Serological Testing for COVID-19 Disease: Moving the Field of Serological Surveillance Forward

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Since the outbreak of COVID-19, there has been a worldwide initiative to develop methods for detecting SARS-CoV-2 in the community. Numerous strategies were developed, but most lack comparative accuracy, which is critical in assessing their utility in clinical practice. Testing strategies for COVID-19 disease can be either molecular or serological. Molecular testing is the preferred diagnostic method for symptomatic patients because it detects the presence of viral particles in the respiratory tract by real-time reverse transcriptase PCR (RT-PCR) or other methodologies (1). Serological methods are less useful for testing recent symptomatic infection because serology relies on the body's immune system to generate antibodies against foreign viral particles—a process that can take up to 3 weeks following the initial infection.

The clinical application of serological testing (*a*) identifies the individual's previous SARS-CoV-2 exposure, (*b*) quantifies the levels and class of antibodies against SARS-CoV-2, and (*c*) assesses the ability of these antibodies to prevent reinfection (i.e., quantifies the physiological response of the antibodies to neutralize the virus's ability to infect host cells). Quantifying the neutralization response of a patient's antibodies requires a specialized laboratory and is time consuming and expensive. Consequently, it is preferable to understand the correlation between inexpensive, high-throughput, readily accessible serological binding assay quantitative testing used in clinical practice and physiological neutralization capability. If correlated, serology testing can enhance our ability to investigate the potential of convalescent plasma donors to treat patients who are acutely ill with COVID-19 and understand the relative immunity after exposure in populations at greatest risk of complications (e.g., older adults, healthcare professionals, patients with multimorbidity) if reinfected.

As of this writing, >50 serological binding assays have emergency use authorization from the US Food and Drug Administration (FDA) (2, 3). Although the emergency use authorization for these methods is justified during the pandemic period, the lack of rigorous evaluation for these tests before FDA approval results in highly variable results specific to their antibody class detection, antigen targets, and format (4). Furthermore,

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although there is much debate about the sensitivity and specificity of these assays, a more compelling issue might be understanding the association of qualitative or quantitative results with the physiological response of the antibodies to neutralize the SARS-CoV-2 virus.

In this issue of *The Journal of Applied Laboratory Medicine*, Rychert and colleagues evaluated the performance of 3 widely utilized quantitative serological binding assays for SARS-CoV-2 IgG (Abbott SARS-CoV-2 IgG, DiaSorin Liaison SARS-CoV-2 S1/S2 IgG, and anti–SARS-CoV-2 IgG ELISA from EUROIMMUN) and whether these assays were associated with neutralizing antibody response. A total of 35 samples from patients confirmed as COVID-19 positive by RT-PCR were compared with 100 samples from 80 adult and 20 pediatric healthy donors. All specimens in each panel were tested using the 3 IgG immunoassays. Although serological tests vary in their antibody class detected, IgG antibodies may have a larger role than IgA and IgM because they persist longer and are thought to have higher neutralizing activity to SARS-CoV-2, which is essential for recovery from COVID-19 (2). Seroprevalence studies indicate that >90% of adults ≥50 years of age hold antibodies to all 4 common circulating coronaviruses; therefore, a serological assay must target antibodies highly specific to a SARS-CoV-2 antigen (2). Analysis of the amino acid sequences of the nucleocapsid protein and spike protein of SARS-CoV-2 (the two most common antibody targets in commercially available serological tests) reveals <30% similarity to the respective homologs in other coronaviruses (2), showing promise for high specificity. In their work, all 3 serological assays showed high sensitivity, differing only in samples collected <5 days after confirmed infection by RT-PCR molecular testing. The 2 chemiluminescent assays showed high specificity, even when testing serum samples from individuals who tested positive for respiratory illnesses other than COVID-19. Given low prevalence in some areas, it is possible that even a test with high specificity will generate high rates of false-positive results in large-scale serosurveys. Assuming a prevalence of 5% and specificity of 99%, the positive predictive values for Abbott and DiaSorin are only 80%–85% (5). To improve positive predictive value in low prevalence settings, the CDC recommends an orthogonal testing algorithm such that positive samples are tested by another assay with a different target antigen or format (6).

A range of testing formats exist, although the most widely used and accepted binding assay tests are ELISA- or chemiluminescent-based. They identify both binding and neutralizing IgG antibodies; however, it is generally accepted that neutralizing antibodies are particularly critical for any protective immunity (7). Neutralization assays exist, but compared with other serological assays, they require significantly more time, money, and laboratory expertise. Nonetheless, neutralization assays provide the most accurate reflection of a protective immune response by identifying the antibody concentration needed to prevent viral replication within cells in vitro. Without rigorous FDA oversight, independent evaluation of the different serological assays is critical in guiding utility.

Compared with the Vyriad neutralizing antibody assay used by Rychert et al., positive IgG detection by any of the 3 serological binding assays predicted the presence of neutralizing antibodies; therefore, their ability to discern neutralizing antibody-positive from neutralizing antibody-negative samples shows promise. However, the concentration of neutralizing antibodies detected by the Vyriad assay did not correlate well with the level of IgG measured by any the 3 serological assays, regardless of the target epitope. This finding means that the strength of the read-out (whether color, fluorescence, or radiance) of these 3 serological tests (based on levels of binding and neutralizing antibodies) should not be used to evaluate the strength of the neutralizing antibody response.

In contrast to this report on 3 specific FDA-authorized serological tests, there have been reports of correlation between titers in other antispike serological assays and titers in some viral neutralization assays (8–11). Although it is clear that differences in the specific serological tests utilized in a study play a role in the level of correlation seen with viral neutralization assays, it is important to note that differences in viral neutralization assay formats also affect the ability to directly compare these results to one other. Furthermore, the variability in the immune response of patients recovering from COVID-19 disease (12) and the extent to which patients mount maladaptive innate immune responses, leading to high plasma cytokine levels (13), may help explain the variability in serological assay vs neutralization assay correlation seen with differing patient cohorts. Rychert and colleagues demonstrated the utility of IgG serological assays in predicting a neutralizing antibody response. It is important to remember that a neutralizing antibody test assesses the ability of antibodies to prevent viral replication in vitro—an environment lacking the complex physiological processes that occur in the human body. Specifically, the inability of in vitro neutralizing antibody tests to account for cellular immunity may underestimate the level of protection a patient may have from reinfection (14). In addition, we have yet to study how neutralizing antibodies are able to penetrate specific tissue where SARS-CoV-2 thrives and how comorbidities such as atherosclerosis and immunodeficiency play a role. Assessment of the exact minimum levels of neutralizing antibody required to elicit a protective immune response is needed. The Vyriad assay uses 1:100 dilution to determine antibody presence; however, the FDA requirements for use of an investigational new drug indicate that donor convalescent plasma should have a neutralizing antibody titer of at least 1:160 (2). The work by Rychert and colleagues provides reassurance that high-throughput, readily accessible serological binding assays reflect the presence of neutralizing antibodies but also a cautionary note that titers of IgG, regardless of epitope, may not reflect the extent of conferred immunity. This conclusion will further challenge the optimal implementation of therapies such as convalescent plasma and speaks to the complexity of acquired immunity to SARS-CoV-2.

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