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REVIEW ARTICLE

Tale of the Titers: Serologic Testing for SARS-CoV-2—Yes, No, and Maybe, With Clinical Examples From the IDSA Diagnostics Committee

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Diagnosis of acute severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection relies on detection of viral antigens or amplified viral nucleic acids. Serology, although invaluable for epidemiology, is not routinely needed clinically. However, in some settings, serologic data may have direct clinical utility: for example, in evaluation of persistent symptoms in patients without a prior diagnosis of acute infection. In contrast, SARS-CoV-2 serologic testing is sometimes used or requested in situations in which existing data do not support it, such as determination of need for vaccination. In this study, we describe available methods of serologic testing and provide cases supported by clinical vignettes of where such tests can be helpful, as well as examples where they are not. These examples may help clarify clinical decision making in this rapidly evolving area.

Keywords. COVID-19; diagnostics; immunity; serology.

In the early months of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic, very little was known regarding humoral or cellular immune response to this novel human pathogen. Serologic testing was widely used for public health and epidemiologic purposes but played a limited role in the diagnosis or management of coronavirus disease 2019 (COVID-19). Now, after over half a billion estimated cases and more than 10 billion vaccine doses given worldwide (https://coronavirus.jhu.edu/map.html), there is increasing interest in ascertaining the clinical utility of testing patients for immune response to SARS-CoV-2 infection and vaccination.

Immunoglobulin (Ig)M antibodies against SARS-CoV-2 antigens are detectable in 85% of immunocompetent patients within 7 days of symptom onset and IgG antibodies become detectable after 8 days. Over 90% of individuals are seropositive for IgG after day 14 of illness and IgG antibodies remain detectable for months to possibly years. Some immunocompromised patients may take longer to seroconvert or may never seroconvert after acute infection with SARS-CoV-2 [1]. As increasing numbers of people receive treatment with SARS-CoV-2 antivirals, an important unanswered question is whether early treatment might decrease overall antibody responses.

Seroprevalence surveys are an integral component of understanding the spread of infectious diseases among populations and in determining the proportion of the population that has mounted a detectable humoral antibody response to vaccination. Since the initial US Food and Drug Administration (FDA) Emergency Use Authorization (EUA) for antibody assays in Spring 2020, SARS-CoV-2 antibody surveillance studies have been conducted by local public health authorities, the US Centers for Diseases Control and Prevention (CDC), clinical laboratories, and independent research teams [2]. After the introduction of COVID-19 vaccinations in December 2020, interest has extended to include evaluation of antibodies generated in response to COVID-19 vaccination. Current serology surveillance studies, including national serology surveillance conducted by the CDC utilizing samples from blood donors, can distinguish between antibodies generated after infection with SARS-CoV-2 versus those generated after COVID-19 vaccination [3].

Outside of population-level seroprevalence studies, the clinical utility of determining an immunologic response to a pathogen varies by the organism of interest. Examples where testing

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for immune response is clinically valuable include measuring humoral responses against hepatitis B virus (HBV) or cellular reactivity in the setting of latent infection by *Mycobacterium tuberculosis*. However, for other infections, tests assessing immune response are of little clinical value (eg, evaluation of influenza immunity) or may play a very limited role (eg, when evaluating response to active immunization with nonconjugated, 23-valent vaccines against *Streptococcus pneumoniae*). In this review, we assess current data evaluating where and how available immunological tests *may* fit into clinical management of COVID-19, where they may not, and where they may be reasonably considered in some cases (Table 1).

OVERVIEW OF AVAILABLE SARS-CoV-2 SEROLOGY TESTS IN THE CLINICAL SETTING

Antibody-Based Tests

All antibody tests currently available in the United States have been brought to market via the FDA's EUA authority. Initially, an umbrella EUA was issued, then subsequently revoked on July 21, 2020 [4]. The FDA now requires individual EUA for each test. At the time of writing, there were 84 EUA-authorized serologic tests available, using a range of methodologies. Currently, the sole indication for these tests under EUA is to aid in identifying individuals with an adaptive immune response to SARS-CoV-2, indicating recent or prior infection. It is notable that although research regarding vaccine efficacy and public health protection is primarily based on detection of neutralizing antibodies, all but 2 tests with EUA are designed to detect binding antibodies, which are easier to measure but not correlated directly with immunologic protection [5]. The FDA has published test performance characteristics for each assay, including sensitivity, specificity, and positive/negative predictive value based on seroprevalence assumptions [2].

