SARS-CoV-2 Antibody Testing: Important but Imperfect

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Pandemic control is daunting and requires a variety of public health and laboratory testing strategies to be effective. Not surprisingly, the COVID-19 pandemic presents several diagnostic challenges.

The gold standard of diagnosis of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection is molecular detection of genomic RNA by nucleic acid amplification or deep-sequencing methods. Although serologic evidence of viral antigens or early (IgM) virus-specific immune response can provide clinically meaningful diagnostic information for some viruses, SARS-CoV-2 IgM antibodies may develop later than typical with other viral infections, and detection of IgM without IgG is rare [1]. Thus, SARS-CoV-2 antibody testing utility rests mainly on identifying prior infection [2, 3].

In contrast, antibody tests provide insights into the epidemiology of infection and are critical for guiding strategies to reduce community transmission. In addition, antibody testing may ultimately provide insights into protective immunity against SARS-CoV-2 infection or disease. Given the pace of COVID-19 spread, and the need to rapidly deploy diagnostic modalities, a plethora of antibody detection methods have been developed. The basic principle of antibody detection involves the incubation of viral proteins or particles with patient serum to capture specific antibodies present in the sample. While many approaches are available to detect or quantify antibodies, many factors influence antibody testing accuracy and performance. These include the platform utilized (enzyme-linked immunosorbent assays [ELISA], lateral flow immunoassays [LFA], chemiluminescent immunoassays [CLIA], etc.), the antibody isotype detected (IgA, IgM, IgG, IgG/IgM total), the specific viral component (antigen) used to bind antibodies (SARS-CoV-2 spike glycoprotein

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[S] or fragment [e.g., S1, S2, RBD, etc.], nucleocapsid protein [NP]), and the specimen type (i.e., serum, plasma, whole blood, finger-stick whole blood). Sample collection time following infection is critical, and subjects should not be tested until at least two weeks from the first day of symptoms. Further complicating antibody testing accuracy, nearly all adults ≥50 years old already have antibodies to all four circulating “common cold” coronaviruses (NL63, 229E, OC43, and HKU1). Though antibody cross-reactivity with SARS-CoV-2 was initially of concern [4], western blot and ELISA studies have not found this to be a significant problem [3].

Despite these complications, several commercial antibody assays demonstrate good sensitivity and specificity (based on studies of samples obtained pre-pandemic and following PCR-proven SARS-CoV-2 infection), and more than fifty test systems have obtained Emergency Use Authorization by the U.S. Food and Drug Administration (FDA). The sensitivity, specificity, and positive and negative predictive value for each is provided on the FDA website http://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/eua-authorized-serology-teste-performance. Recently, IDSA guidelines outlining approaches for antibody testing were published [2].

Using antibody detection assays, information regarding SARS-CoV-2 prevalence is expanding at breakneck speed. For example, a report of a recent meta-analysis of prevalence surveys identified 230 studies (> 1.4 million subjects) that met requirements for analysis. Data interpretation is complicated by the more than 30 different serologic methods used in these studies [5]. Prevalence estimates are also complicated by the finding of a subset of people with documented SARS-CoV-2 infection who fail to generate detectable antibodies following infection, resulting in data gaps. Interestingly, several relatively small cohort studies suggest

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that the likelihood of finding SARS-CoV-2 antibodies following infection correlates with COVID-19 severity [6].

The study by Peterson et al. confirms and refines understanding of "seronegative" SARS-CoV-2 infection. Using a commercially available test, a large (n=2,547) cohort with well-documented infection were studied. One in sixteen individuals (6%) did not have detectable SARS-CoV-2 antibodies two or more weeks following symptom onset [7]. Three additional test methods were compared in a subset of samples with reassuring concordance in results. The study identified variables associated with seronegative test results, with the highest risk occurring in those without COVID-19 symptoms (11%). Among those with symptoms, the likelihood of developing antibodies increased directly with the symptom number and severity [7]. Not surprisingly, immune suppression was associated with lower rates of seroconversion. Antibody detection was more frequent in non-Hispanic black individuals compared to non-Hispanic white individuals and in severely obese individuals compared to those who were underweight [7]. Further studies to validate racial and body weight associations with antibody detection, and to understand the genetic and environmental factors involved in these associations are needed. Nevertheless, these data provide a baseline estimate of seronegative testing following COVID-19 for future epidemiologic studies.

