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Editorial

SARS-CoV-2 Serologic Testing: Facts, Fiction, and Fallacies

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Over the past year, the COVID-19 pandemic placed unprecedented demands on clinical laboratory providers, in vitro diagnostics manufacturers, and public health agencies to respond to the testing needs of an international public health emergency. These efforts included developing and implementing a wide array of testing strategies to detect symptomatic and asymptomatic infections, monitor the acute and convalescent phases, and determine adaptive immune responses to SARS-CoV-2. To date, it is estimated that >400 million SARS-CoV-2 tests have been performed for diagnostic, screening, and surveillance purposes in the United States alone (1).

The reference method for diagnosing a SARS-CoV-2 infection continues to be nucleic acid amplification tests (2). Unfortunately, the initial limited availability of these tests (exacerbated by supply chain shortages), lengthy turnaround times, and expense drove efforts to find other suitable alternatives to increase COVID-19 testing capacity—both inside the clinical laboratory and at the point of care.

Serologic (antibody test) methods that have been used effectively in the management of other infectious diseases were soon introduced worldwide and touted as a potential solution to help address COVID-19 testing challenges. However, the use of these tests and their widespread clinical adoption were almost immediately called into question. These concerns centered on the quality and accuracy of the tests and the absence of data to support analytic and clinical performance claims. There was also a general lack of understanding of how to appropriately utilize and interpret these tests in different target populations. In particular, the impact of disease prevalence on predictive values was underappreciated by most clinicians. Unknowns such as the timing and frequency of antibody testing for early detection and limited information on the duration and interindividual variation of adaptive immune responses were also confounding issues.

Today, >440 different SARS-CoV-2 antibody tests are being marketed globally, but only 75 of these assays have formally received emergency use authorization (EUA) from the US Food and Drug Administration (FDA) (3). These tests range from simple CLIA-waived, single-step, cartridge-based immunochromatographic assays to nonwaived, plate-based enzyme immunosorbent immunoassays and chemiluminescence-based immunoassays on high-throughput, random access autoanalyzers. Many of the initial assay formats qualitatively detected the presence or absence of antibodies directed against one or more of the SARS-CoV-2 viral proteins. The main targets are the nucleocapsid and spike proteins. More recently, semiquantitative and quantitative SARS-CoV-2 antibody assays received EUAs that recognize these viral protein targets with a higher degree of sensitivity and specificity. Some laboratory-developed and commercialized neutralizing antibody tests have also been used to evaluate the function of antibody responses.

One of the major problems that has plagued SARS-CoV-2 antibody tests since their introduction into the US market—and has helped undermine both provider and consumer confidence—was the FDA's initial stance allowing early availability during the public health emergency by using a less rigorous review and oversight process than other COVID-19 diagnostics that required EUA submission and approval. In its March 16, 2020, policy, the FDA indicated that a higher level of flexibility was appropriate for antibody tests than for molecular (and antigen) tests that detect the presence of the virus that causes COVID-19, given that antibody tests were not intended for use in diagnosing active SARS-CoV-2 infections (4). Antibody tests were permitted to be used in the appropriate CLIA setting as long as the tests were properly validated and labeled as outlined in this policy and the developer notified the FDA. Regrettably, many of these tests started to be deployed in unregulated settings and without the necessary validation and safeguards required, including the issuance of EUA. Around the country, including Southern California, many state and local public health agencies had to intervene and issue "cease and desist" orders for opportunistic facilities offering antibody tests to screen and diagnose acute infections. By May 2020, the FDA responded to the mounting concerns and revised its policies to address issues of improved transparency in prioritizing access

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and accuracy of COVID-19 antibody testing (5). At that time, FDA Commissioner Steven M. Hahn acknowledged, "Our action today is an important step the agency has taken to ensure that Americans have access to trustworthy tests . . . and we continue to be available to work extensively with industry to help them with developing accurate tests for the public" (6). Immediately, more than a dozen antibody tests were voluntarily withdrawn from the FDA's notification list. As of April 14, 2021, the FDA's updated removal list includes a total of 262 SARS-CoV-2 antibody procedures that should no longer be used and/or distributed for COVID-19 (7).