Table 1. Serologic Testing for SARS-CoV-2

Potential Value for Serologic Testing	
Assessing potential sequelae of prior infection	Delayed onset cytokine release syndrome
…	PASC ("long COVID")
	MIS-C, MIS-A
Qualifying convalescent plasma	
Serologic Testing Not Indicated	
Assessing need for COVID-19 vaccination	
Testing of T-cell responses	
Indications Where Data Are Evolving	
Determining organ transplant candidacy	
Prioritizing limited monoclonal antibody to patients most likely to benefit]	

Abbreviations: COVID, coronavirus disease 2019; MIS-A, multisystem inflammatory syndrome in adults; MIS-C, MIS in children; PASC, postacute sequelae of severe acute respiratory syndrome coronavirus 2; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

Infection by SARS-CoV-2 typically induces antibodies to both nucleocapsid (*N*) and spike (S) proteins [5]. Vaccines approved and in use in the United States present a portion of the spike protein to the immune system; recipients would thus only mount a humoral immune response with anti-S antibodies [6]. Currently available serologic assays detect total antibody (including IgA, IgG, and IgM) or are specific for IgG and/or IgM antibodies to the N-protein, S-protein, or both. The specific antigenic target may be affected by variants if mutations are present at the target sites. The FDA requires manufacturers to assess and report on the impact of these viral mutations [7]. Test results may be qualitative (ie, detected vs not detected), semiquantitative (ie, relative concentration of antibody using a test-specific scale), or quantitative (ie, numerical result standardized against a reference material) [8]. The majority of available clinical assays are either qualitative or semiquantitative. Healthcare settings and clinical laboratories utilize SARS-CoV-2 antibody assays that detect anti-nucleocapsid antibodies, antispike antibodies, or both.

Tests for Assessing Cellular Immunity

SARS-CoV-2 also elicits a cellular immune response in the form of T cells that respond to specific viral components. Tests to assess the cellular immune response to SARS-CoV-2 are described in research settings. Currently, there is only 1 T-cell assay with FDA EUA approval for use in the clinical setting; the only indication for this test is to aid in identifying individuals with an adaptive T-cell immune response to SARS-CoV-2, indicating recent or prior infection with SARS-CoV-2 [9]. As with antibody tests, T-cell assays can, in principle, distinguish between response to vaccination versus natural infection by targeting viral antigens other than the spike glycoprotein (eg, nucleocapsid).

INDICATIONS WHERE SEROLOGIC TESTING MAY HAVE CLINICAL VALUE

Assessing Sequelae of Prior Infection in the Setting of Negative Nucleic Acid Test Results

Antibody testing is not recommended for acute diagnosis of SARS-CoV-2 infection [5]. However, serologic testing can help in the evaluation of patients with symptoms suggestive of sequelae of prior infection, including cytokine-release syndrome (CRS), long COVID, and multisystem inflammatory syndrome (MIS). With any antibody assay, it is important to understand which antigen(s) were used to design the assay (ie, nucleocapsid or spike) and the type of antibody targeted (ie, IgM, IgG, total).

Clinical vignette (Cytokine Release Syndrome): A 74-year-old, unvaccinated man with diabetes presents with increasing fevers and dyspnea. SARS-CoV-2 polymerase chain reaction (PCR) is negative. C-reactive protein (CRP) is 250 mg/L and chest CT shows diffuse airspace disease. The patient had a low-grade fever

and mild cough ten days prior, beginning five days after attending a family reunion.