In addition to applying antibody testing for prevalence studies, determining the precise role of antibodies as a correlate of protection against SARS-CoV-2 is clearly needed. Commercial antibody tests like those used by Petersen, et al. [7] measure all antibodies bound to the viral antigen used, regardless of the antibody functional importance. To identify antibodies that correlate with protection against re-infection in vivo, it is necessary to specifically test the antibodies ability to neutralize infectivity

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using in vitro methods. For SARS-CoV-2, this has been done using animal models of infection, or for the 229E coronavirus, human challenge studies [8]. Although incompletely characterized, most SARS-CoV-2 neutralizing antibodies are directed against the surface-exposed spike protein [8]. Commercial assays that test for spike-directed antibodies correlate very well with neutralization assays; however, the importance of antibodies directed against other viral proteins, and the relative sensitivities of these assays are not clearly defined. Thus, commercial antibody testing may misrepresent the potential for protection.

In addition to antibody-mediated SARS-CoV-2 neutralization, T cells play a role in protection against SARS-CoV-2 and related coronaviruses [8-10]. Nevertheless, passive immunization with polyclonal sera and neutralizing monoclonal antibodies prevent infection in a variety of in vitro and animal model systems, and may reduce COVID-19 severity in clinical situations [11, 12]. Thus, antibodies alone are capable of offering some protection under these circumstances.

Using a SARS-CoV-2 rhesus macaque infection model, prior infection clearly provided evidence of protection against disease in a subsequent challenge [13]. Protection was mediated by immunologic control, given low levels of viral subgenomic RNA levels and anamnestic immune responses, and viral replication was unlikely prevented [13]. Although neutralizing antibodies were detected in the animals, the relative protective role of these antibodies, compared to cellular and innate immunity, remains to be determined [8, 10]. In addition, animal models do not always predict human outcomes given the many differences in infection parameters between species. Thus, rigorous clinical studies are needed to determine the existence and extent of sterilizing or disease-reducing immunity elicited by SARS-

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CoV-2 infection. Taken together, these data suggest that initial infection reduces subsequent infection and/or disease.

Once present, the rate and extent of antibody decay following infection and/or vaccination likely influences SARS-CoV-2 susceptibility. Arguments suggesting that antibodies do not persist are largely drawn from human challenge studies using low pathogenicity coronaviruses that cause the common cold (e.g. OC43 and 229E coronaviruses)[8], and relatively small serologic SARS-CoV-2 studies [6]. Infection of humans with 229E coronavirus protects against re-infection, though protection decreases within two years and subsequent reinfection is common. Reports of SARS-CoV-2 reinfection are reported, though infrequent. The majority of these appear to be asymptomatic, suggesting that antibodies may reduce disease severity [14]; however, these data illustrate that antibodies do not always confer protection.

On the other hand, immune memory may play a protective role in SARS-CoV-2 infection. While antibody levels following viral infection or vaccination frequently fall over time [15, 16], this does not necessarily imply loss of protective immunity. Despite antibody decline or loss (using commercial antibody detection methods), immune memory documented by virus-specific T cell proliferative and B cell cytokine responses is frequently detected in individuals who have lost antibodies following immunization, and epidemiological studies indicate ongoing protection in seronegative individuals [17]. Thus, the decline in SARS-CoV-2 antibody levels may not reflect a loss of protective immunity. This is seen in other settings. For example, epidemiologic studies proved that pre-exposure intramuscular immune serum globulin (IM-ISG) prevents hepatitis A virus (HAV) infection, yet no HAV antibodies are found in IM-ISG recipients following administration [18]. Using a more sensitive neutralization assay, HAV neutralizing antibody seroconversion was documented,

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providing a relative correlate of HAV immunity that was missed by commercial antibody test methods [18]. It remains to be determined if this will be true in SARS-CoV-2 infection.

The development of antibody testing methods that predict resistance to infection or severe disease would greatly expedite return to societal norms, and allow confident assessment of infection and vaccine responses. If a specific commercial test can be shown to identify antibodies at levels found to correlate with protection against SARS-CoV-2 infection in exposed or vaccinated individuals, this could be used to confer an "immunity passport" or "risk-free" certificate for a period of time that remains to be elucidated. This would enable individuals to travel or return to work with the presumption that they are protected against a second infection. However, much more work is needed to reach this goal.

The issues summarized above emphasize the potential importance of SARS-CoV-2 antibody detection. The caveat to this is that test methods are imperfect, and the many technical variables and findings of seronegative individuals following SARS-CoV-2 infection show that testing standardization and characterization of their role in protection against SARS-CoV-2 are urgently needed.

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