Double-Edged Spike

Are SARS-CoV-2 Serologic Tests Safe Right Now?

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"Validation of new immunologic tests ain't easy," it's been said; validation of new tests for human antibodies to the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a particularly daunting task. While we endure months of disruption to daily life prompted by the ongoing pandemic, serologic testing for antibodies to SARS-CoV-2 has now come to the limelight. SARS-CoV-2 is a highly contagious and acute severe respiratory pathogen that has produced an enormous strain on health care resources. In the United States and many other countries, specific social behavior restrictions have been enacted to moderate the impact of rapid propagation of this contagion (ie, "flatten the curve"). Scientific experts, governmental officials, and other professionals have publicly advocated for SARS-CoV-2 antibody testing to identify individuals who have developed immunity and therefore could potentially reenter the workplace safely despite ongoing high prevalence of the virus. A test for detecting "immune" individuals who will not be reinfected and who will not infect others is an appealing concept, but is it realistic?

The temporary easing of Food and Drug Administration (FDA) marketing/use regulations has enabled the rapid expansion of accurate, fast, and reliable nucleic acid tests to identify acute infection with SARS-CoV-2. Laboratory professionals, diagnostic companies, suppliers, investigators, and hospital administrators have all stepped up to manage acute supply shortages for critical testing components including instruments, test-compatible swabs, and nucleic acid extraction kits, ensuring continued availability of reliable and timely test results. As we approach the peak of severe disease prevalence in several regions (according to comprehensive models developed by epidemiologists and statisticians), we now are faced with a new laboratory crisis: SARS-CoV-2 antibody testing.

Numerous antibody tests have recently become available. Serologic tests for antibodies to SARS-CoV-2 are typically based on lateral flow immunochromatography or enzyme-linked immunosorbent assays (ELISA). Currently available tests predominantly target antibodies to 1 of 2 main surface proteins of the novel coronavirus – the nucleocapsid protein (N) and the spike protein (S). Several assays focus on the S1 subunit of the spike protein, which is somewhat specific to each coronavirus strain.[1,2] The S1 subunits host the binding domain for the angiotensin converting enzyme 2 (ACE2) receptor, which is thought to be the mechanism by which SARS-CoV-2 gains entry into cells.[1] Because the S1 subunit is highly immunogenic and its affinity for the ACE2 receptor appears to correlate with infectivity,[1] it has been the target for SARS-CoV-2 serologic assays with reportedly high sensitivity and specificity.[2,3]

Clinical implementation urgently requires validation of these new assays. Since real-life performance data are scarce, the coronavirus disease 2019 (COVID-19) pandemic has been marked by an inspiring level of interlaboratory collaboration. At Yale-New Haven Hospital, we are particularly grateful for invaluable discussions and sharing of data with Johns Hopkins, Massachusetts General Hospital, Mount Sinai, NYU-Langone, Cornell/Columbia, ARUP, Mayo Clinic, and many others. Scientific journals have contributed via the rapid dissemination of curated studies, and preprint sites offer additional information that can be scrutinized in a shorter time frame, prior to dedicated reviewer analysis. The accumulation and exchange of valuable laboratory evidence has increased our understanding of the serologic testing landscape in a short period of time. As a result, we now know that individuals with symptomatic SARS-CoV-2 infection will generally not have detectable antibodies to SARS-CoV-2 within the first 7 days

of the onset of symptoms.[3,4] The majority of hospitalized SARS-CoV-2-infected individuals with confirmed viral RNA will have detectable IgG antibodies 14 days, and more certainly 28 days, after the onset of symptoms with assay sensitivity and specificity in the high 90 percents.[5] Total antibody concentration appears to rise to detectable levels first; IgM and IgA both rise 1 to 2 days earlier than IgG[3] (unpublished observations). Preliminary data suggests older individuals produce more robust antibody responses. Assays differ in overall performance, but several methods being validated by large laboratories appear comparable. One might therefore ask: "What, exactly, is the problem?"

As valuable as this information is, it may be insufficient to support critical decisions that providers, managers, administrators, and governmental agencies will face, especially regarding immunity in individuals who have remained asymptomatic or minimally symptomatic during the pandemic.

To determine whether an individual is immune to SARS-CoV-2, we must know the pretest probability in the specific population being tested, as well as the sensitivity and specificity for protective antibodies of the assay. A significant challenge is that, to date, serologic data are largely limited to hospitalized, ill patients. There is reason to suspect that serologic findings in asymptomatic or mildly symptomatic exposures may not correlate as well as in hospitalized patients, particularly as anecdotal evidence suggests individuals with low viral loads produce lower antibody titers (unpublished data).

In addition, assessment of antibody *effectiveness* is problematic even in seriously ill patients. Approximately one-third of SARS-CoV-2-infected patients who developed antibodies during hospitalization have been reported to lack antibodies that neutralize virus in plaque growth assays, considered the standard laboratory test for antibody effectiveness.[6] This implies an individual with antibodies may not be immune to reinfection.

Finally, a positive antibody result (in a potentially immune individual) does not guarantee noninfectious status; there may be continuing active viral shedding, particularly if their antibodies are nonneutralizing. The molecular heterogeneity of SARS-CoV-2 subtypes,[7] could also have an effect on the sensitivity and specificity of serologic assays. The imperfect performance of comparable, more established, serologic tests for other diseases (eg, toxoplasma IgM) may be acceptable because we have a much better understanding of the clinical scenarios. Unfortunately, the same confidence does not hold true for SARS-CoV-2 serologic testing.