The patient has a high pretest probability of COVID-19 given his potential exposure, lack of vaccination, and symptoms, and has an elevated risk of severe disease due to age, diabetes, and unvaccinated status. His presentation is suggestive of cytokine release syndrome, which can occur 1–2 weeks after acute SARS-CoV-2 infection, at which point nucleic acid tests may be negative in many patients [10]. At more than 2 weeks out from the likely time of his infection, this patient would be likely to have a positive (likely IgG) serology for SARS-CoV-2 [11]. High-risk patients with worsening clinical status have been shown to benefit from immunomodulators, such as tocilizumab (anti-IL-6 receptor monoclonal antibody) [12] or baricitinib (a Janus Kinase inhibitor) [13] but only if administered early, either before or very shortly after the need for mechanical ventilation [13]. In this setting, a positive SARS-CoV-2 anti-*N* or anti-S IgG would convey a high posttest odds of COVID-19-associated CRS. A positive serologic result for SARS-CoV-2 would add confidence to the clinical intervention, because tocilizumab and baricitinib carry significant risk of secondary infection and should not be used indiscriminately. In this scenario, serologic results are only useful in guiding therapy if obtained promptly. In addition, a positive antibody result may obviate the need for further diagnostic workup, potentially sparing the patient more invasive studies such as bronchoscopy (although obtaining lower respiratory tract samples may still have clinical utility if there is a question of true chronic infection, such as for immunocompromised patients).

Clinical vignette (PASC): A 45-year-old previously healthy and active woman presents with six weeks of debilitating fatigue and difficulty concentrating. She completed COVID-19 vaccination series six months prior. She denies recent serious illness but recalls two days of a mild sore throat shortly before the onset of current symptoms.

Patients with asymptomatic or minimally symptomatic COVID-19 may not undergo testing for SARS-CoV-2 by nucleic acid or antigen tests. These individuals remain at risk of developing "long COVID", also referred to as postacute sequelae of SARS-CoV-2 (PASC), albeit to a lesser degree than those with more severe disease. Postacute sequelae of SARS-CoV-2 can degrade quality of life and impair function, and it may persist for weeks to months after acute infection [14]. These patients will typically have negative SARS-CoV-2 nucleic acid tests by the time they present for evaluation, but >90% are positive for COVID-19 antibodies [15]. In this example, the patient would be expected to have positive anti-S IgG serology due to her vaccination, so nucleocapsid serology would be the relevant test to obtain as evidence of infection.

The utility of serologic testing for PASC has changed over the course of the pandemic. Early in the pandemic, seroprevalence was low and a positive antibody test in the setting of suggestive symptoms had high posttest odds for diagnosis of PASC. By early 2022, US seroprevalence for SARS-CoV-2 antibodies was 57.7% [16], decreasing the positive predictive value of SARS-CoV-2 serologic results for diagnosis of PASC [13]. In contrast, the negative predictive value is high when testing more than 2 weeks out from symptom onset, given the high sensitivity of SARS-CoV-2 antibody tests for detection of past infection and the relatively quick (2–14 day) time to mount detectable IgG antibodies. Negative serology results in this setting should prompt further evaluation of alternative etiologies for the patient's symptoms. Positive serology results may be helpful for patient counseling and support. Although there are currently no approved targeted therapies for PASC, having a diagnosis is helpful for some patients.

Clinical Vignette (MIS-C/A): A 15-year-old male presents with fever, abdominal pain, diarrhea, and a rash, along with an elevated CRP, ferritin, and creatinine. Neither he nor his family recall any preceding illness, but several of his classmates have been diagnosed with COVID-19 in recent weeks.

Authorized serological assays may facilitate diagnosis of MIS in children (MIS-C) and adults (MIS-A). Multisystem inflammatory syndrome is a rare but life-threatening syndrome, more often reported in children and adolescents (<18 years) than adults. Although thought to be associated with recent infection with SARS-CoV-2, waves of MIS cases generally follow peaks of SARS-CoV-2 infection by approximately 4 weeks [17]. Clinically, MIS is a systemic inflammatory condition characterized by fever and a constellation of findings that typically include gastrointestinal symptoms, rash, conjunctivitis, myocarditis, and multiorgan dysfunction [18]. Diagnosis of MIS-C/MIS-A can be quite challenging, because symptoms are nonspecific and highly variable between cases, the differential diagnosis is broad, and there is no single diagnostic biomarker.

