antibodies. Even though antibodies can be easily measured, such testing should not be used to diagnose early infection during the window period, as this could produce a false negative result. Instead, the preferred methods to

diagnose early infection are molecular diagnostic tests that directly detect the presence of viral nucleic acid, performed on nasal swabs or saliva. Rapid antigen tests (detecting expressed viral proteins) may also be used.

If antibody testing is performed during early disease (i.e., less than two weeks after symptoms begin) in lieu of a molecular diagnostic test capable of detecting viral nucleic acid, even in asymptomatic infections, it will very likely miss a true infection and prevent a patient from receiving appropriate care. However, if performed at least two weeks after symptoms begin, appropriately validated antibody tests can accurately identify individuals who have been previously infected with the virus—even those who had only mild symptoms or none at all. Accordingly, for ill and hospitalized patients with COVID-19 who requires immediate treatment even before generating an antibody response, serology testing is of limited value.

However, establishing previous exposure to the virus is currently important for two specific groups: i) younger patients who are suspected to have a severe inflammatory response as a late complication of COVID-19, and ii) patients who have recovered from COVID-19 and want to donate convalescent plasma (a cell-free blood product containing antibodies, which may neutralize the SARS-CoV-2 virus). Convalescent plasma is currently being tested in at least two randomized, controlled clinical trials to determine effectiveness in treating hospitalized COVID-19 patients[2]. Early data from an observational study by the Mayo Clinic indicates that patients who received convalescent plasma early in their disease had better outcomes[3]. It is currently unknown, but important to determine, whether most persons exposed to SARS-CoV-2 and those who become ill with COVID-19 will produce neutralizing antibodies, representing potential convalescent plasma donors, or whether such donors will represent a smaller group of previously infected people

In addition to identifying previous exposure to SARS-CoV-2 for purposes of diagnosing late complications and identifying potential convalescent plasma donors, antibody testing also can be used to perform seroprevalence surveys, which are research studies to determine the proportion of infected individuals in a specific population. However, for antibody tests to generate meaningful seroprevalence data, it is important to estimate the probability of a positive antibody test to be a true infection. This is formally described as the "positive predictive value" (PPV) of antibody testing. PPV is affected by the false positive rate of the test (its specificity), and by the prevalence of infection in the tested population. This aspect of the test—it's PPV—becomes very important when testing a population with a low prevalence. If the test used has a substantial false positive rate, then the chances of a false positive will exceed those of a true positive. For example, consider a test with a 5% false positive rate and 99% true positive rate (the sensitivity of the test at detecting those who truly have the infection). For two different populations with prevalences of 20% and 2% (20 or 2 in 100 who were infected with SARS-CoV-2, respectively), the test's PPV will be approximately 80% and 30%, respectively. This means that for the low-prevalence population, 7 out of 10 positive results will be false positives, essentially rendering a positive result meaningless—or possibly worse, leading to unnecessary additional testing and treatment. Currently, the U.S. prevalence for COVID-19 is highly variable, but the most recent surveillance data from the Center of Disease Control reports a national average of ~2%[4]. Now imagine the scenario where multiple seroprevalence studies across the nation are being performed using multiple test kits for SARS-CoV-2 antibodies with different or inadequately established performance characteristics; this is the situation in which we find ourselves today. With the uneven spread of COVID-19 across the nation, it is likely that complex mathematical

algorithms will be necessary to accurately model regional and local seroprevalence, but this must be done using rigorously validated and highly specific serologic tests for SARS-CoV-2 antibodies.

Perhaps one of the most widely discussed indications for antibody testing is the potential to identify individuals who have developed immunity to SARS-CoV-2 and are presumed to be no longer susceptible to re-infection. This concept of "presumptive immunity" is one that is more commonly used for infectious diseases for which there is an effective vaccine (the process of inducing antibody production by exposing people to purified proteins or inactivated forms of the infectious microbe). The prevailing scientific rationale for presumptive immunity is neutralizing antibodies, which, if present at sufficiently high levels, will inhibit the virus from entering human cells and thus short-circuit the infection. However, not all antibodies produced will have this neutralizing effect. Commercial test kits that are currently available only provide information on whether antibody is present, but do not predict this neutralizing capacity. For COVID-19, a recent study from our own institution has shown that not all individuals who tested positive on commercial antibody kits have high levels of neutralizing antibodies, meaning that these commercial antibody tests are poor indicators of neutralizing antibodies – only slightly more than half of the specimens collected from COVID-19 patients and were positive for antibodies demonstrated high neutralizing capacity[5]. Methods that specifically detect for neutralizing antibodies are laborious and may require advanced biosafety facilities. Hence, they are performed almost exclusively in research labs, or specialized reference labs dedicated for this purpose. Moreover, neutralizing antibodies are not the only immune response our body is capable of generating. While the production of antibodies indicates past exposure and generation of an immune response to SARS-

CoV-2, we do not yet understand whether additional immune responses (e.g., T cell-mediated immunity) are required for clearance of the infection and protection against future infection—that is, whether such antibodies as detected by serologic tests equate to COVID-19 'immunity'.

Crises such as the COVID-19 pandemic represent especially lucrative commercial opportunities because the number of infected and potentially exposed people is extraordinarily high. We need to demand appropriate uses of testing and high standards for testing accuracy, not only to meaningfully address the consequences of the COVID-19 pandemic, but also to mitigate future pandemics. Without adherence to such standards, widespread antibody testing has the potential to cause harmful misinformation and confusion. We should not let supply of test kits alone drive antibody testing, but rather should employ only those tests that are validated and use them only when appropriate to their purpose. Our best outcome from this and future pandemics follows sound science.

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