Quality will play a pivotal role in ensuring we are able to obtain the data required to understand COVID-19 immunity. Some of the serologic tests currently available are simply bound to be inferior and that needs to be documented. The United Kingdom abandoned large-scale purchasing of test kits when the kits failed to satisfy minimum validation metrics.[8] Predictably, online direct-to-consumer tests are being aggressively marketed without any published information to evaluate their clinical performance.[9] While some antigenic targets have shown minimal cross-reactivity with the 4 prevalent non-SARS-CoV-2 coronaviruses,[2] without validation studies there is a real risk that some assays may simply reflect prior exposure to the common cold. Fortunately, reputable commercial entities with experienced scientists, sophisticated equipment, and good manufacturing practices have begun to release serologic assays under FDA guidance. Commercial assays typically undergo extensive prerelease standardization, including testing for interferences and matrix effects, quality control, and test results in large patient cohorts. This sets the stage for acquisition of clinical and epidemiologic data.

But concerns remain when proposals call for testing populations different from those used to validate the assay. What if a health care worker (HCW) who had a fever and no other symptoms 14 days ago wants to return to work and tests positive for SARS-CoV-2 antibodies; can we assume with high confidence that this HCW is both immune and noninfectious? If we are wrong, then we have placed patients and coworkers at risk. A failed prevention is also likely to erode faith in the integrity of laboratory tests for the disease. We have heard the argument that any testing is better than none, providing a path to restoring normalcy, and the lack of which has high ongoing societal costs. As laboratory professionals, we can only respond that for anti-SARS-CoV-2 serology: (1) bad assays will always be counterproductive; (2) good assays have not been proven in the proposed test population; and (3) more experience is needed to help us properly interpret the serologic test results.

Regulatory and health officials appear to recognize these limitations; eg, return to work guidelines from the Centers for Disease Control and Prevention currently do not include serologic testing. The role of serologic testing in identifying potential donors for convalescent plasma remains to be fully investigated (as does the therapeutic benefit of such an intervention in this setting), but other uses for serologic testing may emerge. One such clinical scenario where SARS-CoV-2 serologic assays may be particularly useful is when a positive serology is accompanied by repeatedly negative nucleic acid testing in the setting of a highly suggestive clinical presentation; serology may provide the basis for specific therapies for COVID-19 infection. Still, until we understand the patterns of antibody response to SARS-CoV-2 in asymptomatic individuals, and the correlation of antibody response with susceptibility to

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reinfection, it seems prudent to apply caution to the criteria used to frame economic, social, and corporate policy.

Biological variability is the bane of clinical pathology; in the setting of validation and clinical application of serologic testing, this variability presents a daily struggle. Reputable diagnostic companies and both commercial and academic clinical laboratories have repeatedly demonstrated that the value of dedication to testing quality ensures clinical utility. Health industry manufacturing experts, engineers, quality and regulatory managers, sales professionals, scientists, and physicians have been working diligently under significant duress during the COVID-19 pandemic, to the great benefit of society. As laboratory medicine professionals, we must now leverage these efforts by ensuring that: (1) serologic tests for SARS-CoV-2 antibodies perform as well as intended; and (2) we provide information that enables health care providers, administrators, and health officials to best interpret and apply the available evidence. At this point in the evolution of serologic testing for SARS-CoV-2, we must say in unison "caveat emptor."

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References

1. Cui J, Li F, Shi ZL. Origin and evolution of pathogenic coronaviruses. *Nat Rev Microbiol.* 2019;17:181-192.
2. Okba NMA, Muller MA, Li W, et al. SARS-CoV-2 specific antibody responses in COVID-19 patients. *medRxiv.* 2020;p. 2020.03.18.20038059
3. Lou B, Li T, Zheng S, et al. Serology characteristics of SARS-CoV-2 infection since the exposure and post symptoms onset. *medRxiv.* 2020;p. 2020.03.23.20041707
4. Liu L, Liu W, Wang S, et al. A preliminary study on serological assay for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in 238 admitted hospital patients. *medRxiv.* 2020;p. 2020.03.06.20031856
5. Wölfel R, Corman VM, Guggemos W, et al. Virological assessment of hospitalized patients with COVID-2019. *Nature.* 2020;1-10.
6. Wu F, Wang A, Liu M, et al. Neutralizing antibody responses to SARS-CoV-2 in a COVID-19 recovered patient cohort and their implications. *medRxiv.* 2020;2020.03.30.20047365.
7. Zhao Z, Sokhansanj BA, Rosen G. Characterizing geographical and temporal dynamics of novel coronavirus SARS-CoV-2 using informative subtype markers. *bioRxiv,* 2020;p. 2020.04.07.030759.
8. Bell, J. Trouble in testing land. 2020 4/5/2020 4/9/2020]; https://www.research.ox.ac.uk/Article/2020-04-05-trouble-in-testing-land.
9. https://www.arcpointlabs.com/. 2020 4/10/2020]; https://www.arcpointlabs.com/covid-19-antibody-testing/.

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