Both the CDC [19] and the World Health Organization [20] have established case definitions for MIS-C, and the CDC has a working case definition for MIS-A [21]. Because MIS-C is considered to represent a postinfectious sequela of acute SARS-CoV-2 infection, both case definitions require any of the following evidence of recent or current infection: positive reverse-transcription PCR (RT-PCR) or antigen tests, known exposure to COVID-19, or positive SARS-CoV-2 serology. More than half of MIS-C patients have negative nucleic acid testing (RT-PCR) for SARS-CoV-2, because the inciting acute infection is believed to occur 2–5 weeks before presentation with MIS-C symptoms. For this reason, whereas positive respiratory testing for SARS-CoV-2 may support a diagnosis of MIS, negative testing has limited negative predictive value. In contrast, the vast majority (80%–90% in most studies) of patients with MIS-C are positive for SARS-CoV-2 by serological testing [22].

Serological testing is recommended to facilitate diagnosis of suspected MIS-A and MIS-C. To distinguish prior infection from vaccination, testing for the presence of IgG directed against nucleocapsid antigen is preferred, because vaccinated individuals are expected to have elevated titers of antispike

antibody. As in the PASC example, positive anti-nucleocapsid serologies must now be interpreted with caution due to widespread primary infection during the delta and omicron waves. Recent CDC seroprevalence studies indicate that 57.7% of adult Americans were positive for anti-*N* antibodies as well as approximately 75% of children <17 years [16]. Although MIS-C is a rare complication of primary SARS-CoV-2 infection, estimated to occur in 3 per 10 000 individuals <21 years of age [17], the incidence of MIS-C after reinfections is unknown.

Qualification for Use of Convalescent Plasma

A potential role for authorized serologic assays would be to identify donors for high-titer COVID convalescent plasma (CP). Although the National Institutes of Health (NIH) COVID-19 treatment guidelines recommend eligible patients be considered for enrollment in a clinical trial to assess the efficacy and safety of CP, Infectious Diseases Society of America (IDSA) guidelines suggest consideration of CP for immunosuppressed ambulatory patients with mild to moderate COVID-19 who are at high risk for progression to severe disease and have no other treatment options within 8 days of symptom onset [12, 23]. The FDA amended the EUA on January 10, 2022, authorizing high-titer CP for the treatment of COVID-19 in patients with immunosuppressive disease or receiving immunosuppressive treatment in either the outpatient or inpatient setting [24, 25].

Clinical Vignette (Convalescent Plasma): A 71-year-old man with chronic lymphocytic leukemia (CLL) is admitted to the hospital with persistent fever, worsening cough and shortness of breath two weeks after testing positive for SARS-CoV-2 infection. The patient was vaccinated and boosted prior to initiation of CLL therapy; he received rituximab in combination with chemotherapy. Evaluation for bacterial or fungal superinfection is negative and SARS-CoV-2 antibodies are undetectable. SARS-CoV-2 PCR remains positive at low Cycle Threshold. No anti-S monoclonal antibody (mAb) products are available with neutralizing activity against the currently circulating variant.

This patient is immunocompromised, and he is at high-risk for severe disease and is unlikely to mount an effective humoral immune response on his own, so exogenous antibodies may be beneficial if given early after symptom onset. Although the overall literature is mixed, multiple studies suggest the possible benefit of CP in high-risk COVID-19 if antispike titers are high (2–4). Quantitative measurement of donor plasma anti-S titers may identify CP most likely to be beneficial to particularly vulnerable patients. In some settings, CP may be more available than commercial mAbs. More importantly, thresholds for designation as high-titer CP vary among available serologic assays, with FDA guidance available regarding which assays and cutoffs may be used (https://www.fda.gov/media/141477/download).

A limitation of this approach is that most commercially available SARS-CoV-2 serologic assays measure only binding activity and not neutralization, which would be a closer correlate for protection. In addition, given the rapidly changing distribution of SARS-CoV-2 viral variants, CP samples not used promptly after collection may not be well matched to the dominant circulating viral variant when the antibodies are needed.