In this issue of *Clinical Chemistry*, Wang et al. (8) describe a prospective, case-controlled study of 1080 consecutive individuals tested over a 2-week period (August 2020) with 2 commercially available chemiluminescence assays that are widely used for the detection of IgG antibodies to SARS-CoV-2 nucleocapsid and spike proteins. The authors note a subset of discordant results between the Abbott anti-N IgG and EUROIMMUN anti-S1 IgG methods, with only 52 samples positive by both methods, 61 samples positive or borderline positive for anti-S1 alone, and 2 samples positive for anti-N only. They conclude that the real-world performance of these assays may be lower than previously indicated in other studies, with the Abbott anti-N IgG method being less sensitive but more specific than the EUROIMMUN anti-S1 IgG. They also indicate that these findings have major implications for the interpretation of SARS-CoV-2 serology results, especially when differentiating individuals who had naturally occurring SARS-CoV-2 infection vs seroconversion following vaccination that targeted the S protein. Importantly, the authors mention that one of the limitations of this study is that these results may not be readily generalizable across other methods and platforms.

We and others have also reported, through retrospective studies and prospective studies in more targeted populations including symptomatic, asymptomatic, and vaccinated healthcare workers, that SARS-CoV-2 antibody test performance claims may not be as expected when making real-world assessments (9–11). Even SARS-CoV-2 molecular tests by nucleic acid amplification detection do not measure up to the original manufacturer claims, as much of the early data submitted for EUA approvals were generated using selected patient populations or laboratory-contrived samples. Nonetheless, serologic methods pose additional and unique challenges to result interpretation and appropriate use in specific clinical situations; this has been never been more evident than with the evolution seen with SARS-CoV-2 antibody tests over this past year.

When validating assay performance, both analytic and clinical sensitivity and specificity are generally easy to assess; however, a critical factor that determines clinical utility is disease prevalence, which is often overlooked and sometimes difficult to quantify. If an assay that is 100% sensitive and 99% specific is used to test for SARS-CoV-2 when the prevalence is 1%, the positive predictive value of the test will be 50%. In this situation, half of the positive results will be false positives. Early in the pandemic, when disease prevalence was low, laboratories were surprised when they implemented a screen-and-confirm approach (following the CDC's recommendation) and discovered significant discrepancies between orthogonal assays. In the current study by Wang et al., it is unclear what the disease prevalence was at the time of the finding of discordant results between the anti-N and anti-S1 methods, but relatively low disease prevalence likely contributed to the discrepant results. We will need to continually reevaluate and reassess how we use these tests, especially when interpreting results as the SARS-CoV-2–vaccinated population grows.

In their interim clinical considerations, the CDC advises against the use of laboratory testing to assess for immunity to SARS-CoV-2 following COVID-19 vaccination (12). Citing the variable performance characteristics and lack of established clinical utility or serologic correlates of immune protection, current EUA antibody tests are not specifically authorized for monitoring immune responses after vaccination, determining the need for additional doses of the same or different vaccines, or vaccination of unvaccinated individuals. Although the CDC notes that these tests may be helpful in providing evidence of prior infection in an individual with a history of COVID-19 vaccination, a test that specifically evaluates IgM/IgG to the SARS-CoV-2 nucleocapsid protein should be used to identify past infection. Unfortunately, most patients and some physicians may not know what type of antibody test was used or should be ordered or how to interpret the test results. Last, despite whatever new or extended claims may be issued with next-generation assays, antibody testing does not evaluate the cellular immune response, which likely plays an important role in vaccine-mediated and naturally acquired protection.

Clinical laboratorians—the "real-world" laboratory medical experts—are responsible for understanding how the assays used in their laboratories function in the clinical scenarios for which they are being used. It is not sufficient to rely on test manufacturers' or test developers' claims, for many of the reasons highlighted by Wang et al. During a pandemic caused by a novel virus, analytic and clinical certainty is unobtainable. Nevertheless, with carefully designed protocols, and by sharing results in the peer-reviewed literature, we all gain a

fundamental understanding of the facts, fiction, and fallacies of SARS-CoV-2 serology testing.

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