SCENARIOS WHERE SEROLOGY TESTING IS NOT INDICATED

Determining Response to, or Need for, Vaccination

A frequent request from patients and clinicians is for antibody testing to assess whether they are immune to COVID-19 and/or whether previous SARS-CoV-2 vaccination had been effective.

Clinical Vignette (need for vaccination): An otherwise healthy 55-year-old man requests testing "COVID antibody levels." He had COVID exposure the month prior and developed a week of fever and cough but was not formally tested. He previously received two doses of an mRNA SARS-CoV-2 vaccine. His primary care provider recommends a booster, but the patient feels that he is likely immune at this point, and that demonstration of high titers would show that he would not require another vaccine dose.

Patient requests are one of the current drivers of serologic testing. Patients may desire reassurance that they are protected and may feel that a high anti-S antibody level would indicate that they do not require vaccination or boosting. Many patients will have experiences in which antibody titers to other pathogens (HBV, varicella) are used to infer clinical immunity. Although population-based data do indicate that, on average, higher SARS-CoV-2 antibody titers are associated with reduced risk of severe disease, at this time there are no validated clinical thresholds that can reliably guide individual decisions regarding the potential benefits of a COVID-19 vaccine booster. In additions, as noted above, most commercial assays measure antibody binding rather than neutralization, limiting extrapolation to immune protection. Finally, epidemiological data suggest additional protection by vaccination even in people with high-antibody titers from natural infection, with in vitro studies indicating both higher as well broader neutralization from boosting even in the presence of existing immunity. In this setting, serologic testing should not be used in decision making regarding the need for vaccination or boosting for immunocompetent patients. This practice should be discouraged, although it can be challenging to convey these nuances to patients.

Clinical Vignette (T-cell immunity): A physician has a history of not seroconverting after two series of hepatitis B vaccinations and is concerned that clinically available SARS-CoV-2 antibody tests will not be helpful to either detect prior SARS-CoV-2 infection or response to COVID-19 vaccination.

The physician presents to the infectious disease clinic requesting tests to determine T-cell response to SARS-CoV-2 virus after receiving a primary series and one booster of mRNA COVID-19 vaccinations.

The assessment of T-cell responses to SARS-CoV-2 virus or COVID-19 vaccination is not indicated for routine clinical practice. Furthermore, the availability of assays to detect T-cell responses is limited to research settings. The only SARS-CoV-2 T-cell test with FDA EUA is not widely available in the clinical setting and is only indicated as an aid to identifying individuals with an adaptive T-cell immune response to SARS-CoV-2, indicating recent or prior infection with SARS-CoV-2 or vaccination, and will not help assessment of vaccine response.

INDICATIONS WHERE DATA ARE EVOLVING

Evaluation for Solid Organ Transplant Candidacy

Clinical Vignette (organ donation): A 52-year-old patient with cirrhosis is admitted to the hospital with decompensated liver failure and a high MELD score. The patient completed a COVID-19 vaccine series one month prior, has detectable anti-S antibodies and is deemed an acceptable candidate for liver transplantation. A deceased donor organ offer becomes available several days later. The potential donor has a history of prior COVID-19 diagnosed one month prior, and SARS-CoV-2 RNA is detected by PCR from a nasopharyngeal swab specimen collected as a part of the donor evaluation. The donor had not required COVID hospitalization and chest imaging was normal.

A national shortage of organs combined with high waitlist mortality has led many transplant centers to consider organs (other than lungs) from deceased, SARS-CoV-2 RNA-positive donors. Transmission of SARS-CoV-2 through organ transplantation is uncommon, with only 3 cases of donor-derived infection reported to date, each occurring in lung transplant recipients (https://optn.transplant.hrsa.gov/media/kkhnlwah/sars-cov-2-summary-of-evidence.pdf). Using caution and multidisciplinary expertise, recipients who have been fully vaccinated or have documented antibody evidence of prior infection could be considered for receipt of organs from deceased donors with a history of resolved COVID-19 when the procedure is deemed urgent and potentially lifesaving (patients with documented prior SARS-CoV-2 infection by PCR or antigen testing would not require serologic testing). Several case reports attest to the short-term safety of this approach [26]. Currently, there is insufficient evidence to support donor antibody testing as a means for assessing transmission risk through solid organ transplantation.

Prioritizing Use of Therapeutics With Limited Availability

Clinical Vignette (limited monoclonal antibody availability): A new SARS-CoV-2 variant is rapidly spreading in the population and only one of the approved antispike mAbs shows retained in vitro neutralizing activity. The number of patients meeting inclusion criteria for use of this agent substantially outstrips available supplies.

As new COVID-19 therapeutic agents (and monoclonal antibodies in particular) have become available for treatment and prevention of progression to severe disease, each typically has been in limited supply for at least several months after initial approval. In this setting, it becomes important to prioritize use to those patients most in need of protection. In addition to standard risk factors such as age and medical comorbidities, it is reasonable to consider SARS-CoV-2 serostatus, because patients without significant antispike antibody titers may derive more benefit from exogenous antibody. For example, the American Society of Transplantation suggested assessing serostatus as one factor to consider when allocating limited supplies of prophylactic monoclonal antibody therapeutics such as tixagevimab/cilgavimab [24].

Immunocompromised hosts are at increased risk for developing severe COVID-19. Prolonged SARS-CoV2 infection with viral shedding and breakthrough infection after vaccination have also disproportionately been reported in this patient population. This patient population shows reduced SARS-CoV-2 antibody responses after natural infection or vaccination compared to healthy individuals. However, absolute correlations between antibody levels and immune protection have not been established. When supplies are limited, serologic testing could be considered to prioritize their use toward patients with low or undetectable anti-S titers, who may be most likely to suffer harm without additional protection.

CONCLUSIONS

The mainstay in diagnosis of COVID-19 remains direct detection of viral nucleic acids and/or viral protein antigens. Serologic testing of SARS-CoV-2 infection history is not needed or indicated for routine clinical management. However, there arise several situations as outlined above where testing for anti-SARS-CoV-2 antibodies may provide real value. Clinicians should remain cognizant of where such testing may or may not be useful and of the distinctions between antispike versus anti-nucleocapsid titers, antibody binding versus neutralizing activity, and most importantly in vitro serologic markers versus in vivo protection against severe disease.

Before the deployment of SARS-CoV-2 vaccination, in several studies [27, 28] researchers found protection against reinfection in cohorts of individuals who were seropositive. Analyses of COVID-19 vaccine trial data showed that higher antibody levels (either binding or neutralizing) were associated with lower disease incidence, with a clear dose-response relationship [29]. Subsequent waves of COVID-19 caused by variants of

concern revealed that a given antibody titer provides lower protection against variants compared with the ancestral strain of the virus from early 2020 [30]. Although antibody levels are known to correlate with protection, at this time there is no established threshold of antibody that can be considered protective against infection or severe disease caused by SARS-CoV-2 variants. On the other hand, absence of antibody as determined by a negative antispike antibody test may be helpful as an objective metric to identify patients who have not mounted an immune response to COVID-19 and may benefit from prophylactic or therapeutic antispike monoclonal antibodies.

A major limitation to the clinical utility of SARS-CoV-2 antibody testing is that it does not assess the full spectrum of the immune response, particularly in the critical role of T cell-mediated cellular immunity. Although antibody responses are central to prevention of infection, increasing data indicate that cellular immunity plays a major role in (1) prevention of severe disease, (2) the durability of immune protection, and (3) the breadth of protection against viral variants [31].

Serologic testing can also be diagnostically helpful for patients with persistent symptoms consistent with recent SARS-CoV-2 infection but negative tests for viral RNA or antigen. It is notable that as the population prevalence of previous SARS-CoV-2 infection rises, the positive predictive value of a positive serology falls, whereas the negative predictive value of a negative serology rises. As both SARS-CoV-2 and our understanding of its clinical correlates continue to evolve, optimal strategies for use of serologic analyses will progress as well. Consideration of specific clinical cases, as presented here, may be helpful to providers and patients trying to navigate the complex and evolving landscape of the pandemic.

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